32nd Annual Conference of the European Society for Biomaterials

September 4 – 8 2022

Bordeaux, France

European Society for Biomaterials

www.ESBbordeaux2022.org

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ORGANIZED ON BEHALF OF

European Society for Biomaterials
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BIOMAT – Association for the development of Biomaterials
https://biomat.fr

WELCOME MESSAGES

Welcome Message by the ESB President
Ana Paula Pêgo

Dear colleagues and friends,

It is my pleasure to welcome you to ESB2022, the 32nd Annual Conference of the European Society for Biomaterials (ESB), to be held between the 4th and the 8th of September 2022 in the beautiful and historical city of Bordeaux!

Hosted in France for the 5th time, we know it will be another success!

For many of us this will be one of the first events we will attend live since the start of the COVID19 pandemic, and for ESB this means to meet face-to-face after 3 long years. I am sure this will be a thrilling event, where we will have the opportunity to reconnect with collaborators and friends, as well as meet new members, all bonded by our interest in Biomaterials. Therefore, it was no surprise for us when we learned about the massive support from our community to this event. Both researchers and companies have strongly engaged in so many ways.

This would not have been possible without the commitment of our hosts. Joëlle Amédée, Jean Christophe Fricain, and Didier Letourneur, have been relentless in putting together an amazing program and a showcase for the best French hospitality. The Conference motto – "Innovative Biomaterials and Translational Research" – is a demonstration of their will to look into what lies ahead, promoting a forum to discuss the present and the future of our Society's contributions to the improvement of the patient's quality of life. So, make the best out of the great plenary, ESB 2022 Awards and keynote talks that have been organized, as well as the exciting program that will give voice to researchers, clinicians, entrepreneurs, and industry.

Last but not least, our hosts included in the program different moments for all of us to network in both formal, e.g., Meet the Industrials Lunch, and informal ways during the social events where the French "Art of Living" will be at display.

Seize the opportunity to reach any of us in the ESB Council to get to know better our Society, how you can engage and to discuss any suggestions.

Together with the rest of the ESB Council, I look forward to meeting you at ESB2022. Let's make it memorable!

Kindest regards,

Ana Paula Pêgo
ESB President

WELCOME MESSAGES

Welcome Message by the President of the Region Nouvelle-Aquitaine and Mayor of the City of Bordeaux
Alain Rousset and Pierre Hurmic

Welcome Message

Dear delegates,

Welcome to Bordeaux and the region Nouvelle-Aquitaine!

Welcome to the 32nd Annual Conference of the European Society for Biomaterials in the city of Bordeaux, in the heart of the Nouvelle-Aquitaine. You have chosen an excellent location for this conference.

Bordeaux, which belongs to the UNESCO world heritage is well known for its finest wines, for cosmopolitan flair and for being one of the world leading congress destinations. Also, the Region's climate of openness and understanding makes it an ideal backdrop for all kinds of meetings such as scientific conferences, technical symposia, corporate and major international conferences.

The City's and Region's activity, commitment to research, its prospering economy, the leading vineyards, and excellent conference facilities, combined with professional experience of highly skillet management staff, are the best guaranties for a successful continuation of the tradition.

Bordeaux and the region Nouvelle-Aquitaine are also one of the most important French and European biomaterials research locations. Bordeaux has become a creative and innovative business center with 11 international centers of excellence more than 60 sectors of excellence in the Nouvelle Aquitaine region, and 175 000 students. The University of Bordeaux has a major program called the Bordeaux Initiative of Excellence – Idex Bordeaux, a competitive research university in tune with its environment.

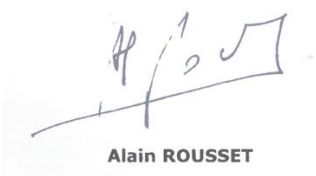
Bordeaux and the region Nouvelle-Aquitaine enthusiastically welcome events of national and international significance.

We are confident that Bordeaux and the region Nouvelle-Aquitaine attractions, their rich cultural live, their refined lifestyle will provide an enjoyable and valuable complement to your congress. With its vast experience hosting world-class events and strong local support, Bordeaux and the region Nouvelle-Aquitaine provide a perfect venue for European delegates.

We are proud to welcome you and hope your conference will be a stimulating exchange of ideas. And please come back another time. Bordeaux and the region Nouvelle-Aquitaine are always worth a visit!

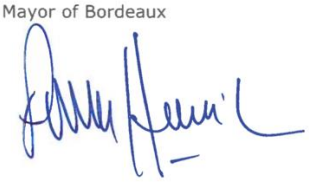
Yours Sincerely,

President of the region Nouvelle-Aquitaine



Alain ROUSSET

Mayor of Bordeaux



Pierre HURMIC

WELCOME MESSAGES

Welcome Message by the ESB 2022 Chair, Dr Joëlle Amédée, and Co-Chairs, Dr Didier Letourneur and Pr Jean-Christophe Fricain

Dear Friends, Dear Colleagues

On behalf of the Organizing Committee, we would like to cordially welcome you to the 32nd Annual Conference of the European Society of Biomaterials which will take place from September 4 to 8, 2022 in Bordeaux, France, as a face-to-face event. After these difficult years with restricted travels, we are excited to host this meeting by re-engaging in rich scientific and informal exchanges between attendees.

The motto of the ESB 2022 Conference is "Innovative Biomaterials and Translational Research". All the ESB meetings attracted a remarkably multidisciplinary group of participants as they bring together at a same and unique place, researchers from various fields including chemistry, physics, biology, biomaterial science, tissue engineering and medicine, companies end clinicians. It is also a unique opportunity to share the most recent technological developments in terms of tools and innovative methods for the production of biomaterials. Despite the uncertain pandemic situation, war in Europe, lack of long-term visibility, the support of our community has been massive, and this confirms our willingness and determination to be together for this ESB 2022 conference. We have received more than 1000 abstracts. On mid-July, we have already more than 950 registered participants from 30 different countries, proving the truly international character of the event.

The program includes 5 plenary talks, given by international renowned invited speakers, and 4 presentations by the ESB 2022 awardees. In addition, it also includes 33 Keynote lectures, 17 symposia, 184 oral communications, 65 Flash poster communications and 840 posters. We want to warmly thank the international scientific committee members for the thorough evaluation of the abstracts, permitting the selection of the oral communications. To stimulate and encourage the visit of the posters, some have been selected as Flash Posters and will be presented at the end of the respective sessions. We have an exciting program including 4 technological sessions, 2 translational sessions and 2 clinical sessions, that really leaves to our motto with the perspective of clinical applications of biomaterials. We also have a session dedicated to the presentation of 5 ongoing European projects.

Best oral and poster presentations made by young researchers will also be awarded. We have also involved the YSF and the young researchers of the French Association for Biomaterials (BIOMAT) in this event.

We want to express our gratitude to all sponsors, exhibitors, and organizations for their support, and we encourage all participants to visit the industrial exhibition in which 27 companies will inform you about their products and their new technologies for the development of innovative biomaterials. You will have the opportunity to meet also the 7 startups within the dedicated start-up's Village. "Meet the companies" and "Meet the Professors" will be organized as lunch meetings to allow dynamic interactions with the attendees especially with young researchers.

Beyond the scientific program, we suggest you to take time to enjoy Bordeaux and its surroundings and of course the famous gastronomy of the south west of France and the emblematic Bordeaux wines.

On behalf of the Organizing Committee, we are now waiting for you.
Enjoy the ESB 2022 Conference!

Joëlle Amédée, Chair

Jean-Christophe Fricain, Co-chair

Didier Letourneur, Co-chair

AWARD WINNERS

George Winter Award 2022

The George Winter award was established to recognize, encourage and stimulate outstanding research contributions to the field of biomaterials.

Professor Abhay Pandit

George Winter Award 2022

Date: Tuesday, 6 September – 9:15-10:00 *(Room A)*

Professor Abhay Pandit is the Established Professor in Biomaterials. He is the Director of a Science Foundation Ireland funded Centre for Research in Medical Devices (CÚRAM) at the the National University of Ireland, Galway. He obtained a PhD from the University of Alabama at Birmingham, where his postgraduate work focused on the modification of a fibrin scaffold to deliver a therapeutic biomolecule and resulted in a clinical trial at the Burn Centre.

Professor Abhay Pandit has over twenty-five years of experience in the field of biomaterials. After a seven-year stint in industry he has worked in academia for the last twelve years. His research is funded by Science Foundation Ireland, the 7th EU Framework programme, Enterprise Ireland, Health Research Board, the AO Foundation and industry sources, and in excess of €78 million. He is the author of 4 patents and has licensed three technologies to medical device companies. He has published more than 180 manuscripts in high-impact factor publications.

He was inducted as an International Fellow in Biomaterials Science and Engineering by the International Union of Societies for Biomaterials Science and Engineering (IUSBSE). He has been elected as a Council Member of the Tissue Engineering and Regenerative Medicine and the International Society and European Society for Biomaterials.

AWARD WINNERS

International Award 2022

The International award was established as a recognition of scientists who have generally spent their career outside Europe, who have been internationally recognized, have a high scientific profile, and have made major contributions to the field of biomaterials.

Professor Joachim Kohn

International Award 2022

Date: Monday, 5 September – 14:30-15:15 *(Room A)*

Professor Kohn is the Director of the New Jersey Center for Biomaterials, an interdisciplinary research center - based at Rutgers - the State University of New Jersey - that spans academia, industry and government. Professor Joachim Kohn leads a scientific staff of approximately thirty research faculty, postdoctoral researchers and graduate students, with expertise in chemistry and chemical engineering, material science, biomedical engineering, cellular and molecular biology, animal studies, and computational technology.

Support comes from multiple grants generously provided by the National Institutes of Health, the National Science Foundation, and the Department of Defense. His research topics: Design and synthesis of innovative biomaterials, the effect of material properties on cell behavior, biocompatibility, development of medical implants and devices for drug delivery and regenerative medicine.

The Kohn Lab has furthered the field of tissue engineering by creating the first combinatorial library of polymers in which the chemical, mechanical and physical properties can be varied incrementally and predictably. In addition to basic studies, his lab has helped to translate our technologies into clinical or pre-clinical products, including surgical meshes, cardiovascular stents, bone regeneration scaffolds, and ocular drug delivery systems. The ultimate goal is to increase the number of viable biomaterials candidates and to accelerate their development into medical devices that alleviate the pain and suffering of patients throughout the world.

AWARD WINNERS

Jean Leray Award 2022

The Jean Leray award was established to recognize, encourage and stimulate outstanding research contributions to the field of biomaterials by early-career scientists.

Doctor Khoon Lim

Jean Leray Award 2022

Date: Monday, 5 September – 9:15-10:00 *(Room A)*

Associate Professor Khoon Lim is a biomedical engineer with specialization in polymer chemistry. His research focus is on adopting a class of polymers known as hydrogels as tissue engineering matrices for a variety of applications. His research technology platform involves photo-polymerizable hydrogel bioinks for 3D bioprinting of functional tissues and also delivery of bioactive molecules to promote tissue regeneration. He has been awarded a total of >$4.5M research grant funding ($3.5M as PI). He is also involved in a number of collaborative projects involving national and international collaborators (Germany, Netherlands, Australia, China and Scotland).

Associate Professor Khoon Lim is also Council member – New Zealand Association of Scientists; Vice President – Australasian Society for Biomaterials & Tissue Engineering (ASBTE); Editorial Board member: *Biomedical Physics & Engineering Express* (IOP Science); Editorial Board member: *Journal of Materials Science: Materials in Medicine*; Associate Editor: *Frontiers in Bioengineering and Biotechnology*; Guest Editor: *Biofabrication*, *Acta Biomaterialia, Advanced Healthcare Materials*; Web and Membership sub-committee member - International Society for Biofabrication.

AWARD WINNERS

Klaas de Groot Award 2022

The Klaas de Groot award was established as a prestigious recognition of scientists who have shown a distinct ability to provide excellent mentorship and guidance to young researchers, helping them to establish their own independent career.

Professor Rui Reis

Klaas de Groot Award 2022

Date: Wednesday, 7 September – 8:30-9:15 *(Room A)*

Professor Rui L. Reis is President of I3Bs (Research Institute on Biomaterials, Biodegradables and Biomimetics), Founding Director of the 3B´s Research Group, Director of the ICVS/3B´s Associate Laboratory (PT Government Associate Laboratory), Full Professor of Tissue Engineering, Regenerative Medicine and Stem Cells, Dept. of Polymer Engineering, School of Engineering, U. Minho. He is also CEO of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, President/Chairman and Chief Scientific officer of Stemmatters, Editor in Chief of the *Journal of Tissue Engineering and Regenerative Medicine,* Wiley-Blackwell, and Director of the PhD program on Tissue Engineering, Regenerative Medicine and Stem Cells of University of Minho. He is in the board of several companies and associations. He was from 2017 to 2019 the President of TECMINHO, the technology transfer office of University of Minho.

Professor Rui L. Reis has been involved in biomaterials research since 1990. His main area of research is the development of biomaterials from natural origin polymers for a range of biomedical applications, including different medical devices, bone replacement and fixation, biodegradable stents and drug eluting stents, drug delivery carriers, partially degradable bone cements and tissue engineering scaffolding. In the last two decades the research of his group has been increasingly focused on tissue engineering (TE), regenerative and precision medicine, stem cells and drug delivery applications. His research group has been working on the engineering of bone, cartilage, osteochondral, skin, intervertebral discs, meniscus, tendons and ligaments, liver and neurological tissues regeneration, as well as on TE approaches for different 3D disease models, including different cancer models, and therapies for diabetes and Alzheimer.

PLENARY SPEAKERS

Pr. Molly Stevens

Professor of Biomedical Materials and Regenerative Medicine and the Research Director for Biomedical Material Sciences in the Department of Materials, Department of Bioengineering and the Institute of Biomedical Engineering at Imperial College London

Date: Sunday, 4 September – 18:00-19:00 *(Room A)*

Molly Stevens is Professor of Biomedical Materials and Regenerative Medicine and the Research Director for Biomedical Material Sciences in the Department of Materials, in the Department of Bioengineering and the Institute of Biomedical Engineering at Imperial College London.

Prof Stevens' multidisciplinary research balances the investigation of fundamental science with the development of technology to address some of the major healthcare challenges. Her work has been instrumental in elucidating the bio-material interfaces. She has created a broad portfolio of designer biomaterials for applications in disease diagnostics and regenerative medicine. Her substantial body of work influences research groups around the world with over 30 major awards for the groups research and Clarivate Analytics Highly Cited Researcher in Cross-Field research.

Prof. Stevens holds numerous leadership positions including Director of the UK Regenerative Medicine Platform "Smart Acellular Materials" Hub and Deputy Director of the EPSRC IRC in Early-Warning Sensing Systems for Infectious Diseases.

PLENARY SPEAKERS

Professor Matthias Lutolf

Professor of Bioengineering at the Swiss Federal Institute of Technology Lausanne (EPFL). Founding Scientific Director of the newly established Roche Institute for Translational Bioengineering, Basel, Switzerland

Date: Monday, 5 September – 8:30-9:15 *(Room A)*

Matthias Lutolf is Professor of Bioengineering at the Swiss Federal Institute of Technology Lausanne (EPFL) and founding Scientific Director of the newly established Roche Institute for Translational Bioengineering. His highly innovative and crossdisciplinary research focuses on the development of advanced bioengineering strategies to produce next-generation organoids with improved reproducibility and physiological relevance for basic research and real-world applications in drug discovery and precision medicine. For example, he pioneered the generation of organoids in fully controllable hydrogel matrices and novel approaches to control organoid patterning and morphogenesis through microfluidics, bioprinting, and microfabrication.

Professor Kristi Anseth

Professor of Chemical and Biological Engineering, and Head of Academic Leadership of the BioFrontiers Institute at the University of Colorado, USA

Date: Tuesday, 6 September – 8:30-9:15 *(Room A)*

Kristi Anseth is the Tisone Professor of Chemical and Biological Engineering, a Distinguished Professor, and Associate Faculty Director of the BioFrontiers Institute at the University of Colorado at Boulder. Dr. Anseth came to CU-Boulder after earning her B.S. degree from Purdue University, her Ph.D. degree from the University of Colorado, and completing post-doctoral research at MIT as an NIH fellow. Her research interests lie at the interface between biology and polymer science where she designs new biomaterials for applications in drug delivery and regenerative medicine. Dr. Anseth's research group has published over 350 peer-reviewed manuscripts, and she has trained more than 110 graduate students and postdoctoral associates. She is an elected member of the US National Academy Engineering, National Academy of Medicine, and National Academy of Sciences. In 2020, she received the L'Oreal-UNESCO for Women in Science Award in the Life Sciences.

PLENARY SPEAKERS

Professor José-Alain Sahel

Vision Research Institute, Sorbonne University, Paris, France

Date: Tuesday, 6 September – 14:30-15:15 *(Room A)*

Professor José-Alain Sahel, MD, is Distinguished Professor and Chairman of the Department of Ophthalmology at the Pittsburgh School of Medicine, and Exceptional Class Professor of Ophthalmology at Sorbonne Université, Paris. He is a clinician-scientist who developed a groundbreaking neuroprotective therapeutic strategy to rescue sight and launched translational first-in-man studies with electronic retinal implants, gene and cell therapies, and optogenetics to restore vision in patients with untreatable blinding retinal degenerations. He co-authored over 660 peer-reviewed articles and 40 patents.

Professor Marja A. Boermeester

Professor of Surgery. Surgeon and clinical epidemiologist. Department of Surgery, Amsterdam University Medical Centers, Amsterdam, the Netherlands

Date: Wednesday, 7 September – 13:45-14:30 *(Room A)*

Marja Boermeester is Professor of surgery and a clinical epidemiologist at the Amsterdam University Medical Centers (AUMC), and principal investigator of many multicenter trials on diagnostics and treatment of abdominal infections. She received many grants, (e.g., > 10 National Health Care & Efficacy Research Grants) and trials were published in international high-ranked journals (NEJM, JAMA, Radiology, BMJ, Lancet, Ann Surg). Her core business is surgery of abdominal infections, complex abdominal wall repair (CAWR), and intestinal failure surgery. She chairs a successful Intestinal Failure Team and CAWR unit in Amsterdam in the AUMC, with referrals from all over the country and is an international expert center on intestinal failure surgery. She has 15 PhD fellows under her supervision, and more than 35 previous PhDs. Pr Marja Boermeester is member or chair of several national and international guideline committees (Antibiotics in Sepsis, Chronic Pancreatitis, Peri-operative Patient Safety, Diagnostics of Acute Abdominal Pain, Emergency Surgery, Incisional hernia surgery).
Previous editor of British Journal of Surgery, Pr Marja Boermeester is member of the Editorial Board of JAMA Surg. She is the President of the Surgical Infection Society Europe (SIS-E) (2019-2022).

KEYNOTE SPEAKERS

Doctor Zaida Alvarez

Ramon y Cajal and leading investigator of the biomaterials for neural regeneration group at the Institute for Bioengineering of Catalonia (IBEC), Spain

Talk: KL2 SFB ESB - Artificial Extracellular Matrix Scaffolds Enhance Maturation of Human Stem Cell-Derived Neurons

Date: Monday, 5 September – 17:45 *(Room F)*

Dr. Zaida Alvarez is currently a Ramon y Cajal and leading investigator of the biomaterials for neural regeneration group at the Institute for Bioengineering of Catalonia (IBEC), Spain. She earned her PhD degree in Biomedical Engineering with Prof. Elisabeth Engel at Polytechnic University of Catalonia in 2014. In 2015, as a self-funded postdoc she joined Professor Samuel Stupp's laboratory, at Northwestern University in Chicago to work on peptide amphiphiles for neural regeneration where she published more than 15 papers in high impact factor journals such as Science, Nature nanotechnology, nature communications, Advanced Science or Nanoletters. In 2019, she was promoted as an assistant professor at the department of medicine, at Feinberg Medical school at Northwestern University where she continued her research in spinal cord regeneration and in vitro platforms for iPs modelling. She is also consulting engineering in a couple of companies in the USA, she has 4 patents already transferred to AmphixBio Incorporation and got numerous awards such as Young Baxter investigator award in 2019, and Rafael Hervada award in 2021.

Doctor Maria Asplund

Head of the research group "Bioelectronic Microtechnology" at the University of Freiburg, Germany

Talk: SYMP-09 - Electrical stimulation and conductive biomaterials in tissue engineering: Advances and challenges

Date: Wednesday, 7 September – 9:45 *(Room H)*

Dr. Maria Asplund is heading the research group "Bioelectronic Microtechnology" at the University of Freiburg, Germany. She develops micro-devices for future bioelectronic medicine, with applications ranging from artificial vision via implantable brain interfaces, to tissue regeneration of skin and spinal cord. Her work contributes to making future bioelectronics smaller, more energy efficient, and seamlessly integrated with biological tissue. With a background in Applied Physics and Electrical Engineering (MSc, Linköping University 2003) and a PhD from the Royal Institute of Technology (Stockholm, 2009) she started her own research group in Freiburg as a Junior Fellow of Freiburg Institute for Advanced Studies

(FRIAS) in 2011, and continued as a junior group leader with the Cluster of Excellence BrainLinks-BrainTools. In 2017, she was awarded with an ERC Starting Grant for her development of an electroactive wound dressing. Since 2019 she is, in addition to her position in Freiburg, appointed as guest professor at the Luleå University of Technology, Sweden and is furthermore appointed as Senior Fellow of FRIAS.

Emilie Attiogbe

Third year PhD Student in the Laboratory of experimental organogenesis LOEX, Québec, Canada

Talk: KL2 Canadian Society & ESB - Innovative 3D Autologous and Immunocompetent Skin Model Reconstructed by Tissue Engineering

Date: Monday, 5 September – 17:30 *(Room E)*

Passionate about tissue engineering, **Emilie Attiogbe** has completed a bachelor and master degree in biochemistry and biomaterials for health as well as an engineering degree at university de Cergy Pontoise (France). Currently a third year PhD student in the laboratory of Pr Véronique Moulin, she is working on the development of an immunocompetent skin model for the study of human wound healing in the Laboratory of experimental organogenesis LOEX (Québec, Canada). A curious person, Emilie is always looking for new challenges. She likes to share science with the community and is involved in various scientific popularization activities. She is also involved in a network in the province of Quebec that brings together students working on stem cells. Willing to be a difference-maker, she also participates in a student venture capital project that aims to invest in the best student high-tech start-ups.

Irem Bayindir-Buchhalter

Editor-in-Chief of Advanced NanoBiomed Research, Weinheim, Baden-Württemberg, Germany

Talk: KL Wiley - Scientific Publishing with Impact

Date: Monday, 5 September – 15:30 *(Room E)*

Irem Bayindir-Buchhalter, Editor-in-Chief of Advanced NanoBiomed Research, studied Molecular Biology and Genetics at the Middle East Technical University, Ankara. She focused on hematopoietic differentiation of embryonic stem cells during her MSc at Heidelberg University and the German Cancer Research Center (DKFZ). She obtained her PhD working on

adipose tissue plasticity and metabolic homeostasis at the same institute. Her passion for science communication and strong interest in continuous learning led Irem to apply for a position in scientific editing at Wiley-VCH. She is based in Weinheim working as a peer review editor for Advanced family of journals.

Doctor Christophe Bureau

Vice-President of Strategic Innovation at Sinomed, Lausanne, Switzerland

Talk: KL Translational I - From Quantum Chemistry to Drug Eluting Stents: a Historical Narrative of How The Electro-Grafting of Vinylic Polymers Ended Up in Patients

Date: Tuesday, 6 September – 15:30 *(Room E)*

Dr. Christophe Bureau obtained his Ph.D in Quantum Physics at Ecole Normale Supérieure in Paris, "Xtof" spent 10 years at the French Atomic Energy Commission (CEA), where he invented a process to coat metals with nanometric polymer brushes, termed "electro-grafting" (eG™). He spun-off in 2007 to create AlchiMedics, raising a total of 12M€ from venture capital. AlchiMedics was acquired in 2012 by Sinomed, a Chinese unicorn in interventional cardiology, neurology and structural heart, listed on the Shanghai stock exchange (688108.SH). As of 2022, more than 1 million patients are implanted worldwide with the BuMA™, HT Supreme™ and NOVA™ stents bearing the eG™ technology, which promotes early re-endothelialization in interventional cardiology and neurology procedures. Since 2019, Xtof is also Vice-President of Strategic Innovation at Sinomed

Doctor Caroline Caradu

Department of Vascular Surgery, Bordeaux University Hospital, France

Talk: KL Clinical cardiovascular - Biomaterials in Vascular Surgery: Clinical Issues and (Endo)Vascular perspectives

Date: Tuesday, 6 September – 15:30 *(Room A)*

Dr. Caroline Caradu obtained her M.D. in 2015 by defending a thesis on the comparison of F-EVAR and Ch-EVAR in complex aortic aneurysms. She then obtained her specialty degree in vascular surgery in 2018 and her Ph.D. in 2020 in collaboration with the Inserm unit 1034, by defending a thesis on the role of endothelial cell dysfunction in the pathophysiology of CLTI. She holds university degrees in ultrasound techniques, wound healing and endovascular

surgery. She completed the majority of her training at the Bordeaux University Hospital in the vascular surgery department of Prof. Eric Ducasse and she is a former Fellow of London St Mary's Hospital. Her research interests include therapeutic approaches to CLTI, endothelial dysfunction, complex aortic aneurysms, vascular graft and endograft infections and the implication of A.I. in the field of vascular pathology.

Professor Gianni Ciofani

Senior Researcher Tenured at the Instituto Italiano di Tecnologia (IIT), Genova, Italy

Talk: KL Antioxidant - Nanozymes in Biomedicine: Route towards Clinical Applications

Date: Monday, 5 September – 10:30 *(Room E)*

Pr. Gianni Ciofani is Senior Researcher Tenured at the *Istituto Italiano di Tecnologia* (IIT), where he is Principal Investigator of the Smart Bio-Interfaces Research Line and Coordinator of the Center for Materials Interfaces (Pontedera, Italy). His main research interests are in the field of smart nanomaterials for nanomedicine, bio/non-bio interactions, and biology in altered gravity conditions. He is coordinator or unit leader of many grants/projects, and he was awarded a European Research Council (ERC) Starting Grant and an ERC Proof-of-Concept Grant in 2016 and 2018, respectively. Gianni Ciofani is author of about 160 papers on international journals, 3 edited books, and 16 book chapters, and delivered about 50 invited talks/lectures in international contexts. He serves as Editorial Board Member of *Nanomedicine UK*, *Scientific Reports*, *International Journal of Nanomedicine*, *Journal of Physics: Materials*, and *Bioactive Materials*. He is Specialty Chief Editor (Nanobiotechnology) for *Frontiers in Bioengineering and Biotechnology*.

KEYNOTE SPEAKERS

Professor Magali Cucchiarini

Group Leader and Vice-Director of the Center of Experimental Orthopaedics at Saarland University Medical Center, Germany

Talk: KL Gene activated - Scaffold-Mediated Viral Gene Delivery for Cartilage and Osteochondral Repair

Date: Tuesday, 6 September – 10:30 *(Room H)*

Pr. Magali Cucchiarini, Group Leader, and Vice-Director of the Center of Experimental Orthopaedics at Saarland University Medical Center (Germany). She graduated from the University of Nice-Sophia Antipolis where she received her PhD Thesis with summa cum laude. She was a senior post-doctoral fellow at Harvard Medical School and worked at the University Hospital Inselsspital (Switzerland). Her major research interest lies in generating novel cell-, gene-, and tissue engineered-based systems to treat orthopaedic disorders. She made over 138 national and international presentations, co-authored 13 books chapters and edited one book (Regenerative therapy for the musculoskeletal system using recombinant adeno-associated viral vectors), and is an author of over 185 peer-reviewed articles. She sits on the Editorial Boards for OAC Open, Journal of Experimental Orthopaedics, BioMed Research International, and OJSM. She serves as a Board Member of the OARSI, was a Board Member of the ORS and is involved at the ICRS and American Society of Gene & Cell Therapy.

Professor Paolo Decuzzi

Senior Scientist and Professor of Biomedical Engineering at the Italian Institute of Technology (IIT), Genova, Italy

Talk: KL Nanoparticles & Theranostic - Engineering Conformable Polymeric microMESH for the Delivery of Combination Therapies against Gliomas

Date: Wednesday, 7 September – 9:45 *(Room B)*

Pr. Paolo Decuzzi is a Senior Scientist and Professor of Biomedical Engineering at the Italian Institute of Technology (IIT). In 2015, he founded the Laboratory of Nanotechnology for Precision Medicine after 10 years of activities conducted in different Clinical Research Institutions in Houston (USA). He is a board member of multiple scientific associations, institutions, and advisory panels, including the Controlled Release Society, IIT, ERC, and the biotech accelerator program SPARK at Stanford. His multidisciplinary research focuses on the rational design of nanoconstructs and implantable devices for the treatment and imaging of various diseases (cancer, cardiovascular and chronic inflammatory diseases), the development of AI-based models for optimizing the therapeutic efficacy of drug delivery systems. In this

context, he has published more than 200 scientific contributions and generated more than 10 patents. His activities have been funded by multiple organizations, in the US and EU, and private companies totaling over $15 million.

Doctor Miguel Dias Castilho

Assistant Professor at the Eindhoven University of Technology. Adjunct appointment at the University Medical Center Utrecht, Netherlands

Talk: KL1 Biohybrid materials - Electrowriting of functional biomaterials

Date: Wednesday, 7 September – 9:45 *(Room F)*

Dr. Miguel Dias Castilho is a tenured Assist. Prof. at the Eindhoven University of Technology and holds an adjunct appointment at the University Medical Center Utrecht, The Netherlands. His research is focused on design and processing of biomaterials that can instruct functional restoration of damaged& diseased tissues, with a particular focus on mineralized tissues. He authored more than 50 publications in peer-reviewed journals and is currently supervising 5 PhD students and 1 post-doc fellow. He is involved in the EU-funded H2020 project BRAV3 (874827) as a WP co-leader and expert on 3D printing and design of microfiber polymeric scaffolds, and as PI in the Eurostars - CHIRON project (E!114399) focusing on development of bioactive bone substitutes. For his contributions on 3D printing for regenerative medicine, he was conferred several awards, including the 2017 Wake Forest Institute Young Scientist Award and the 2021 innovation in Biofabrication award. Since the start of his academic trajectory, he has been able to secure competitive national and international consortium grants

Doctor Agnes Dobos

Cofounder and application specialist at BIO INX, Ghent, Belgium

Talk: KL2 Production methods - Gelatin: From Biomaterial to BIO INK

Date: Tuesday, 6 September – 12:00pm *(Room F)*

Dr. Agnes Dobos earned her BSc in Biochemical Engineering at the Technical University of Budapest in 2014. She continued her studies in Sweden and Austria and received MSc double degree in Tissue Engineering and Medical Biosciences at FH Wien and the University of Linköping in 2016. She joined the Ovsianikov Lab at the Vienna University of Technology (TU

Wien) in 2016 where she is pursued her PhD in the field of multiphoton lithography. She has employed this technology to enable high resolution fabrication of biomimetic in vitro models for organ-on-chip applications. After her PhD she joined the group of Prof Sandra Van Vlierberghe at University of Ghent as a postdoc where she was working on a project to turn the biofabrication technology into reality via the launch of the spin-off company BIO INX where she is a cofounder as application specialist. Her research interests lie in the area of biofabrication and organ-on-chip devices.

Doctor Rui M. A. Domingues

Senior Researcher and Invited Assistant Professor at I3Bs – Research Institute for Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal

Talk: KL Tissue models - Writing 3D In Vitro Models of Human Tendon Within a Biomimetic Fibrillar Support Platform

Date: Wednesday, 7 September – 14:45 *(Room B)*

Dr. Rui M. A. Domingues is a Senior Researcher and Invited Assistant Professor at I3Bs – Research Institute for Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal. He holds a PhD in Chemical Engineering from the University of Aveiro (Portugal), and has studied and worked at different institutions in Europe and US, namely Institute of Chemical Technology (Czech Republic), ENCE (Spain) and University of Texas at Austin (US). His research has focused on the development of functional biomaterials and biofabrication concepts for tissue engineering and regenerative medicine applications (TERM). His main research interest is on exploring different types of nanoparticles, in particular cellulose nanocrystals, magnetic and molecularly imprinted nanoparticles to produce nanostructured biomaterials with biomimetic features to control cell fate in TERM strategies. During his career he has widely applied these concepts on the development of bioengineered systems, particularly targeting tendon tissue regeneration and in vitro modeling.

KEYNOTE SPEAKERS

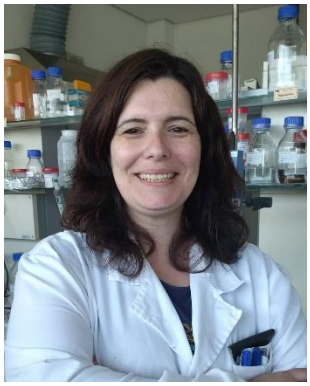
Doctor Audrey Ferrand

Research group leader at the Institut de Recherche en Santé Digestive, Toulouse, France

Talk: KL OoO - Biomaterials, Organoids And Microfluidics: A Winning Trio For Organ-On-Chips

Date: Tuesday, 6 September – 10:30 *(Room A)*

Dr. Audrey Ferrand obtained in 2004 a PhD in Pharmacology from the University of Toulouse. Her research interest in the laboratory of Digestive Biology (INSERM U531) was to decipher the signaling pathways involved in the initiation of colon and pancreas cancers. In 2005, she joined the laboratory of Jeffrey Settleman at the Massachusetts General Hospital (MGH) Cancer Center (Harvard Medical School,USA) where she characterized the differential drug response of mutated EGFR in non-small cell lung cancer. In 2006, she moved to the University of Melbourne (Australia) where she identified the crucial role of gastrin precursors in the tumor-initiating capacity of CD133-positive colon cancer cells. In 2009, she has been recruited as a junior assistant professor (INSERM CRCN) to establish a research program aiming to identify therapeutic targets in colorectal. Since 2018, she leads a research group at the Institut de Recherche en Santé Digestive studying the interactions between the intestinal epithelium and the environment.



Doctor Albina R. Franco

Researcher at I3Bs – Research Institute for Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal

Talk: KL Biomimetics I - Engineered spider silk hybrid proteins with transforming growth factor b3 modulates stem cell response and anticipate new prospects for tendon repair

Date: Thursday, 8 September – 9:00 *(Room A)*

Dr Albina R. Franco is a Researcher at the I3Bs Research Institute of the University of Minho (Portugal). She obtained her PhD (Biotechnology) in 2014 from the Portuguese Catholic University. Her search for better Medical Healthcare has driven her to pursue a career envisioning new solutions by engineering innovative biomaterials for tissue engineering and stem cell differentiation. Her research is currently focused on the exploitation of new recombinant proteins for different biomedical applications, in particular designing new drug-free materials using bioengineered spider silk polymers incorporating different bio-instructive

and functional domains (antimicrobial, tenogenic). She has over 35 peer-reviewed publications with over 555 citations.

Julien Gautrot

Professor in Biomaterials and Biointerfaces in the School of Engineering and Materials Science and Engineering at Queen Mary, University of London, UK

Talk: KL Protein surface interactions - Competitive Binding and Molecular Crowding Regulate the Cytoplasmic Interactome of Non-Viral Polymeric Gene Delivery Vectors

Date: Wednesday, 7 September – 14:45 *(Room C)*

Julien Gautrot is Professor in Biomaterials and Biointerfaces in the School of Engineering and Materials Science and Engineering at Queen Mary, University of London. His research focuses on the development of biointerfaces and microengineered biomaterials for stem cell technologies and the design of advanced cell culture platforms. In particular, his group has been exploring the physico-chemical properties of polymer brushes, the regulation of interactions of biomacromolecules at these interfaces and pioneered their application for the design of stem cell microarrays and for gene delivery. He has published over 90 research articles, including in journals such as Chem. Rev., Nat. Mater., Nat. Cell Biol., Nat. Commun., Angew. Chem. Int. Ed., ACS Nano, Advanced Functional Materials and Nano Letters. In 2017, he was awarded an ERC consolidator grant to explore the design of bioemulsions for stem cell technologies. Since 2021, he is Director of the CDT in Molecular Biochemical Engineering at QMUL

Professor Ronette Gehring

Professor and Chair of Veterinary Pharmacotherapeutics and Pharmacy at Utrecht University, Netherlands

Talk: KL Tissue & Organ engineering - The role of physiologically based kinetic modeling in interpreting the results of in vitro cell-based toxicity assays

Date: Wednesday, 7 September – 9:45 *(Room A)*

Pr. Ronette Gehring is Professor and Chair of Veterinary Pharmacotherapeutics and Pharmacy at Utrecht University (The Netherlands). Her research focuses on the use of mechanistic mathematical models to analyze, interpret and extrapolate pharmacological and toxicological

data with an emphasis on understanding intra- and inter-species differences in kinetics and dynamics. She is also interested in exploring ways to reduce the use of animals in biomedical research through replacement with in vitro and in silico models. She has (co-) authored more than 100 peer-reviewed scientific publications and contributed book chapters to several veterinary textbooks. She is a diplomate of the American College of Veterinary Clinical Pharmacology (ACVCP) and the European College of Veterinary Pharmacology and Toxicology (ECVPT), and is currently serving on the steering committee of VetCAST.

Professor Manuela E. Gomes

Associate Professor and President of the I3Bs Research Institute of the University of Minho, Portugal

Talk: KL Physical forces - New tools in magnetic tissue engineering

Date: Tuesday, 6 September – 10:30 *(Room C)*

Pr. Manuela E. Gomes is Associate Professor and President of the I3Bs Research Institute of the University of Minho (Portugal). Her research interests currently focus on tendon tissue engineering strategies, namely in the development of scaffold materials and bioinks based on biodegradable natural origin polymers, stem cells sourcing and differentiation (using biochemical and physical methods). She has been involved in numerous European and national/regional projects as PI or team member. She currently coordinates a Consolidator Grant from the European Research Council (ERC CoG, MagTendon) and a EC funded Twinning Project (Achilles). She is editor 2 books and co-editor of the Tissue Engineering Encyclopaedia (Elsevier, 2019) and author of 225 full papers, 45 book chapters, 8 patents and has delivered more than 50 lectures in international conferences. Her work has been cited more than 10.706 times, with an h-index of 55 (Scopus).

KEYNOTE SPEAKERS

Sei Kwang Hahn

SeokCheon Chair Professor in the Department of Materials Science and Engineering at POSTECH, South Korea

Talk: KL1 BIOMAT & Human Repair - Wireless Theranostic Smart Contact Lens for Monitoring and Control of Intraocular Pressure in Glaucoma

Date: Monday, 5 September – 17:15 *(Room C)*

Sei Kwang Hahn is the SeokCheon Chair Professor in the Department of Materials Science and Engineering at POSTECH. He obtained his B.S. (1991), M.S. (1993) and Ph.D. (1996) at KAIST. He did his post-doctoral research with Prof. Allan Hoffman at the University of Washington for 2001-2002. After that, he worked at the Hoffman-La Roche group, Chugai Pharmaceutical Co. in Japan for 2002-2005. Since 2005, he has worked as a professor at POSTECH. He was a visiting professor at Harvard Medical School for 2012-2013 and at Stanford University for 2019-2020. He won the prestigious Song-Gok Science and Technology Award in 2022, the Controlled Release Society Award in 2018, the Minister of Health and Welfare Award in 2017, and the Korean President Award in 2015. He was the Samsung Future Technology Committee, the Presidential Advisory Council on Science and Technology, and is the National Academy of Engineering Korea. He is the editorial board member of ACS Biomaterials Science and Engineering, ACS Applied Bio Materials, Biomacromolecules, the associate editor of Biomaterials Research, and the Guest Editor of APL Materials and Advanced Drug Delivery Reviews.

Doctor Christophe Hélary

Research engineer at Laboratory of Condensed Matter Chemistry of Paris (LCMCP), Paris, France

Talk: KL1 Wound healing - Dense Collagen/PLGA Composite Hydrogels Generated by In Situ Nanoprecipitation as Novel Medicated Wound Dressings: In Vitro and In Vivo Evaluation

Date: Monday, 5 September – 10:30 *(Room B)*

Dr. Christophe Hélary got his Ph.D degree in Tissue Engineering and Biomaterials from Sorbonne University, Paris, France in 2010. In 2012, he became a research engineer at Laboratory of Condensed Matter Chemistry of Paris (LCMCP). The Christophe's research activities in the past 10 years have been focused on the development of dense collagen hydrogels for biomedical applications. He uses collagen in dense phase to improve stability,

physical and mechanical properties of hydrogels. Since 2013, his research orientation is towards the development of composite hydrogels to deliver biomolecules (drugs, therapeutic genes) in a controlled manner. He associates dense collagen with synthetic polymers (PLGA, PCL, PLA), natural polymers (alginate, hyaluronic acid), silica nanoparticles or hydroxyapatite. He has published 58 articles and is an inventor on 3 patents. He is a member of the Biohydrogel Society and BIOMAT, the French society for biomaterials.

Doctor Andreas Lendlein

Fellow of the Materials Research Society (2021), the American Institute for Medical and Biological Engineering (2021) & the Controlled Release Society (2020). Founding Editor-in-Chief of the journal Multifunctional Materials and serves on the Executive Advisory Board of Wiley-VCH´s Macromolecular Journals

Talk: KL Nanofibrous materials - Design and Fabrication of Multifunctional Micro-/Nanofiber Meshes for Healthcare

Date: Wednesday, 7 September – 9:45 *(Room E)*

Dr. Andreas Lendlein received his doctoral degree in Material Science from Swiss Federal Institute of Technology (ETH, Switzerland). His research interests comprise the creation of material functions by design and implementation of multifunctionality in polymer-based materials for biofunctional implants, controlled drug release systems, healthcare technologies and soft robotics. He published 745 papers, is an inventor on 338 issued patents and published patent applications in 63 patent families, and received 23 awards for his scientific work and his achievements as an entrepreneur. He is elected fellow of the Materials Research Society (2021), the American Institute for Medical and Biological Engineering (2021) & the Controlled Release Society (2020), founding Editor-in-Chief of the journal *Multifunctional Materials* and serves on the Executive Advisory Board of Wiley-VCH´s Macromolecular Journals.

KEYNOTE SPEAKERS

Doctor Riccardo Levato

Associate Professor of Translational Bioengineering and Biomaterials at the Department of Clinical Sciences (Utrecht University), and at the Department of Orthopedics (University Medical Center). Principal Investigator at the Regenerative Medicine Center, Utrecht, Netherlands

Talk: KL Bioprinting technologies - Advances in Light-based Bioprinting: Layerwise and Layerless Volumetric Technologies for Organoid Culture and Tissue Engineering

Date: Thursday, 8 September – 9:00 *(Room C)*

Dr. Riccardo Levato is Associate Professor of Translational Bioengineering and Biomaterials at the Department of Clinical Sciences (Utrecht University), and at the Department of Orthopedics (University Medical Center), and Principal Investigator at the Regenerative Medicine Center. He worked in several research groups across Europe: 3Bs, University of Minho (Portugal); BioMatLab, Technical University of Milan (Italy), Institute for Bioengineering of Catalonia (IBEC, Spain), and he holds a cum laude PhD in Biomedical Engineering from IBEC and the Technical University of Catalonia. In 2020 he was awarded a Starting grant from the ERC on the development of a novel volumetric bioprinting technology for organoid research and to engineer functional bone marrow analogues in vitro. Since 2021, he is coordinator of a European consortium (ENLIGHT), on biofabricated pancreas to study treatments for diabetes. He published more than 55 papers, and he was conferred several awards including the 2016 Wake Forest Institute for Regenerative Medicine Young Investigator Award, and the 2021 Jean Leray award from the European Society for Biomaterials.

Professor João F. Mano

Professor at the Department of Chemistry of the University of Aveiro. Vice-director of CICECO, Aveiro Institute of Materials, Portugal

Talk: KL Multifunctional & Cell-instructive hydrogels - Design of hydrogels using natural macromolecules with improved structural and functional properties

Date: Thursday, 8 September – 9:00 *(Room F)*

Pr. João F. Mano is a full professor at the Department of Chemistry of the University of Aveiro (Portugal). He is vice-director of CICECO – Aveiro Institute of Materials where he is directing the COMPASS Research Group (compass.web.ua.pt). His current research interests include the use of biomaterials and cells towards the development of multidisciplinary concepts especially aimed at being used in regenerative and personalised medicine. He is the Editor-in-Chief of

Materials Today Bio (Elsevier). He has been coordinating several national and European research projects, including two advanced grants from the European Research Council. João F. Mano has received different honours and awards, including two honoris causa doctorates (Univ. of Lorraine and Univ. Utrecht) and was elected fellow of the European Academy of Sciences (FEurASc), Biomaterials Science & Engineering (FBSE) and American Institute of Medical and Biological Engineering (FAIMBE).

Doctor Christophe Marquette

Research Director and Deputy Director at the Institut de Chimie et Biochimie Moléculaires et Supramoléculaires (ICBMS, UMR5246, CNRS-Université Lyon1), Lyon, France

Talk: KL1 Bioinks - 3D Bioprinting: in vitro model, implantable bioink and intraoperative methods

Date: Monday, 5 September – 10:30 *(Room A)*

Dr. Christophe Marquette received the Doctorat in Biochemistry (1999) from the Université Claude Bernard-Lyon 1. After a two years post-doctoral fellowship at the Concordia University (Canada, Qc), he integrated the Centre National de la Recherche Scientifique (CNRS) in 2001. He is presently permanent Research Director and Deputy Director at the Institut de Chimie et Biochimie Moléculaires et Supramoléculaires (ICBMS, UMR5246, CNRS-Université Lyon1) and is in charge of the Biochips and Micro-arrays Group, dealing with biology/surface interaction and 3D printing of living cells. Since 1998, he is author or co-author of more than 150 articles (H index: 39), 15 book chapters, 18 patents and more than 120 communications. He is also the founder the Nano-H, AXO Science, HealShape and 3Deus Dynamics Companies. He is also the founder of the unique 3d.FAB platform, specialised in additive manufacturing technologies for Life Science (http://fabric-advanced-biology.univ-lyon1.fr/).

KEYNOTE SPEAKERS

Professor Lorenzo Moroni

Head of Complex Tissue Regeneration (CTR). Head of MERLN Institute, Maastricht, Netherlands

Talk: KL1 Sustainability of biomaterials - Sustainability of Additive Manufacturing Technologies to create Smart Scaffolds for Regenerative Medicine

Date: Thursday, 8 September – 9:00 *(Room B)*

Pr. Lorenzo Moroni received his PhD cum laude in 2006 from Twente University on 3D scaffolds for osteochondral regeneration. In 2014 he joined Maastricht University, as a founding member of the MERLN Institute for Technology-Inspired Regenerative Medicine. In 2016, he became full professor in biofabrication for regenerative medicine, and is chair of the Complex Tissue Regeneration Department and director of MERLN. His research group aims at developing biofabrication technologies to control cell fate, with applications spanning from skeletal to vascular, neural, and organ regeneration.

Doctor Erik Nilebäck

Senior application scientist at Biolin Scientific

Talk: KL in situ monitoring - QCM-D as a powerful tool to design and evaluate Biomaterials

Date: Tuesday, 6 September – 10:30 *(Room F)*

Dr. Erik Nilebäck is a senior application scientist at Biolin Scientific and has been active in the world of surface science, life science and innovation for over 15 years. He has a PhD in bioscience from Chalmers University of Technology in Sweden on the topic "QCM-D – With Focus on Biosensing in Biomolecular and Cellular Systems" following his MSc studies in Engineering Biology at Linköping University. Biomaterials is the scientific area that sparked Erik's interest for research and during his PhD project he studied how QCM-D can be used to study specificity in enzyme- and/or cell-carbohydrate interactions relevant for tissue engineering. Today, in the application development team at Biolin Scientific, Erik is providing technical expertise for the QSense product range to customers, colleagues and collaborators.

KEYNOTE SPEAKERS

Doctor Jos Olijve

Scientific Support Manager at Rousselot, Eindhoven, Netherlands / Gent, Belgium

Talk: KL1 Production methods - Influence of Endotoxin on Cellular Activity

Date: Tuesday, 6 September – 11:30 *(Room F)*

Dr Jos Olijve is Scientific Support Manager at Rousselot and among others responsible for biomedical co-developments with academia, CRO's, Institutes and customers. He is also involved in product developments and creating background knowledge on the behavior of physical, chemical and purity properties of gelatin in various biomedical and pharmaceutical applications. Mr. Olijve has an educational background in biochemistry. Before joining Rousselot he worked for FujiFilm, where he was responsible for the development of photographic gelatins. He was also involved in the development of recombinant gelatin and gelatin based peptides for both photographic and biomedical applications. Dr. Olijve is the holder of 18 patents/patent applications, and (co-)author of 15 scientific papers.

Doctor Valentina Onesto

Senior Postdoctoral Researcher at CNR-Nanotec in Lecce, Italy

Talk: KL Scaffold - 3D sensing scaffold for extracellular pH mapping at single cell level in tumor models

Date: Monday, 5 September – 10:30 *(Room C)*

Dr. Valentina Onesto received a Master degree in Biomedical Engineering from the Polytechnic of Milan, Milan (Italy) and a PhD in biomedical, system and applied nanotechnologies engineering from University Magna Graecia, Catanzaro (Italy). She has been a Visiting Researcher at the Italian Institute of Technology (IIT) of Genova (Italy) and a Visiting Research Associate at King's College London, London (UK). From January 2019 she was a Senior Research Fellow Scientist at the CRIB@IIT of Naples (Italy). In September 2020 she started a Postdoctoral Researcher position at CNR-Nanotec in Lecce (Italy) within the ERC-Starting Project INTERCELLMED (No. 759959) and in July 2021 she became a Senior Postdoctoral Researcher in the same institute.

KEYNOTE SPEAKERS

Doctor Maria Pereira

Co-founder and Chief Innovation Officer at TISSIUM, Paris, France

Talk: KL Translational II - Shinning a light on tissue repair: from the bench to the bedside

Date: Tuesday, 6 September – 17:15 *(Room E)*

Dr. Maria Pereira is a co-founder and Chief Innovation Officer at TISSIUM, a start-up focused on leveraging its polymer platform for atraumatic tissue reconstruction. Maria holds a PhD in Bioengineering from the MIT-Portugal program and an MBA from INSEAD. She co-invented TISSIUM's core technology during her PhD studies in the laboratory of Prof. Jeff Karp, where she focused on the development of biomaterial-based solutions for unmet medical needs in the fields of tissue adhesion and drug delivery. Maria managed several multi-center collaborations leading to high impact publications in journals such as Science Translational Medicine and Advanced Materials. The first product from this core technology reached clinical stage in 2016. In 2014, she was named one of MIT Technology Review Magazine's Innovators Under 35 Award and in 2015, she was recognized by Forbes Magazine as part of the 30 under 30 list in Healthcare.

Doctor Massimo Perucca

Co-owner and scientific manager at Project HUB 360, Avigliana, Italy

Talk: KL3 Sustainability of biomaterials - Are bio-based and circular solutions more sustainable? A scientific perspective to assess environmental, economic and social sustainability of bio-circular options

Date: Thursday, 8 September – 9:00 *(Room B)*

Dr. Massimo Perucca, MD Physics PhD in FluidDymamics, starting from fundamental research in fusion plasmas at MIT, Boston-MA, he devoted his efforts to the industrial applications of plasma technologies and nanotechnologies, targeting to safe and sustainable-by-design (SSbD) solutions. With more than 20 years' experience in Life Cycle Assessment (LCA) Dr. Perucca is involved in the investigation of circular models for bio-based and nano-composite materials obtained by agro-forest and fish industry sidestreams. In 2005 he started the company Project HUB 360. Currently maintaining the role of co-owner and scientific manager, with the support of his team of ten experts, he is developing custom tools for sustainability assessment and multi-criteria decision quantitative models for SSbD decisions support tools.

KEYNOTE SPEAKERS

Since 2010 he is technical director of the Italian magazine "Trattamenti e Finiture". He actively contributed to more than 20 European large research projects and several national and transnational projects; he counts several scientific and technical publications in plasma technology, nano-composite coatings, environmental and economic sustainability.

Doctor Mauro Petretta

Senior Scientific Advisor at REGENHU, Villaz-Saint-Pierre, Switzerland

Talk: KL2 Bioinks - Extending the capabilities of bioprinting with the Dynamic Formulation Module (multi-material patterning, mixing and core-shell) in a single dispensing process

Date: Monday, 5 September – 11:00 *(Room A)*

Dr. Mauro Petretta is the Senior Scientific Advisor at REGENHU and is responsible for the development of scientific applications using the bioprinting platform and for interactions with the scientific community. His work focuses on the processing of biomimetic and functionalized biomaterials, ranging from polymeric composites to cell-laden hydrogels, through additive manufacturing technologies for use in fields such as hard and soft tissue regeneration, drug discovery, personalized pharmaceuticals or bioelectronics. He brings years of research experience on tissue engineering and regenerative medicine combined with an in-depth know-how of REGENHU's bioprinting platforms.

Doctor Jens Puschhof

Group leader of the Epithelium Microenvironment Interaction Laboratory in the Microbiome and Cancer Division at German Cancer Research Center in Heidelberg, Germany

Talk: KL Biomaterials for cancer - Applications of organoids in bioengineering

Date: Thursday, 8 September – 9:00 *(Room E)*

Dr. Jens Puschhof is group leader of the Epithelium Microenvironment Interaction Laboratory in the Microbiome and Cancer Division at German Cancer Research Center in Heidelberg, Germany. His group studies the role of microenvironmental factors in colorectal carcinogenesis using organoid- and organ-on-a-chip co-cultures. Dr. Puschhof graduated with a MSc in Oncology from the University of Oxford and obtained his PhD cum laude from Utrecht University. During his PhD and postdoc in Hans Clevers's group at the Hubrecht Institute, he

and his colleagues developed organoid models of the snake venom gland and made seminal contributions to the understanding microbial impacts on colorectal cancer development.

Professor John A. Rogers

Director of the Querrey Simpson Institute for Bioelectronics at Northwestern University, USA

Talk: KL Electroactive - Soft Electronics as Interfaces to Living Tissues

Date: Tuesday, 6 September – 10:30 *(Room E)*

Pr. John A. Rogers obtained BA and BS degrees in chemistry and in physics from the University of Texas, Austin, in 1989. From MIT, he received SM degrees in physics and in chemistry in 1992 and the PhD degree in physical chemistry in 1995. From 1995 to 1997, Rogers was a Junior Fellow in the Harvard University Society of Fellows. He joined Bell Laboratories as a Member of Technical Staff in the Condensed Matter Physics Research Department in 1997, and served as Director of this department from the end of 2000 to 2002. He then spent thirteen years on the faculty at University of Illinois, most recently as the Swanlund Chair Professor and Director of the Seitz Materials Research Laboratory. In the Fall of 2016, he joined Northwestern University as the Louis Simpson and Kimberly Querrey Professor, where he is also Director of the recently endowed Institute for Bioelectronics. He is a member of the National Academy of Engineering, the National Academy of Sciences, the National Academy of Medicine, the National Academy of Inventors and the American Academy of Arts and Sciences.

Professor Ipsita Roy

Professor of Biomaterials, Department of Materials Science and Engineering, University of Sheffield, UK

Talk: KL2 Sustainability of biomaterials - Sustainable Biomaterials of bacterial origin and their use in Biomedical Applications

Date: Thursday, 8 September – 9:00 *(Room B)*

Pr. Ipsita Roy, Professor of Biomaterials, Department of Materials Science and Engineering, University of Sheffield, is an expert in microbial biotechnology, natural biomaterials and their biomedical applications. Professor Roy was awarded the prestigious Inlaks Scholarship to

study for her Ph.D. at the University of Cambridge, UK. At Cambridge, she was awarded scholarships including the Churchill College Scholarship and the Cambridge University Philosophical Society Fellowship Award. Her postdoctoral work was at the University of Minnesota, USA. She has published over 100 papers in high 'Impact Factor' journals such as Biomaterials, ACS Applied Materials Interfaces, with an H index of 40. Her group is focussed on the production of novel natural and sustainable polymers such as Polyhydroxyalkanoates, Bacterial cellulose and their biomedical and environmental applications. She is an editor of Scientific Reports and Biomedical Materials. Her total grant portfolio is more than 10 million pounds.

Professor Alberto Saiani

Professor of Molecular Materials at the University of Manchester, UK

Talk: KL1 Supramolecular - b-sheet peptide based supramolecular scaffolds: from design to application

Date: Tuesday, 6 September – 10:30 *(Room B)*

Pr. Alberto Saiani is currently Professor of Molecular Materials at the University of Manchester. He graduated with a PhD in Polymer Physics from the University Louis Pasteur (Strasbourg, France). Subsequently, he held several post-doctoral positions in Japan, UK and Belgium before joining in 2002 the University of Manchester where he established the Polymers & Peptides Research Group (polymersandpeptides.co.uk). His work includes fundamental, industrial, and translational research and spans a wide area of experimental polymer and biopolymer science. In 2014 he co-founded a start-up company, Manchester BIOGEL (manchesterbiogel.com), which develops and commercialises advanced gel-based combination products for the life science and biomedical sector based on the technology developed in his group.

KEYNOTE SPEAKERS

Doctor Jochen Salber

Head of the Department of Experimental Surgery at the Center for Clinical Research at the Ruhr University Bochum (RUB). Senior physician at the Department of Surgery at the University Hospital RUB, Germany

Talk: KL1 Advances in characterization - New advances in preclinical validation tools through biofabricated human 3d tissue equivalents-on chips integrated with innovative nmr spectroscopy

Date: Monday, 5 September – 11:30 *(Room F)*

Dr. Jochen Salber holds a PhD in polymer chemistry and biomedical sciences of the RWTH Aachen University (Germany) and is a board-certified orthopedic surgeon with 14 years of clinical experience working as emergency doctor and trauma surgeon. He is a full instructor of the European Resuscitation Council (ERC) and educates future ER specialists. Currently, he is head of the Department of Experimental Surgery at the Center for Clinical Research at the Ruhr University Bochum (RUB) and a senior physician at the Department of Surgery at the University Hospital RUB, Germany. His clinical interests are focused on Foreign Material-Associated Infections (FMAI), sepsis and multiple trauma immunology. His research group is working in the fields of clinically applied biomedical research with focus on biocompatibility assessment, tissue engineering and regenerative medicine. Refereed Journal Publications >60; h-index: 21; i10-index: 29; Citations: 1747; RG-score: 34.82

Maryam Tabrizian

Professor at the Biomedical Engineering Department and Faculty of Dental Medicine and Oral Health Sciences at McGill University, Montreal, Canada

Talk: KL1 Canadian Society & ESB - Targeted Gene Delivery of Interleukin-10 via Polymer Nanoparticles to Reduce the Inflammation in Atherosclerosis

Date: Monday, 5 September – 17:15 *(Room E)*

Maryam Tabrizian is full professor at the Biomedical Engineering Department and Faculty of Dental Medicine and Oral Health Sciences at McGill University. She holds a Canada Research Chair Tier I in Regenerative Medicine and Nanomedicine. She has received many prestigious awards and fellowships including the FRQS-Chercheur National, Guggenheim Foundation, Biomaterials Science & Engineering - International, RSC Academy of Sciences, ADEA Leadership Institute, the Canadian Academy of Health Sciences fellowships and James McGill professorship. Her research program is built on four main pillars: 1. Biomimetic materials for

immunoengineering, angiogenesis and osteogenesis, 2. Nanoplexes tissue targeting and cell imaging, 3. Real-time monitoring of molecular and cellular events at biointerfaces, and 4. Microfluidics and Lab-on a-chip devices. M. Tabrizian has over 250 peer-reviewed publications with over 16700 citations and h-index of 67. She has been the Editor-in-Chief of Materials since 2011.

Doctor Tiziano Serra

Head of Sound Guided Tissue Regeneration Focus Area at AO Research Institute Davos, Switzerland

Talk: KL Contactless - Contactless Bioassembly Processes For Controlling Tissue Organization

Date: Monday, 5 September – 10:30 *(Room E)*

Dr. Tiziano Serra is the Head of Sound Guided Tissue Regeneration Focus Area at AO Research Institute Davos, ARI. His team is focusing on the development of contactless biofabrication technologies, based on extrinsic fields, for modeling and regeneration. He received a MSc in Materials Science and Engineer from University of Salento (IT), a PhD at the Institute for Bioengineering of Catalonia (ES) and was postdoctoral fellow at University College London (UK). He was the recipient of the ISBF Young Investigator Award from the International Society of Biofabrication, BRIDGE Fellowship from SNSF-Innosuisse, and Julia Polak European Doctoral Award from European Society of Biomaterials. Tiziano is the inventor of Sound Induced Morphogenesis (SIM) technology and Chief Scientific Officer of mimiX Biotherapeutics.

Professor Mark Tibbitt

Assistant Professor of Macromolecular Engineering in the Department of Mechanical and Process Engineering at ETH Zurich, Switzerland

Talk: KL Dynamic materials - Dynamic covalent hydrogels for advanced biomaterials design

Date: Thursday, 8 September – 9:00 *(Room H)*

Pr. Mark Tibbitt joined ETH Zurich as an Assistant Professor of Macromolecular Engineering in the Department of Mechanical and Process Engineering in June 2017. Previously, he was an NIH Postdoctoral Fellow in the laboratory of Prof. Robert Langer in the Koch Institute for Integrative Cancer Research at the Massachusetts Institute of Technology. He received his B.A.

in Integrated Science and Mathematics from Northwestern University, and his Ph.D. in Chemical Engineering at the University of Colorado Boulder under the supervision of Prof. Kristi S. Anseth. His research integrates concepts from chemical engineering, synthetic chemistry, materials science, and biology to rationally design and assemble soft matter. A recent focus of the lab has been on the design and understanding of dynamic polymer materials for biomedical applications, including as printable bioinks, injectable drug delivery systems, and viscoelastic 3D culture platforms.

Doctor Nihal Engin Vrana

CEO and co-founder of SPARTHA MEDICAL, Strasbourg, France

Talk: KL Immunomodulatory - Medical device personalisation via multifunctional, immunomodulatory biomaterials; decreasing the complications and improving the healing

Date: Monday, 5 September – 10:30 *(Room F)*

Dr. Nihal Engin Vrana is CEO and co-funder of SPARTHA MEDICAL. He holds a B.Sc. (Biology) and a M.Sc. (Biotechnology) from Middle East Technical University and obtained his PhD in 2009 at Dublin City University as a Marie Curie ESR fellow. His major research interests are immunomodulatory and multifunctional coatings, medical implants, tissue engineering, biomaterial assessment and cell biomaterials interactions. He has coordinated several EU projects (BiMot, IMMODGEL and PANBioRA). He has published more than 100 peer reviewed articles and 7 book chapters, holds 6 patents and has edited 2 books.

Doctor Nathalie Weber

Clinical trial leader at Institut Straumann AG, Basel, Switzerland

Talk: KL Osteoarticular repair - Clinical Applications of Enamel Matrix Derivative, a Biomimetic and Inflammation-modulating Biomaterial

Date: Tuesday, 6 September – 17:15 *(Room B)*

Dr. Nathalie Weber has been a clinical trial leader at Institut Straumann AG since 2018 where she manages clinical studies evaluating dental implant surfaces and regenerative dental biomaterials such as bone grafts, collagen membranes and enamel matrix derivative. She was previously a global product manager for five years at the same company responsible for the

worldwide sales and marketing of a portfolio of regenerative dental biomaterials. Nathalie Weber holds a B.Sc. in Life Science and Technology from the Swiss Federal Institute of Technology in Lausanne (EPFL) and a M.Sc. in Biomedical Engineering from the Swiss Federal Institute of Technology in Zurich (ETH Zurich).

Professor Régine Willumeit-Römer

Director of the Institute of Materials Research, Division Metallic Biomaterials, Geesthacht, Germany

Talk: KL2 Wound healing - The comparability of in vitro and in vivo experiments for degradable Mg-implants: basis for a digital twin of implant degradation

Date: Monday, 5 September – 11:00 *(Room B)*

Pr. Régine Willumeit-Römer started her career as physicist specialized on ribosomal structure research. After her PhD in physics she habilitated in biochemistry and moved from the ribosome towards membrane active molecules such as peptide antibiotics. This was also the link to become interested in antibacterial implant surfaces and biomaterials as such. To date, as Director of the Institute of Materials Research, Division Metallic Biomaterials she is responsible for the development of degradable Mg-based implant materials.

Francis Max Yavitt

PhD Candidate in the lab of Dr. Kristi Anseth at the University of Colorado Boulder, USA

Talk: KL1 SFB ESB - Photoinduced Hydrogel Network Reorganization Modulates Intestinal Organoid Epithelial Shape to Template Crypt Formation

Date: Monday, 5 September – 17:15 *(Room F)*

Francis Max Yavitt is a PhD Candidate in the lab of Dr. Kristi Anseth at the University of Colorado Boulder. He earned a B.Eng in Chemical Engineering with a minor in biosciences from McMaster University and a M.Sc. in Chemical Engineering from the University of Colorado Boulder. Generally, his research interests involve the development of novel hydrogel chemistries to study cellular mechanobiology. His current graduate research focuses on the use of photoadaptable hydrogels to investigate the role of mechanotransduction in intestinal organoid development. He was awarded an NIH F31 Predoctoral Fellowship to pursue this work. More information about his research contributions and interests can be found at https://www.colorado.edu/ansethgroup/ or on Twitter @LabMaxYav.

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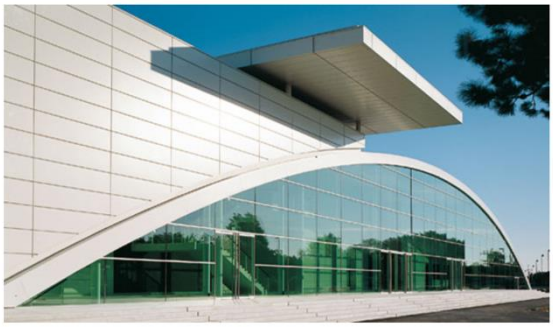
CONFERENCE VENUE

The Palais des Congrès, Bordeaux is located in front of the Bordeaux Lake district, which offers an environment between nature and conviviality. This congress center is directly accessible by tramway from the St Jean Rail Station in 30 minutes and from the City Centre in 20 min. Accommodations, more than 1400 rooms, are within walking distance of Conference venue.



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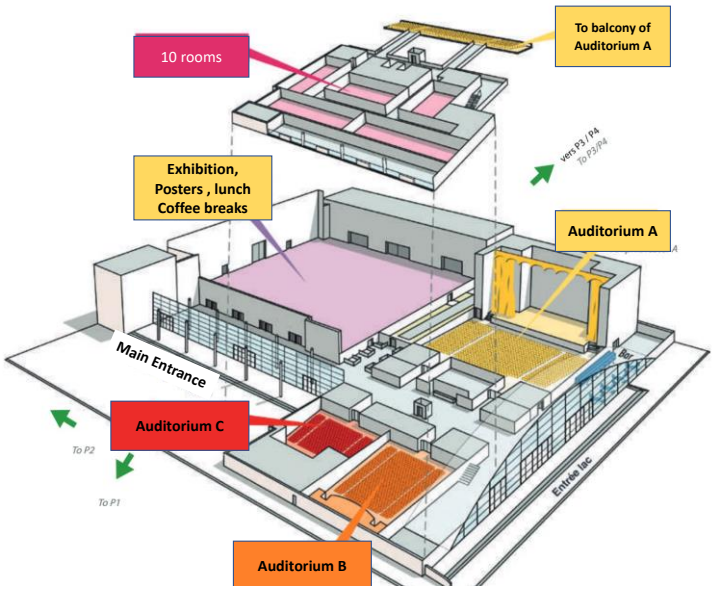
The Palais des Congrès offers all facilities and technical requirements for hosting large international conferences and promoting interactive discussions in various halls at the two levels including three auditoriums and 10 rooms for meetings.

ADDRESS:

PALAIS DES CONGRÈS

Avenue Jean-Gabriel Domergue
33300 BORDEAUX, France

CONFERENCE VENUE



Ground level: Auditorium A, B and C

- 1080 sqm for the Lobby and access to the auditorium and the first level
- **Auditorium / Room A:** Plenary session for up to 1290 participants
- **Auditorium/ Room B:** Parallel session for up to 350 participants
- **Auditorium C / Room C:** Parallel session for up to 190 participants

Exhibition Hall: booths, posters, catering and lunch in a 3 000 sqm area gross, directly located close to the Rooms A, B and C. This spacious Exhibition area will be located close to Plenary Session and all Parallel sessions.

It will host the exhibitor stands, the coffee and lunch break area as well as part of the poster presentations area. Thus, a constant frequentation at the booths will be guaranteed to maximize networking opportunities. In order to promote visits in the exhibition hall, we also plan to organize specific meetings and lunch meetings between company's representatives and young researchers during the lunch break.

First level: Rooms for the other Parallel Sessions or dedicated meetings (D, E, F, G, H)

Meeting rooms (80 to 180 seats) for the 3 other parallel sessions (Rooms E, F, H) for European projects symposia, technological and sessions clinical sessions; ESB Council Committee (Room G), Post-graduate lunch symposia organized by *Acta Biomaterialia* (Room D).

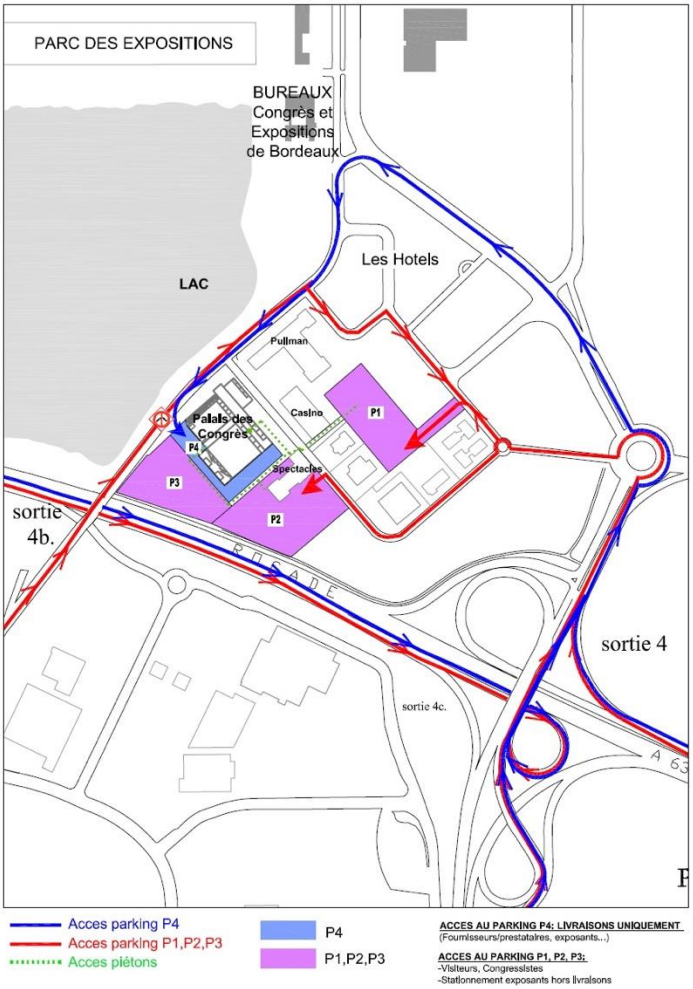
ROAD MAP

ACCESS

Bordeaux is located in Southwest of France; it is accessible by visitors from all countries from Europe.

By plane: The international airport Bordeaux Merignac is connected to 5 of the biggest European hubs (Paris Charles de Gaulle (1:20 hour by flight), Amsterdam, London, Madrid, Lisbon, Rome). It provides more than 80 direct connections in Europe and a direct flight to Canada.

By train: Bordeaux can be easily reached by train from everywhere in France (2 hours from Paris) (https://www.oui.sncf/)



Public transportation in Bordeaux: Within Bordeaux, a modern and efficient public transportation system with trams and buses is available. (https://www.infotbm.com/en)

- From the airport, guests can take the bus connecting the airport with the City Centre (30 min).
- From the City Centre: Tramway C and stop at "Parc des Expositions / Nouveau stade".
- From the rail station: Tramway C and stop at "Parc des Expositions / Nouveau stade"

Free TMB tickets will be offered to all delegates for the whole conference (Sept 4-9). They will be provided at the registration desk of the Congress Center. These tickets are valid day and night.

PARKING

A car park is available during the sessions at the Congress Center

GENERAL INFORMATION

ABSTRACT BOOK

The abstracts will be published solely as an electronic abstract book.

BANKING AND CURRENCY EXCHANGE

Euro is the official currency. Opening times differ from one bank to the next, with most open weekdays from 9 am to 4 pm. Most bank branches have cash machines (ATMs) that allow you to withdraw cash and get bank statements 24 hours/day.

CERTIFICATE OF ATTENDANCE

Delivery of the Certificates of Attendance will take place after the conference via email.

CLOAKROOM

A cloakroom is located at the Ground level, close to the registration desk of the Congress Center.

DISCLAIMER

The organizers are not liable for damages and/or losses of any kind which may be incurred by the conference delegates or by any other individuals accompanying them, both during the official activities as well as going to/from the conference. Delegates are responsible for their own safety and belongings.

ELECTRICITY

Electric sockets are 230 volts AC, 50 Hz. European-style round two-pin plugs are in use. You might need a transformer and a plug that fits the French socket.

EMERGENCY TELEPHONE NUMBERS

Calls to 112 are free of charge and can be made from a landline, pay phone or mobile phone, even without a SIM card. Dialing the number will direct you to an operator who will notify the appropriate service, typically the local fire and rescue service. It can be used for any life-threatening situation, including serious medical problems, fire-related incidents, crimes and life-threatening situations. You can also call an ambulance through this number. In addition to French, calls can be answered in English.

GENERAL INFORMATION

ESB COUNCIL ROOM

ESB Council Committee room will be accommodated in room G (1st level) during the days of the conference (from Sunday, 4 September 9:00 to Thursday, 8 September 13:00).

GENERAL ASSEMBLIES

The General Assembly of YSF and ESB will take place in Auditorium A.

- **General Assembly YSF:** Tuesday, 6 September, 18:30-19:30
- **General Assembly ESB:** Wednesday, 7 September, 11:30-12:30

INDUSTRIAL EXHIBITION

The Industrial Exhibition will take place throughout the whole conference.

Opening hours

Sept 4, 2022	10:00-16:00 *Set-up* 16:30-19:00
Sept 5, 2022	8:30-19:00
Sept 6, 2022	8:30-19:00
Sept 7, 2022	8:30-19:00
Sept 8, 2022	8:30-13:00 13:00-18:00 *Dismantling*

In order to have an excellent interaction between participants and exhibitors, coffee breaks, poster sessions, lunches and exhibition are held in the same level (Ground level: Exhibition Hall and main hall).

INTERNET

Public Wi-Fi is available for the participants of the ESB 2022 conference free of charge. The Wi-Fi access code is:

- Network: ESB2022
- Password: Bordeaux2022

Conference homepage: www.esbbordeaux2022.org

LOST AND FOUND

Lost and found items can be recovered at the registration desk.

GENERAL INFORMATION

NAME BADGES

Participants and accompanying persons are required to wear the official conference name badge on all conference occasions. Without the name badge, admission cannot be granted!

OFFICIAL LANGUAGE

The conference language is English.

ORAL CONTRIBUTIONS

Please check date and time of your contribution at the scientific program.
Please prepare your presentations in MS Powerpoint (ppt or pptx, format 16:9) or Adobe Acrobat (pdf) format. Please take care that special fonts/characters and videos are properly integrated.

Bring a copy of your presentation on a USB flash drive to the Media Check at Ground level (Preview room) as early as possible, **at the end of the sessions of the day before** or **at the latest during the break immediately preceding your session** so that it can be uploaded onto the central computer system to ensure a smooth change over between speakers.
A technician will be available to assist you with the upload.

Please, note that it is not possible to use your own notebook or any other file format for the presentations.

Duration of talks:

Keynote Lectures	Check on the dedicated program the duration of the presentation *3 to 5 min of discussion should be included in the time duration (check on the website)*
Plenary Lectures	40 min *+ 5min discussion*
ESB 2022 Awardees	45 min
Oral Presentation	12 min *+ 3 min discussion*
Flash Poster	5 min / 4 slides (*no discussion*)

GENERAL INFORMATION

POSTER PRESENTATIONS

The poster sessions will be held at the Exhibition Hall and Hall 1.

The sessions have been scheduled for Monday, 5 September 2022 from 13:30 –14:30 and for Tuesday, 6 September 2022 from 13:30 –14:30.

Posters should be put up latest by the end of Monday, 5 September during the coffee break at 10:00 and be on display until the end of the conference on Thursday, 8 September at 13:00 (at the latest after the closing session on Thursday, 8 September, 12:45).

The size of the poster panels is 120 cm (height) x 80 cm (width), and comfortably accommodates posters in A0 portrait (upright) format. Posters can only be fixed on poster boards using the fixing material provided at the poster desk.
No responsibility will be assumed for posters not removed at the abovementioned times!

During the two poster sessions (Monday, 5 September 2022 from 13:30-14:30 and Tuesday, 6 September 2022 from 13:30-14:30), authors are kindly asked to be present in the area of their posters in order to answer questions that interested viewers may have.
A jury dedicated to the evaluation of posters will examine those that have applied for the "Best Poster Presentation". **The authors will have to present their poster in 5 min and will have to answer the questions of the jury for 3 min.**
The auteurs will be awarded during the Closing Ceremony on Thursday, 8 September 2022 12:15-12:45, together with the prizes for the "Best Oral Presentation".

PROGRAM UPDATES

Program updates will be included in the online system of the conference as accessible via the conference web pages (www.esbbordeaux2022.org).

REGISTRATION DESK

The registration desk will be located at the Hall Foyer and will be open at the following hours:

Sept 4, 2022	14:00-17:15
Sept 5, 2022	8:00-8:30
Sept 6, 2022	8:00-8:30
Sept 7, 2022	8:00-8:30
Sept 8, 2022	8:00-9:00

GENERAL INFORMATION

Registration fees on site

Regular	900€
Students	600€

Conference registration fees include for Student and Full Conference only (from Sunday 4th to Thursday 8th):

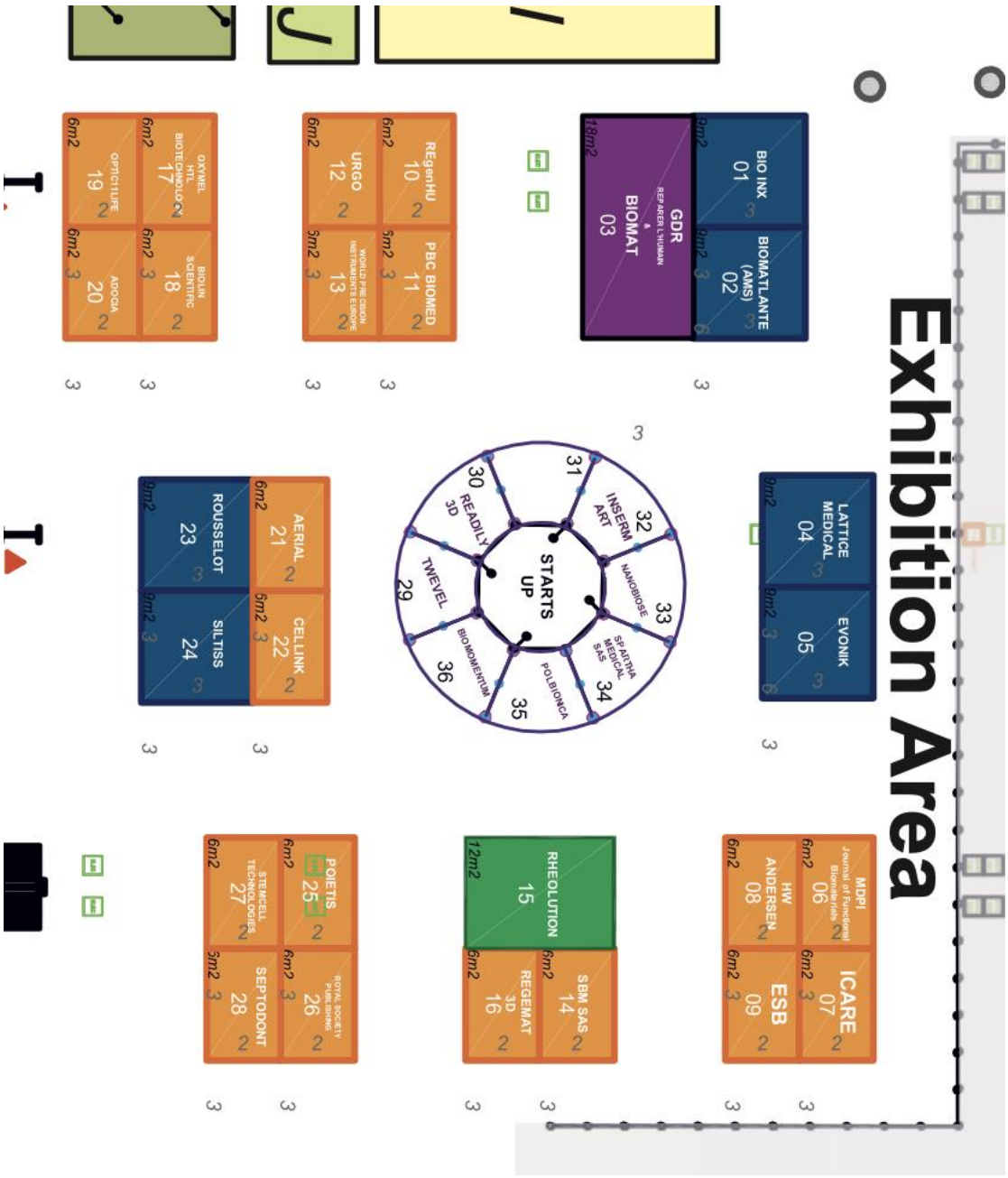
- Admission to scientific program with oral and poster presentation
- Admission to exhibition
- Access to abstracts in electronic form
- Coffee breaks
- The Welcome Reception on Sunday, 4 September
- Lunches (Monday, Tuesday, Wednesday and Thursday).

TIME ZONE

The time zone in France is Central European Summer Time (UTC+02:00) at the time of the conference.

TECHNICAL EXHIBITION & POSTER EXHIBITION

SCHEDULE – SUNDAY, 4 SEPTEMBER 2022



SCHEDULE – SUNDAY, 4 SEPTEMBER 2022

2:00 - 4:00pm | Registration

>> YSF and Young Researchers BIOMAT - Opening Workshop

Chairpersons: YSF and Young Researchers BIOMAT
Location: Room A

14:05 | Tips for successful science communication
Elodie CHABROL, Freelance science communicator and director of Pint of science international & France

14:50 | Wonder & synergies at the service of knowledge
Renaud POURPRE, Freelance science communicator / facilitator, France

15:35 | From lab to market: a giant leap
Paul DELTROT, Readily3D SA, EPFL Innovation Park, Lausanne, Switzerland

16:00 - 17:15 | Registration & Coffee Break

17:15 - 18:00 | Welcome Ceremony

Location: Room A

18:00 - 19:00 | PL1 - Opening Lecture
Pr Molly Stevens, Imperial College London, UK

Chairpersons: Sébastien Lecommandoux & Sandra Van Vlierberghe
Location: Room A

19:00 - 22:00 | Welcome Reception

SCHEDULE – MONDAY, 5 SEPTEMBER 2022

08:00 - 08:30 | Registration

08:30 - 09:15 | PL2 - Plenary Lecture
Matthias Lutolf, Swiss Federal Institute of Technology Lausanne, Switzerland
Chairpersons: Joëlle Amédée & Matteo D'Este
Location: Room A

09:15 - 10:00 | Jean Leray Award
Pr Khoon Lim, University of Otago, Christchurch, New Zealand
Chairpersons: Ana Pego & Luigi Ambrosio
Location: Room A

10:00 - 10:30 | Coffee Break & Posters

--- 10:30 - 12:30 | Parallel Sessions ---

>> 10:30 - 12:30 | PSOP-01 - SCAFFOLDS

Chairpersons: Marc Bohner & Cecilia Persson
Location: Room C

10:30 | KL Scaffold - 3D sensing scaffold for extracellular pH mapping at single cell level in tumor models
Valentina ONESTO, Institute of Nanotechnology, National Research Council (CNR-Nanotec), Italy

11:00 | O1 Scaffold - Cell-Derived Extracellular Matrices for Establishment of 3D in vitro Tumor Models for Cancer Research
Gülsün BAGCI, 1Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain. 2 CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.

11:15 | O2 Scaffold - Composite Scaffolds Enriched with Nano-hydroxyapatite and Strontium-nano-hydroxyapatite Promote Osteogenic Differentiation
Maria CHATZINIKOLAIDOU, Foundation for Research and Technology Hellas and University of Crete, Heraklion, Greece

11:30 | O3 Scaffold - Engineered Porous Microshuttles for Drug, Nanomaterials and Cell Delivery
K. SONG, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

11:45am | O4 Scaffold - Nanoneedles for Targeted siRNA Transfection of the Human Corneal Endothelium
Ciro CHIAPPINI, Centre for Craniofacial and Regenerative Biology, King's College London, London, United Kingdom; London Centre for Nanotechnology, King's College London, London, United Kingdom

12:00 | FP01 Scaffold - Fabrication of 6-BromoIndirubin-3'-Oxime Incorporated Guanosine Diphosphate Crosslinked Chitosan Scaffolds to Promote Osteogenic Differentiation in Myoblastic C2C12 Cells

Celine J. AGNES, Department of Biomedical Engineering, McGill University, Montreal, Quebec, Canada

12:05 | FP02 Scaffold - Hybrid Core-Shell Scaffolds for Bone Regeneration and a 3D Printing Approach to Tune their Properties

Chiara PASINI, Department of Mechanical and Industrial Engineering, University of Brescia, Brescia, Italy

12:10 | FP03 Scaffold - Deposition of a polymer-based honeycomb-like membrane on 3D printed bioactive glass scaffold and decellularized bone matrix as scaffolds for bone tissue engineering

Michel BOISSIERE, Laboratoire ERRMECe, CY Cergy Paris Université, Neuville sur Oise, France

>> 10:30 - 12:30 | PSOP-02 - WOUND HEALING

Chairpersons: Emilie Attiogbe & Arn Mignon
Location: Room B

10:30 | KL1 Wound healing - Dense Collagen/PLGA Composite Hydrogels Generated by In Situ Nanoprecipitation as Novel Medicated Wound Dressings: In Vitro and In Vivo Evaluation.

Christophe HELARY, Sorbonne University, CNRS, UMR 7574 – Chemistry of Condensed Matter Laboratory, Paris, France

11:00 | KL2 Wound healing - The comparability of in vitro and in vivo experiments for degradable Mg-implants: basis for a digital twin of implant degradation

Regine WILLUMEIT-RÖMER, Institute for Metallic Biomaterials, Helmholtz-Zentrum Hereon, Max-Planck-Str. 1, 21502 Geesthacht, Germany

11:30 | O1 Wound healing - Wound Dressings Based on Gellan Gum, Alginate and Lipid Nanoparticles Loaded with Antibacterial Peptide Nisin

Elzbieta PAMULA, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Kraków, Poland

11:45 | O2 Wound healing - Augmented siloxane-based rapid action foams for hemostatic treatment

Pritha SARKAR, Department of Materials Science and Engineering, University of Central Florida, United States

12:00 | O3 Wound healing - Effect of local administration zoledronic acid and PRP with bone allograft on bone defect healing

Elyarbek TASHMETOV, Surgical diseases department, Karaganda medical university, Karaganda, Kazakhstan

12:15 | FP01 Wound healing - Three Fungal Exopolysaccharides as New Biomaterials for Wound Healing

Masoud HAMIDI, Université libre de Bruxelles (ULB), École polytechnique de Bruxelles – 3BIO-BioMatter, Brussels, Belgium

12:20 | FP02 Wound healing - Multifunctional hydrogel-based patch for targeting inflammation and regeneration in chronic intestinal wounds

Marco ARAÚJO, i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

12:25 | FP03 Wound healing - Development of Intelligent Hydrogel-Based Burn Wound Dressings

Manon MINSART, Polymer Chemistry & Biomaterials Research Group, CMaC, Ghent University, Ghent, Belgium

>> 10:30 - 12:30 | SYMP-01 - NEW BIOINKS FOR COMPLEX 3D PRINTED TISSUE SUBSTITUTES: FROM NATIVE FUNCTIONALITY TO DISEASE MODELLING

Chairpersons: Alexandra P Marques & Christophe Marquette
Location: Room A

10:30 | KL1 Bioinks - 3D Bioprinting: in vitro model, implantable bioink and intraoperative methods

Christophe MARQUETTE, 3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Villeurbanne, France

11:00 | KL2 Bioinks - Extending the capabilities of bioprinting with the Dynamic Formulation Module (multi-material patterning, mixing and core-shell) in a single dispensing process

Mauro PETRETTA, REGENHU SA, Villaz-St-Pierre, Switzerland

11:30 | O1 Bioinks - Novel Approach for Guiding Stem Cell Organization and Tissue Growth by Multi-head 3D Bioprinting to Mimic Human Corneal Stroma

Paula PUISTOLA, Eye Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

11:45 | O2 Bioinks - Development of a novel hybrid bioink containing living microfactories

Maryam GHASEMZADEH-HASANKOLAEI, CICECO–Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

12:00 | O3 Bioinks - Drop-on-demand micropatterning of the novel amphiphilic peptide I3K on regenerated silk fibroin substrates to guide and promote adhesion and proliferation of neuronal cells

Ana JIMENEZ-FRANCO, Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK

12:15 | FP01 Bioinks - A Collagen-Glycosaminoglycan Scaffold Coating Enhances the Bio-Lubrication of a 3D-Printed Framework for Cartilage repair

Austyn MATHESON, Tissue Engineering Research Group, Dept. of Anat.& R.M., Royal College of Surgeons in Ireland, Dublin, Ireland; Trinity Centre for Biomedical Engineering, Trinity College Dublin, Ireland Advanced Materials & Bioengineering Research Centre, RCSI and TCD, Dublin, Ireland Biomedical Engineering, School of Dental Medicine, UConn Health, USA

12:20 | FP02 Bioinks - Micellar nitric oxide-releasing hydrogels for DLP-based 3D printing

Marcelo GANZAROLLI DE OLIVEIRA, Institute of Chemistry, University of Campinas, UNICAMP, Campinas, SP, Brazil

12:25 | FP03 Bioinks - Clickable Dynamic Bioinks

Vianney DELPLACE, Université de Nantes, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

>> 10:30 - 12:30 | SYMP-02 - ANTIOXIDANT NANOMATERIALS FOR BIOMEDICAL APPLICATIONS

Chairpersons: Gianni Ciofani & Matteo Battaglini
Location: Room H

10:30 | KL Antioxidant - Nanozymes in Biomedicine: Route towards Clinical Applications

Gianni CIOFANI, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), Italy

11:00 | O1 Antioxidant - Bionic tissue with antioxidant microreactors reduce oxidative stress on HepG2 cells

Stefan PENDLMAYR, (1) Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark. (2) Sino-Danish Center for Education and Research, University of Chinese Academy of Sciences, Beijing, China

11:15 | O2 Antioxidant - Engineering Biomimetic Epigenetically-Activated Extracellular Vesicles to Promote Bone Formation

Kenny MAN, School of Chemical Engineering, University of Birmingham, Birmingham, UK

11:30 | O3 Antioxidant - Establishment of Selenium-incorporated Mesoporous Silica Nanoparticles (MSNs) for Osteosarcoma Therapy

Lei HE, Department of Instructive Biomaterials Engineering (IBE), MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, the Netherlands

11:45 | O4 Antioxidant - ALTERNATIVE Horizon 2020 project for building an innovative platform to assess the cardiotoxicity of chemicals

Gianluca CIARDELLI, Politecnico di Torino, Torino, Italy

12:00 | FP01 Antioxidant - Designing a nanointelligent bioartificial pancreas to treat type I Diabetes

Joana MOREIRA MARQUES, i3S - Institute for Research & Innovation in Health, University of Porto, Porto, Portugal; Faculty of Pharmacy of the University of Porto, Porto, Portugal

12:05 | FP02 Antioxidant - Firefly-Bioinspired Hydrogels with Redox-responsiveness as Cell-encapsulating Injectable Matrices

Minye JIN, INM – Leibniz Institute for New Materials. Campus D2-2, Saarbrücken, Germany; Chemistry Department, Saarland University, Saarbrücken, Germany; University of Twente, Enschede, The Netherlands.

>> 10:30 - 12:30 | SYMP-03 - CONTACTLESS BIOASSEMBLY PROCESSES: NEW AVENUES IN THE BIOFABRICATION WORLD

Chairpersons: Hugo Oliveira & Catherine Le Visage
Location: Room E

10:30 | KL Contactless - Contactless Bioassembly Processes For Controlling Tissue Organization

Tiziano SERRA, AO Research Institute, Davos Platz, Switzerland

11:00 | O1 Contactless - Novel Electro-Assisted Printing of Soft Hydrogels by Electrochemistry

Arua DA SILVA, Implantable Bioelectronics Laboratory. Department of Automatic Control and Systems Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

11:15 | O2 Contactless – Additive Manufacturing for the Development of Microneedle Arrays as Minimally Invasive Drug Delivery Systems

Nikoletta SARGIOTI, 1 School of Mechanical and Manufacturing Engineering, Dublin City University, Ireland. 2 School of Mechanical Materials Engineering, University College Dublin, Ireland

11:30 | O3 Contactless - Magnetic and Matrix-assisted 3D Bioprinting for Biomimetic Tendon Tissue Model

Syeda Mahwish BAKHT, 13B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Braga/Guimarães, Portugal

11:45 | O4 Contactless - A micro physiological system recapitulating inflammation in a microtissue

Martin FRAUENLOB, Institut Pasteur, Biomaterials and Microfluidics core facility, C2RT, Paris, France & Bioassays, Microsystems and Optical Engineering Unit, BIOASTER, Paris, France

12:00 | FP01 Contactless - In vitro and in vivo characterization of a novel tricalcium silicate-based ink for bone regeneration using Laser-Assisted Bioprinting
Nicolas TOUYA, INSERM U1026 BIOTIS

12:05 | FP02 Contactless - Physical and biological behaviour of flowable fiber reinforced composite compared to alternative bulk filling composites
Nina ATTIK, Université de Lyon — Université Claude Bernard Lyon 1, UMR CNRS 5615, Laboratoire des Multimatériaux et Interfaces, Lyon, France; Universitéde Lyon, Université Claude Bernard Lyon 1, Faculté d'Odontologie, Lyon, France

12:10 | FP03 Contactless - Hybrid HMSCs-Microcomposite Building Blocks for Bottom-Up Engineering of Bone Tissue
K. SONG, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

--- 10:30 - 12:30 | Technological Sessions ---

>> 10:30 - 11:30 | TECH S1 - BIOENGINEERING IMMUNOMODULATORY BIOMATERIALS

Chairpersons: Jacek Wychowaniec & David Eglin
Location: Room F

10:30 | KL Immunomodulatory - Medical device personalisation via multifunctional, immunomodulatory biomaterials; decreasing the complications and improving the healing
NIHAL ENGIN VRANA, SPARTHA MEDICAL, Strasbourg, France

10:45 | O1 Immunomodulatory - Introducing Controlled Topography onto 3D Scaffolds via Digital Light Processing to Modulate the Immune Response
Sandra CAMARERO-ESPINOSA, POLYMAT, University of the Basque Country UPV/EHU, Donostia / San Sebastián, Gipuzkoa, Spain; IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

11:00 | O2 Immunomodulatory - Nanoscale spatio-mechanical regulation of the immune signaling in cytotoxic lymphocytes
Mark SCHVARTZMAN, Ben-Gurion University of the Negev,Beer-Sheba, Israel

11:15 | O3 Immunomodulatory - Bridging the Gap Between the Immune Response and Mineralization During Fracture Healing
William Arthur LACKINGTON, Biointerfaces Lab, Empa, St. Gallen, Switzerland

SCHEDULE – MONDAY, 5 SEPTEMBER 2022

>> 11:30 - 12:30 | TECH S2 - NEW ADVANCES IN BIOMATERIALS CHARACTERIZATION FOR TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Chairpersons: Valeria Chiono & Jochen Salber
Location: Room F

11:30 | KL1 Advances in characterization - New advances in preclinical validation tools through biofabricated human 3d tissue equivalents-on chips integrated with innovative nmr spectroscopy

Jochen SALBER, Department of Experimental Surgery/Centre for Clinical Research, Ruhr University, Bochum, Germany

11:45 | O1 Advances in characterization - Real time imaging of local O2 for quantitative analysis of O2 distribution and consumption rates during early degradation of Magnesium alloys

Ashwini Rahul AKKINENI, Centre for Translational Bone, Joint and Soft Tissue Research, University Hospital Carl Gustav Carus and Faculty of Medicine of Technische Universität Dresden, Germany

12:00 | O2 Advances in characterization - CHROMATIN COMPACTION DECREASES CELL ADHESION STRENGTH: A QUANTITATIVE APPROACH BY FLUIDIC FORCE MICROSCOPY

Julie BUISSON, Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg (CRBS), Strasbourg, France

12:15 | O3 Advances in characterization - How the physicochemical characteristics and protein corona modulate the activity of nanostructured lipid carriers (NLC) against Helicobacter pylori

Rute CHITAS, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

12:30 - 13:30 | Lunch Break & Meeting Industrials and Professors

13:30 - 14:30 | PS1 - Poster Session 1

14:30 - 15:15 | International Award

Pr Joachim Kohn. Rutgers University, New Jersey, USA

Chairpersons: Liz Tanner & Ana Pego
Location: Room A

SCHEDULE – MONDAY, 5 SEPTEMBER 2022

--- 15:30 - 16:30 | Parallel Sessions ---

>> 15:30 - 16:30 | WS - WILEY

Chairpersons: Irem Bayindir-Buchhalter & Nicola Contessi Negrini
Location: Room E

15:30 | KL Wiley - Scientific Publishing with Impact
Irem BAYINDIR-BUCHHALTER, Wiley-VCH, Weinheim, Germany

16:00 | O1 Wiley - Mechanotransduction in High Aspect Ratio Nanostructured Meta-biomaterials
Khashayar MODARESIFAR, Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, Delft, The Netherlands

16:15 | O2 Wiley - Development of a Vascularized Tissue Model: Focus on the Stromal/Vascular Interaction and on 3D Imaging
Alessandra DELLAQUILA, Université Paris Cité, INSERM U1148, Paris, France

>> 15:30 - 16:30 | PSOP-03 - OSTEOARTICULAR TISSUE ENGINEERING

Chairpersons: Jérôme Guicheux & Cristina Martins
Location: Room A

15:30 | O1 Osteoarticular - Comparison of the degradation behavior of WE43-based magnesium screws with and without PEO-surface modification in a miniature pig model
Heilwig FISCHER, 1 Center for Musculoskeletal Surgery, Charité – Universitätsklinikum Berlin, Berlin, Germany 2 Department of Oral and Maxillofacial Surgery, Charité – Universitätsklinikum Berlin, Berlin, Germany 3 Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany

15:45 | O2 Osteoarticular - Effects of Nanostructured and Microstructured Calcium Phosphates on In Vitro Osteoblast Response
Edgar B. MONTUFAR, Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

16:00 | O3 Osteoarticular - Multiscale Characterization and Biological Assessment of Pyrophosphate-Stabilized Amorphous Calcium Carbonate Doped with Bioactive Ions
Christèle COMBES, CIRIMAT, Université de Toulouse, CNRS, TOULOUSE INP - ENSIACET, Toulouse, France

16:15 | O4 Osteoarticular - Targeting HIF Pathway for Personalised Bone Regeneration in Diabetic Patients
Adriana-Monica RADU, Division of Surgery and Interventional Sciences, University College London, London, United Kingdom

>> 15:30 - 16:30 | PSOP-04 - BIODERIVED MATERIALS

Chairpersons: Emmanuel Pauthe & Karine Anselme
Location: Room H

15:30 | O1 Bioderived - Bioinks derived from human amniotic membrane and proof-of-principle application to bioprinting

Léo COMPERAT, Inserm U1026, Tissue Bioengineering, ART BioPrint, F-33076, Bordeaux, France

15:45 | O2 Bioderived - Engineering a Tendon-Bone Enthesis-Mimicking Hybrid Matrix for Repair of Irreparable Rotator Cuff Tears

Chaozong LIU, Department of Orthopaedics & Musculoskeletal Science, Division of Surgery & Interventional Science, University College London (UCL), Royal National Orthopaedic Hospital, London, United Kingdom

16:00 | O3 Bioderived - Production of mineralised collagen films using electrophoretic deposition

Katrina STAUNTON, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

16:15 | O4 Bioderived - Tuning the bioactivity of MSC-secreted ECM through control of substrate stiffness

Daniel HEATH, Department of Biomedical Engineering, University of Melbourne, Parkville, Australia

>> 15:30 - 16:30 | PSOP-05 - SURFACE FUNCTIONALIZATION

Chairpersons: Marie-Christine Durrieu & Aldo Boccacini
Location: Room B

15:30 | O1 Surface - Bone Adhesive with High Mechanical Strength and Natural Bone Replacing Capabilities Shows Clinical Potential in Implant Fixation to Osteoporotic Bone.

Gerard INSLEY, PBC Biomed, Shannon, Ireland

15:45 | O2 Surface - Development of tough 3D-printed biomimetic calcium phosphate scaffolds for bone regeneration

Maria-Pau GINEBRA, Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering, Universitat Politècnica de Catalunya; Barcelona Research Centre for Multiscale Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain

16:00 | O3 Surface - Bone sialoprotein immobilized in collagen type I induces bone regeneration in vitro and in vivo

Ulrike RITZ, Department of Orthopedics and Traumatology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

16:15 | O4 Surface functionnalization - Surface Modifications on Titanium Alloy : A Promising Way to Understand and Fight Implant- Related Infection

Frédéric VELARD, University of Reims Champagne-Ardenne, Reims, France

>> 15:30 - 16:30 | PSOP-06 - BIOMATERIALS PROCESSING

Chairpersons: Michael Gelinski & Riccardo Levato
Location: Room C

15:30 | O1 Processing - Fetuin A functionalization of biodegradable nonwovens towards biomimetic guided bone regeneration

Stefan OSCHATZ, Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany

15:45 | O2 Processing - The freeze-casting process: a promising tool for the fabrication of macroporous composite scaffolds for bone substitution

Jérémy SOULIE, CIRIMAT, Université de Toulouse, CNRS, Toulouse INP - ENSIACET, Toulouse, France

16:00 | O3 Processing - Three-dimensional Architectural Design and Assessment of Ice-templated Collagen Scaffolds for Tissue Engineering Applications

Huijie Lillian ZHANG, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

16:15 | O4 Processing - Versatile method for high-throughput fabrication of microparticles with defined geometries and size for biomedical applications

Marta MACIEL, CEB -Center of Biological Engineering, University of Minho, Braga, Portugal; CICECO – Institute of Materials of Aveiro, University of Aveiro, Aveiro, Portugal

>> 15:30 - 16:30 | PSOP-07 - SOFT TISSUE ENGINEERING

Chairpersons: Alexandra P. Marques & Micheal Doser
Location: Room F

15:30 | O1 Soft tissue - Pathogen-Resistant Dermal Clay Dressings for Healing Burns, Wounds, and Scars

Kausik MUKHOPADHYAY, Department of Materials Science and Engineering, University of Central Florida. Orlando. USA

15:45 | O2 Soft Tissue - Porous Poly(glycerol sebacate)-based Scaffolds for Adipose Tissue Engineering Applications

Rachel FURMIDGE, Materials Science and Engineering, Kroto Research Institute, The University of Sheffield, Sheffield, UK

16:00 | O3 Soft Tissue - Advanced Synthetic Materials for 3D Printing Applications and its Use as Scaffolds for Airway Regeneration.

Luis SORIANO, School of Pharmacy and Biomolecular Sciences, RCSI, Dublin, Ireland; Tissue Engineering Research Group, RCSI, Dublin, Ireland; SFI Centre for Research in Medical Devices (CÚRAM), NUI, Galway, Ireland

16:15 | O4 Soft Tissue - A PGSAA-based adhesive filler for perianal fistulas

Elodie LLUSAR, TISSIUM, Paris, France

16:30 - 17:15 | Coffee Break & Posters

--- 17:15 - 18:45 | Parallel Sessions ---

>> 17:15 - 18:45 | CANADIAN SOCIETY / ESB

Chairpersons: Maryam Tabrizian & Nicolas L'Heureux
Location: Room E

17:15 | KL1 Canadian Society & ESB - Targeted Gene Delivery of Interleukin-10 via Polymer Nanoparticles to Reduce the Inflammation in Atherosclerosis

Maryam TABRIZIAN, Department of Biomedical Engineering; Faculty of Dentistry and Oral Health Sciences, Montreal, Canada

17:30 | KL2 Canadian Society & ESB - Innovative 3D Autologous and Immunocompetent Skin Model Reconstructed by Tissue Engineering

Emilie ATTIOGBE, Centre de Recherche en Organogénèse Expérimentale de l'Université Laval (LOEX), Québec, QC, Canada / Centre de Recherche du CHU de Québec-Université Laval, Québec, QC, Canada

17:45 | O1 Canadian Society & ESB - Non-woven Textiles for Medical Implants: Mechanical Performances Improvement

Frederic HEIM, Laboratoire de Physique et Mécanique Textiles (LPMT), ENSISA, Mulhouse, France

18:00 | O2 Canadian Society & ESB - Effect of Synthetic INGAP-P on human islet insulin secretion and gene expression

JAMES PORTER, Dept. of Biological and Biomedical Engineering, McGill University, Montreal, Canada

18:15 | O3 Canadian Society & ESB - Proteomics as a tool to gain next level insights into photo-crosslinkable biopolymer modifications

Nele PIEN, Polymer Chemistry and Biomaterials Research Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Ghent, Belgium and Laboratory for Biomaterials and Bioengineering, CRC-I, Laval University, Quebec, Canada

18:30 | O4 Canadian Society & ESB - Method for Covalent Bonding of Heparin to Carbon Nanotube-Coated Metal Surfaces

Lynn HEIN, Mechanical Engineering, McGill University, Montreal, Canada; Chemical Engineering, McGill University, Montreal, Canada

>> 17:15 - 18:45 | SFB / ESB

Chairpersons: Maria Grazia Raucci & Joachim Kohn
Location: Room F

17:15 | KL1 SFB ESB - Photoinduced Hydrogel Network Reorganization Modulates Intestinal Organoid Epithelial Shape to Template Crypt Formation

Max YAVITT, Chemical and Biological Engineering, University of Colorado Boulder, Boulder, Colorado, USA; BioFrontiers Institute, Boulder, Colorado, United States

17:45 | KL2 SFB ESB - Artificial Extracellular Matrix Scaffolds Enhance Maturation of Human Stem Cell-Derived Neurons

Zaida ALVAREZ PINTO, Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, USA//Institute for Bioengineering of Catalonia, IBEC, Barcelona, Spain

18:15 | O1 SFB ESB - Hydrogel Viscoelasticity Modulates Fusion of Mesenchymal Stem Cell Spheroids

Mani DIBA, Laboratory for Cell and Tissue Engineering, John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA

18:30 | O2 SFB ESB - Investigating the impact of Ti3C2TX (MXene) within an accommodative intraocular lens design on the development of posterior capsular opacification

Grace COOKSLEY, School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK; Rayner Intraocular Lenses, Limited., The Ridley Innovation Centre, Worthing, UK

>> 17:15 - 18:45 | BIOMATERIALS AWARDS - ELSEVIER

Chairpersons: Abhay Pandit & Ana Pego
Location: Room B

17:15 | O1 - Design of Advanced Polymer Biomaterials for the Treatment of Autoimmune Disease

Nicholas A PEPPAS, Department of Biomedical Engineering, Department of Chemical Engineering, Department of Pediatrics, Surgery and Perioperative Care, Dell Medical School, and Division of Molecular Pharmaceutics and Drug Delivery, The University of Texas at Austin, Austin, USA

17:45 | O2 - Engineered biomaterials for lymph node drug delivery and disease modeling enable next-generation approaches in cancer immunotherapy

Susan N. THOMAS, Woodruff School of Mechanical Engineering and Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA

18:15 | O3 - "Smart" Biodegradable Materials with Desired Shapes, Structures and Electroactive Functions at Nano/Micro-Scales For Medical Applications

Thanh DUC NGUYEN, Department of Mechanical Engineering, Department of Biomedical Engineering, Institute of Materials Science, University of Connecticut, USA

>> 17:15 - 18:45 | BIOMATERIALS SCIENCE - THE ROYAL SOCIETY OF CHEMISTRY

Chairpersons: Cristina Barrias & Laura Ghandi
Location: Room A

17:15 | Award RSC - Lipid nanoparticles for mRNA therapeutics, genome editing, and cell therapy

Yizhou DONG, Division of Pharmaceutics and Pharmacology, College of Pharmacy, The Ohio State University, Colombus, United States

17:45 | O1 RSC - Novel mucoadhesive chitosan-methylcellulose buccal patches with broad antibacterial activity

Lorenzo BONETTI, Dept. of Chemistry, Materials, and Chemical Engineering "G. Natta", Politecnico di Milano, 20131, Milan, Italy

18:00 | O2 RSC - Functionalization of 3D printed PLLA/PLCL bioresorbable stents with endothelial cell adhesive peptides

Victor CHAUSSE, Biomaterials, Biomechanics and Tissue Engineering Group, Dept. of Materials Science and Engineering, Barcelona East School of Engineering (EEBE), Universitat Politècnica de Catalunya (UPC), Barcelona, Spain. Barcelona Research Center in Multiscale Science and Engineering, UPC, Barcelona, Spain

18:15 | O3 RSC - Injectable Biomimetic Mussel – Based Adhesive for Bone Repair and Remodeling

Antzela TZAGIOLLARI, School of Mechanical and Manufacturing Engineering, Dublin City University, Ireland

18:30 | O4 RSC - An iodine labeled injectable hyaluronic acid hydrogel for an in vivo bicolor x-ray monitoring of a biomimetic scaffold in brain regeneration cell therapy

Moustoifa SAID, Univ Grenoble Alpes, Inserm, U1216, Grenoble Institut Neurosciences, Grenoble, France; Univ. Grenoble Alpes, Centre des Recherches sur les Macromolécules Végétales, CNRS UPR 5301, Grenoble, France

SCHEDULE – MONDAY, 5 SEPTEMBER 2022

>> 17:15 - 18:45 | BIOMAT & HUMAN REPAIR FRENCH NETWORK

Chairpersons: Jérôme Chevalier & Pierre Weiss
Location : Room C

17:15 | KL1 BIOMAT & Human Repair - Wireless Theranostic Smart Contact Lens for Monitoring and Control of Intraocular Pressure in Glaucoma

Hahn SEI KWANG , Department of Materials Science and Engineering, Pohang University of Science and Technology (POSTECH), Nam-gu, Pohang, Gyeongbuk, Korea

17:45 | O1 BIOMAT & Human Repair - DIAPID project : an innovative prosthesis dedicated to children

Ariane DAVID, FIMATHO, University Hospital of Lille, France, Lille

18:00 | O2 BIOMAT & Human Repair - Breast reconstruction using a bioabsorbable Tissue-Engineering Chamber for the regeneration of autologous adipose tissue: A Long Term pre-clinical review on minipigs

Julien PAYEN, Lattice Medical, Loos, France

18:15 | O3 BIOMAT & Human Repair - Fucoidan: from brown algae to a novel radiotracer in cardiovascular imaging

Cédric CHAUVIERRE, Université Paris Cité, UMRS1148, INSERM, Paris, France

18:30 | O4 BIOMAT & Human Repair - Bone Substitutes: from lab to bed

Didier MAINARD, CHRU, Nancy, France

18:45 - 19:00 | End of the Sessions

SCHEDULE – TUESDAY, 6 SEPTEMBER 2022

08:00 - 08:30 | Registration

08:30 - 09:15 | PL3 - Plenary Lecture
Pr Kristi Anseth, University of Colorado, USA
Chairpersons: Matteo Santin & Didier Letourneur
Location: Room A

09:15 - 10:00 | George Winter Award
Pr Abhay Pandit, National University of Ireland, Galway, Irland
Chairpersons: Ana Pego & Luigi Ambrosio
Location: Room A

10:00 - 10:30 | Coffee Break & Posters

--- 10:30 - 12:30 | Parallel Sessions ---

>> 10:30 - 12:30 | SYMP-04 - USE OF PHYSICAL FORCES FOR BIOMATERIALS DESIGN OR CHARACTERIZATION
Chairpersons: Agnès Drochon & Rui Domingues
Location: Room C

10:30 | KL Physical forces - New tools in magnetic tissue engineering
Manuela E. GOMES, University of Minho, Guimaraes, Portugal

11:00 | O1 Physical forces - Architectured mechano-hybrid-scaffold for endochondral healing of critical size bone defects
Ansgar PETERSEN, BIH Center for Regenerative Therapies at Charité – Universitätsmedizin Berlin, Berlin, Germany; Julius Wolff Institut, Charité – Universitätsmedizin Berlin, Berlin, Germany

11:15 | O2 Physical forces - High-throughput Generation of Soft Dissolvable Cell Microcarriers using In-air Microfluidics
Luanda LINS, Leijten Lab, Dept. of Developmental BioEngineering, TechMed Centre, University of Twente, Enschede, The Netherlands

11:30 | O3 Physical forces - Biological Evaluation of an In Vitro Biomimetic Platform Based on a Parallel Perfusion Bioreactor Designed to Test and Promote the Osteogenic Commitment of Cells and Biomaterials
Farah DAOU, Dept. of Health Sciences, Center for Translational Research on Autoimmune and Allergic Diseases (CAAD), Università del Piemonte Orientale (UPO), Novara, Italy

11:45 | O4 Physical forces - A novel approach for vascularization in 3D in vitro adipose tissue models
Matteo PITTON, Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy

12:00 | FP01 Physical forces - Perfusable Microvessel Substitutes Integrated in a Tailored Bioreactor Enable the Investigation of Chemotaxis in vitro

Mattis WACHENDÖRFER, Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany

12:05 | FP02 Physical forces - Synthesis of aligned hollow polymeric microfibers by coaxial electrospinning for the development of 3D in vitro models in perfusion bioreactors

Nazely DIBAN, Departamento de Ingenierias Química y Biomolecular, Universidad de Cantabria, Santander, Spain

12:10 | FP03 Physical forces - Photo-crosslinkable gelatin-based bio-inks as strategy towards patient-specific breast reconstruction

Lana VAN DAMME, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium

>> 10:30 - 12:30 | SYMP-05 - GENE-ACTIVATED 3D SCAFFOLDS FOR CARTILAGE AND OSTEOCHONDRAL REPAIR

Chairpersons: Arlyng Gonzalez Vazquez & Fergal J O'Brien
Location: Room H

10:30 | KL Gene activated - Scaffold-Mediated Viral Gene Delivery for Cartilage and Osteochondral Repair

Magali CUCCHIARINI, Center of Experimental Orthopaedics, Saarland University and Saarland University Medical Center, Homburg/Saar, Germany

11:00 | O1 Gene activated - Are gelatin nanoparticles suitable non-viral vectors for the delivery of mRNA?

Lea ANDRÉE, Department of Dentistry - Regenerative Biomaterials, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, The Netherlands

11:15 | O2 Gene activated - The activity of fucoidan/dendrimer nanoparticles regarding angiogenesis

Filipe OLIM, CQM-Centro de Química da Madeira, University of Madeira, Campus da Penteada, 9020-105 Funchal, Portugal

11:30 | O3 Gene activated - Toward an innovative cement formulation combining calcium phosphate and lipid-oligonucleotide to address bacterial resistance

Clémentine AUBRY, ARNA/Inserm U1212, CNRS 5320, University of Bordeaux, Bordeaux, France

11:45 | O4 Gene activated - Novel cancer gene-therapy carriers based on the recombinant fusion of polycationic Elastin-Like Polymers and single-chain variable antibody fragments

Sara ESCALERA-ANZOLA, Smart Biodevices for Nanomedicine, University of Valladolid, Edificio LUCIA, Valladolid, Spain

12:00 | FP01 Gene activated - Decellularisation of whole human condyles for osteochondral repair
Hazel FERMOR, School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

12:05 | FP02 Gene activated - Bone Regeneration with Antibiotic Delivery – A New Approach to Osteomyelitis and Infected Joint Replacements
Gerard INSLEY, Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

>> 10:30 - 12:30 | SYMP-06 - ELECTROACTIVE SCAFFOLDS IN BIOMATERIALS

Chairpersons: Markus Rottmar & Sahba Mobini
Location: Room E

10:30 | KL Electroactive - Soft Electronics as Interfaces to Living Tissues
John A. ROGERS, Department of Materials Science and Engineering and Biomedical Engineering, Northwestern University, Evanston, USA

11:00 | O1 Electroactive - Biomimetic Electroconductive PEDOT Nanoparticle Scaffold for Spinal Cord Injury Repair
Aleksandra SERAFIN, Bernal Institute, School of Engineering, University of Limerick, Ireland

11:15 | O2 Electroactive - Dissolution Behaviour and Biocompatibility of Combinatorially Sputtered SiFeCN Coatings for Spinal Implants
Estefanía ECHEVERRI, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden

11:30 | O3 Electroactive - Collagen/Pristine Graphene as an Electroconductive Interface Material for Neuronal Medical Device Applications
Jack MAUGHAN, Chemical Physics of Low Dimensional Nanostructures Group, School of Physics, Trinity College Dublin & Tissue Engineering Research Group, Royal College of Surgeons in Ireland

11:45 | O4 Electroactive - Bioelectronics to study and regenerate bone
Donata IANDOLO, INSERM, U1059 Sainbiose, Université Jean Monnet, Saint-Étienne, FR.

12:00 | FP01 Electroactive - Nutlin-loaded ultrasound-activated piezoelectric nanovectors: Modulation of anti-angiogenic activity
Ozlem SEN, Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pontedera, Italy

12:05 | FP02 Electroactive - Design of a fully-resorbable electronic device for neural stimulation and monitoring
Isabelle TEXIER, University Grenoble Alpes, CEA, LETI-DTBS, Grenoble, France

12:10 | FP03 Electroactive - A Closed-Loop Soft Robotic Drug Delivery System to Overcome the Foreign Body Response
Lucien H.J. SCHREIBER, Anatomy and Regenerative Medicine Institute (REMEDI), School of Medicine, NUI Galway, Galway, Ireland

>> 10:30 - 12:30 | SYMP-07 - BIOMATERIALS FOR ORGANOIDS AND ORGAN-ON-CHIPS

Chairpersons: Vincent Flacher & Stéphanie Descroix
Location: Room A

10:30 | KL OoO - Biomaterials, Organoids And Microfluidics: A Winning Trio For Organ-On-Chips
Audrey FERRAND, Team 'Environment and Intestinal Epithelium', Institut de Recherche en Santé Digestive (IRSD), Toulouse, France

11:00 | O1 - Using Collagen Microfibers to Unlock in vitro Vascularized Mature Adipose Tissue Regeneration: Applications to Patients-derived Drug-screening Models and Breast Reconstruction
Fiona LOUIS, Joint Research Laboratory (TOPPAN) for Advanced Cell Regulatory Chemistry, Graduate School of Engineering, Osaka University, Japan

11:15 | O2 - 3D Bioprinted Glioblastoma Microenvironment
Jessica SENIOR, University of Huddersfield, UK

11:30 | O3 - Stratified Living Units for Modeling Pancreatic Tumor-Stroma Microenvironment
Maria MONTEIRO, CICECO-Aveiro Institute of Materials, Aveiro University, Aveiro, Portugal

11:45 | O4 - In vitro alveolar-capillary barrier model for the study of particulate matter toxicity
Chiara Emma CAMPIGLIO, Department of Management, Information and Production Engineering, University of Bergamo, Dalmine (BG), Italy

12:00 | FP01 OoO - Cabbage leaves as 3D platform for in vitro adipose tissue model
Lina ALTOMARE, Dipartimento di Chimica, Materiali, Ing. Chimica 'G Natta' Politecnico di Milano, Milan, Italy

12:05 | FP02 OoO - Organ-on-a-chip to evaluate biomaterials
Oscar CASTANO LINARES, University of Barcelona (UB), Barcelona, Spain; Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain; CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain

12:10 | FP03 OoO - Development of optically-tuned bioresins for the rapid volumetric bioprinting of liver organoid-laden metabolic biofactories
Marc FALANDT, Department of Orthopedics, UMC Utrecht, Utrecht, the Netherlands

12:15 | FP04 OoO - sciPULSE ULTRA LOW VOLUME - Dispensing of small droplets in the range of 18 -180 pL
Dietrich KOESTER, SCIENION GmbH, Berlin, Germany

>> 10:30 - 12:30 | SYMP-08 - BIO-INSPIRED SUPRAMOLECULAR SCAFFOLDS AS CELL NICHES FOR BIOMEDICAL APPLICATIONS

Chairpersons: Chris Sammon & Dammy Olayanju
Location: Room B

10:30 | KL1 Supramolecular - b-sheet peptide based supramolecular scaffolds: from design to application
Alberto SAIANI, Department of Materials, University of Manchester, Manchester, United Kingdom

11:00 | O1 Supramolecular - Heparin-Guided Binding of VEGF to Supramolecular Biomaterial Surfaces to Create Dual-Functionalized Hemocompatible Devices
Dina IBRAHIM, Eindhoven University of Technology, Eindhoven, The Netherlands

11:15 | O2 Supramolecular - Biodegradable dendrimers for mRNA therapeutics: crossing the BBB of the ischemic brain
Marília TORRADO, i3S/INEB - Institute for Research and Innovation in Health/Institute of Biomedical Engineering, University of Porto, Porto, Portugal; ICBAS - Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal

11:30 | O3 Supramolecular - Hybrid supramolecular and photoresponsive gelatin hydrogels as dynamic cell culture matrices and volumetrically printable bioresins
Marc FALANDT, Dept. of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

11:45 | O4 Supramolecular - Corneal endothelial tissue engineering using smart, multi-layered polymer sheets
Jasper DELAEY, Polymer Chemistry & Biomaterials group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent (Belgium)

12:00 | FP01 Supramolecular - Bioinspired Composite Paste-type Inks For 3D Printing Scaffolds with Bone Regeneration Potential
Izabela-Cristina STANCU, Advanced Polymer Materials Group, University Politehnica of Bucharest, Bucharest, Romania

12:05 | FP02 Supramolecular - 3D Bioinspired Hydrogels with Molecularly Imprinted Nanoparticles Sequester Endogenous Growth Factors and Synergistically Direct Stem Cell Fate Commitment
Simão P. B. TEIXEIRA, 3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Braga/Guimarães, Portugal

12:10 | FP03 Supramolecular - Dual-crosslinked degradable elastomer with self-healing properties
Mathilde GROSJEAN, Department of Polymers for Health and Biomaterials, IBMM, University of Montpellier, Montpellier, France

SCHEDULE – TUESDAY, 6 SEPTEMBER 2022

--- 10:30 - 12:30 | Technological Sessions ---

>> 10:30 - 11:30 | TECH S3 - CHARACTERIZATION OF BIOMATERIALS: IN SITU MONITORING

Chairpersons: Erik Nilebäck & Philippe Lavalle
Location: Room F

10:30 | KL in situ monitoring - QCM-D as a powerful tool to design and evaluate Biomaterials
Erik NILEBÄCK, Biolin Scientific AB, Västra Frölunda, Sweden

10:45 | O1 In situ monitoring - Real-Time Monitoring and Optimization of Hydrogel Photocrosslinking in Volumetric Printing
Sammy FLORCZAK, Department of Orthopedics, UMC Utrecht, Utrecht, the Netherlands

11:00 | O2 In situ monitoring - Sono-responsive Hybrid TiO2/Polymer Nanomaterials for Actively Targeted Sonodynamic Therapy of Cancer
Ivan ZLOTVER, Department of Material Science and Engineering, Technion – Israel Institute of Technology, Haifa, Israel

11:15 | O3 In situ monitoring - Bio-impedance and Microscopy Monitoring of Spheroid Inside a Microfluidic Device for Electro-Chemotherapy Applications
Pauline BREGIGEON, Univ Lyon, Ecole Centrale de Lyon, INSA Lyon, Université Claude Bernard Lyon 1, CNRS, Ampère, UMR5005, 69130 Ecully, France

>> 11:30 - 12:30 | TECH S4 - PRODUCTION METHODS

Chairpersons: Elzbieta Pamula & Jasper Van Hoorick
Location: Room F

11:30 | KL1 Production methods - Influence of Endotoxin on Cellular Activity
Jos OLIJVE, Rousselot Biomedical, Gent, Belgium

12:00 | KL2 Production methods - Gelatin: From Biomaterial to BIO INK
Jasper VAN HOORICK, BIO INX, Ghent, Belgium

12:15 | O1 Production methods - Cellularized MPL-produced microscaffolds as building blocks for cartilage defects repair.
Olivier GUILLAUME, 3D Printing and Biofabrication Group, Institute of Materials Science and Technology, TU Wien, Vienna, Austria

12:30 - 13:30 | Lunch Break & Meeting Industrials and Professors

13:30 - 14:30 | PS2 - Poster Session 2

14:30 - 15:15 | PL4 - Plenary Lecture
Pr José-Alain Sahel, Sorbonne University, Paris, France
Chairpersons: Didier Mainard & Maria Chatzinikolaidou
Location: Room A

--- 15:30 - 16:30 | Parallel Sessions ---

>> 15:30 - 16:30 | PSOP-08 - ANTIMICROBIAL BIOMATERIALS

Chairpersons: Lia Rimondini & Philippe Lavalle
Location: Room C

15:30 | O1 Antimicrobial - Chitosan-DEAE Nanoparticles for the Development of Antipneumococcal Therapeutics
María Rosa AGUILAR DE ARMAS, Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC), Madrid, Spain; Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales, y Nanomedicina (CIBER-BBN), Madrid, Spain

15:45 | O2 Antimicrobial - Evaluation of the Staphylococcus Aureus Bacteriophages Lytic Activity Depending on the Biopolymer Carrier
Liga STIPNIECE, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga Technical University, Riga, Latvia; Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

16:00 | O3 Antimicrobial - Antibiotic-free Collagen-Hydroxyapatite Scaffolds Reinforced with 3D Printing to Treat Infection and Support Bone Regeneration in Load-Bearing Defects
Katelyn GENOUD, Tissue Engineering Research Group (TERG), RCSI University of Medicine and Health Sciences, Dublin, Ireland

16:15 | O4 Antimicrobial - Biological characterization of antibacterial Ag-doped calcium titanate layer on titanium implants
David PIÑERA AVELLANEDA, Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering, Technical University of Catalonia (UPC), Barcelona East School of Engineering (EEBE), 08019, Barcelona, Spain

>> 15:30 - 16:30 | PSOP-09 - NANOBIOMATERIALS

Chairpersons: Gianni Ciofani & Maria Pau Ginebra
Location: Room A

15:30 | O1 Nanobiomaterials - 3D-printed Plasmonic Scaffolds for SERS Sensing and Imaging of Cancer Models
Clara GARCIA-ASTRAIN, CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), 20014 Donostia - San Sebastián, Spain; Centro de Investigación Biomédica en Red de Bioingeniería Biomateriales, y Nanomedicina (CIBER-BBN), 20014 Donostia-San Sebastián, Spain

15:45 | O2 Nanobiomaterials - Surface-functionalized nanomedicines with FcRn-targeted ligands for intestinal delivery of semaglutide
Soraia PINTO, Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal

16:00 | O3 Nanobiomaterials - General features of metal sulfide biomineralization in microorganisms and particularity in intracellular biomineralization of copper sulfide
Yeseul PARK, Aix-Marseille Université, CEA, CNRS, BIAM, Saint Paul lez Durance, France

16:15 | O4 Nanobiomaterials - Development of Virus-Like Particle platform for the control of cell behavior
Hasna MAAYOUF, Université de Haute-Alsace, IS2M-CNRS 7361, Mulhouse, France

>> 15:30 - 16:30 | PSOP-10 - NEURAL TISSUE ENGINEERING
Chairpersons: Zaida Alvarez & Abhay Pandit
Location: Room F

15:30 | O1 Neural tissue - Design of a Fibre-based Neuronal Guidance Scaffold for the Inner Ear
Peter BEHRENS, Institute of Inorganic Chemistry, Leibniz University Hannover, Hannover, Germany; Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School, Hannover, Germany; Cluster of Excellence Hearing4all, Hannover, Germany

15:45 | O2 Neural Tissue - Anisotropic Interfacial Complexes of Collagen and Glycosaminoglycans as Mimics of Neural Tissues
Rui R. COSTA, 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Avepark – Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

16:00 | O3 Neural Tissue - Fabrication of Living Electrodes Using Multiphoton Induced Degradation in a Photoresponsive Hydrogels for Neural Interface Applications
Jasper Van Hoorick, CÚRAM SFI Research Centre for Medical Devices, National University of Ireland Galway, Galway, Ireland

16:15 | O4 Neural Tissue - The mechanical side of the brain: a tissue engineered model to explore the role of mechanical properties alterations in CNS demyelination
Eva Daniela CARVALHO, i3S – Instituto for Health and Research in Innovation, University of Porto, Porto, Portugal / INEB – Institute of Biomedical Engineering, University of Porto, Porto, Portugal / FEUP – Faculty of Engineering, University of Porto, Porto, Portugal

--- 15:30 - 16:30 | Clinical Sessions ---

>> 15:30 - 16:30 | CS1 - CLINICAL SESSION I: CARDIOVASCULAR CLINICAL APPLICATIONS

Chairpersons: Laurence Bordenave & Aldo Boccaccini
Location: Room B

15:30 | KL Clinical cardiovascular - Biomaterials in Vascular Surgery: Clinical Issues and (Endo)Vascular perspectives
Caroline CARADU, Bordeaux University Hospital, Bordeaux, France

15:45 | O1 Clinical cardiovascular - Preclinical Evaluation of a Novel Nanofibrous Resorbable Synthetic Vascular Stent up to 12 Months in Rabbits
Anthal SMITS, Eindhoven University of Technology, Eindhoven, The Netherlands

16:00 | O2 Clinical cardiovascular - CHARACTERIZATION OF A CELL-ASSEMBLED EXTRACELLULAR MATRIX (CAM) SUTURE MATERIAL
Paul BORCHIELLINI , Univ. Bordeaux, Inserm, BioTis, UMR1026, Bordeaux, France ; CHU de Bordeaux, Bordeaux, France

16:15 | O3 Clinical cardiovascular - Extent of Calcification in Heart Valve Tissue Engineering; a Systematic Review and Meta-Analysis of Large-Animal Studies of Pulmonary Valve Replacement.
Dewy VAN DER VALK, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

--- 15:30 - 16:30 | Translational Sessions ---

>> 15:30 - 17:30 | TRANS S1 - TRANSLATIONAL SESSION I

Chairpersons: Yves Bayon & Maria Pereira
Location: Room E

15:30 | KL Translational I - From Quantum Chemistry to Drug Eluting Stents: a Historical Narrative of How The Electro-Grafting of Vinylic Polymers Ended Up in Patients
Christophe BUREAU, AlchiMedics S.A.S., Paris, France

16:00 | O1 Translational I - Novel Fermentation based functional bio-material advances - GMP compliant non animal derived Collagen and bacterial Cellulose
Tim SMONIK, Evonik, Essen, Germany

16:15 | O2 Translational I - Development of new tissue-engineered vascular grafts: implantation, conservation and sterilization.
Diane POTART, BioTis INSERM U1026, Université de Bordeaux, Bordeaux, France

16:30 - 17:15 | Coffee Break & Posters

>> 17:15 - 18:15 | YSF AND YOUNG RESEARCHERS BIOMAT – WORKSHOP II

Chairpersons: YSF and Young Researchers BIOMAT
Location: Room C

17:15 | How to keep young researchers in a good mental shape?
An BOGAERTS, HSE Department, KU Leuven, Leuven, Belgium

17:45 | Navigating The Early Academic Career, With a Smile
Vianney DELPLACE, Nantes Université, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, Nantes, France

--- 17:15 - 18:15 | Parallel Sessions ---

>> 17:15 - 18:15 | PSOP-11 - CARDIOVASCULAR TISSUE ENGINEERING

Chairpersons: Nicolas L'Heureux & Teresa Simon-Yarza
Location: Room F

17:15 | O1 Cardiovascular - 3D in situ ECM deposition: a new class of biohybrid material for cardiac repair
Albane CARRÉ, Université Paris Cité, INSERM U1148, Paris, France

17:30 | O2 Cardiovascular - Poly(alkylene terephthalate)s for Cardiovascular Applications: Effect of Molar Mass and Alkyl Chain Length on Surface Properties and Biocompatibility
Lenny VAN DAELE, Polymer Chemistry and Biomaterials Group (PBM), Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium

17:45 | O3 Cardiovascular - High-throughput Screening to Mimic Tunica Media SMCs Alignment in Porous Blood Vessel Scaffolds
Klaudia JURCZAK, University of Groningen, Groningen, The Netherlands

18:00 | O4 Cardiovascular - First Implantation of an Allogeneic Woven Tissue-Engineered Blood Vessel Made of Cell-Assembled Extracellular Matrix
Fabien KAWECKI, Univ. Bordeaux, Inserm, BioTis, UMR1026, Bordeaux, France

>> 17:15 - 18:15 | PSOP-12 - HYDROGEL I

Chairpersons: Joao Mano & Sylvia Fare
Location: Room A

17:15 | O1 Hydrogels I - A Universal Nanogel-based Coating Approach For Medical Implants
Devlina GHOSH, University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, Groningen, The Netherlands

17:30 | O2 Hydrogels I - Cell-instructive 3D Printed Multilayered Biomaterials embedded in Photocrosslinkable Hydrogels for Vascular Tissue Engineering
João BORGES, Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

17:45 | O3 Hydrogels I - Ex vivo and in vivo evaluation of injectable and porous hydrogels as support for muscle repair
Cloé PARET, PGNM-INMG, CNRS UMR 5310 - INSERM U1217, Claude Bernard university, Lyon, France

18:00 | O4 Hydrogels I - Thermosensitive shrinking behavior of biopolymer-based hydrogels for high resolution printing
Martina VIOLA, Department of Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, The Netherlands; Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands

--- 17:15 - 18:15 | Clinical Sessions ---

>> 17:15 - 18:30 | CS2 - CLINICAL SESSION II: OSTEOARTICULAR REPAIR
Chairpersons: Jean-Christophe Fricain & Michael Doser
Location: Room B

17:15 | KL Osteoarticular repair - Clinical Applications of Enamel Matrix Derivative, a Biomimetic and Inflammation-modulating Biomaterial
Nathalie WEBER, Institut Straumann AG, Basel, Switzerland

17:45 | O1 Osteoarticular repair - Influence of glenoid microdrilling on tissue remodeling at the pyrocarbon hemi-implant interface
Rémy GAUTHIER, Univ Lyon, CNRS, INSA Lyon, UCBL, Matéis, Lyon, France

18:00 | O2 Osteoarticular repair - Preclinical study design and validation of a model of intervertebral disc degeneration for the evaluation of glyco-functionalised biomaterials
Kieran JOYCE, CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland, Galway, Ireland

18:15 | O3 Osteoarticular repair - 3D-printed Biocompatible and Biodegradable PLA Scaffolds with Optimized Architecture and BMP-2 Dose to Repair a Sheep Metatarsal Critical-size Bone Defect
Charlotte GAROT, U1292 Biosanté, Equipe CNRS EMR 5000 BRM, Université Grenoble-Alpes, INSERM, CEA, Grenoble, France

--- 17:15 - 18:15 | Translational Sessions ---

>> 17:15 - 18:30 | TRANS S2 - TRANSLATIONAL SESSION II
Chairpersons: Yves Bayon & Christophe Bureau
Location: Room E

17:15 | KL Translational II - Shinning a light on tissue repair: from the bench to the bedside
Maria PEREIRA, Tissium, Paris, France

17:45 | O1 Translational II - Inguinal Hernia Mesh with a Drug Delivery System
François AUBERT, Cousin Surgery, Wervicq, France

18:00 | O2 Translational II - Industrialization of mesenchymal stem cell derived extracellular vesicle manufacturing
Adam HARDING, Process Development Department, Lonza Cell and Gene, Geleen, The Netherlands

18:15 | O3 Translational II - A Novel Resorbable Polyester, Tailor-Made to Support as a Knitted Temporary Support for Primary Ventral & Incisional Hernia Repair
Robert VESTBERG, Medtronic – Sofradim Production, Trévoux, France

18:30 - 19:30 | General Assembly Young Scientist Forum
Location: Room A

19:30 - 19:45 | End of the Sessions

18:15 - 23:59 | YSF Night

SCHEDULE – WEDNESDAY, 7 SEPTEMBER 2022

08:00 - 08:30 | Registration

08:30 - 09:15 | Klaas de Groot Award
Pr Rui Reis, University Minho, Portugal
Chairpersons: Ana Pego & Liz Tanner
Location: Room A

09:15 - 09:45 | Coffee Break & Posters

--- 9:45 - 11:15 | Parallel Sessions ---

>> 09:45 - 11:15 | EUR S - EUROPEAN PROJECTS SYMPOSIA
Chairpersons: Nicolas Blanchemain & Cristina Barrias
Location: Room C

9:45 | Eur S 01 - Open innovation test beds, a tool for the effective validation of new technologies on medical devices
Iraida LOINAZ, CIDETEC, Basque Research and Technology Alliance (BRTA), Donostia - San Sebastián, Spain, Donostia - San Sebastián, Spain

10:00 | Eur S 02 - cmRNAbone project: 3D Printed-Matrix Assisted Chemically Modified RNAs Bone Regenerative Therapy for Trauma and Osteoporotic Patients
Matteo D'ESTE, AO Research Institute Davos, Davos, Switzerland

10:15 | Eur S 03 - From the Teaming Phase 2 project Baltic Biomaterials Centre of Excellence consortium
Dagnija LOCA, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of Riga Technical University, Riga, Latvia

10:30 | Eur S 04 - From pathobiology to synovia on chip: driving rheumatoid arthritis to the precision medicine goal (FLAMIN-GO)
Annalisa CHIOCCHETTI, Department of Health Sciences, Interdisciplinary Research Center of Autoimmune Diseases-IRCAD and Center for Translational Research on Autoimmune and Allergic Disease-CAAD, Università del Piemonte Orientale, 28100 Novara, Italy; Trusteck s.r.l. (TRUSTECK, Italy); Max Planck Institute (MPG, Germany); Science on the Street (SoS, Slovenia); REGENHU (reH, Switzerland) and EU CORE Consulting Srl (EUCORE, Italy) , Italy, Italy

10:45 | Eur S 05 - EVPRO - Development of Extracellular Vesicles loaded hydrogel coatings with immunomodulatory activity for Promoted Regenerative Osseointegration of revision endoprosthesis
Claudia SKAZIK-VOOGT, Precision engineering and Automation, Fraunhofer Institute for Production Technology, Aachen, Germany

11:00 | Eur S 06 - Background, Success and Failures to get an ERC
Riccardo LEVATO, Utrecht University, Utrecht, The Netherlands

>> 09:45 - 11:15 | SYMP-09 - ELECTRICAL STIMULATION AND CONDUCTIVE BIOMATERIALS IN TISSUE ENGINEERING: ADVANCES AND CHALLENGES

Chairpersons: Sahba Mobini & María Ujué González
Location: Room H

09:45 | KL Conductive biomaterials - Conducting biomaterials for regenerative bioelectronics – a story of electrochemistry
Maria ASPLUND, Department of Microsystems Engineering (IMTEK) , University of Freiburg; BrainLinks-BrainTools Center, University of Freiburg; Division of Nursing and Medical Technology, Luleå University of Technology; Department of Microtechnology and Nanoscience, Chalmers University of Technology; Freiburg Institute for Advanced Studies (FRIAS), University of Freiburg , Freiburg, Germany

10:15 | O1 Conductive biomaterials - Elongation-induced Crystallization by One-step Melt-spinning of Poly (L-lactic Acid) Fibers for Future Piezoelectric Bioapplications
Richard SCHÖNLEIN, 1) POLYMAT and Polymers and Advanced Materials: Physics, Chemistry and Technology, Faculty of Chemistry, University of Basque Country UPV/EHU, Donostia-San Sebastian, Spain; 2) POLYMAT and Department of Mining-Metallurgy Engineering and Materials Science, Faculty of Engineering in Bilbao, University of Basque Country UPV/EHU, Bilbao, Spain

10:30 | O2 Conductive biomaterials - Electroconductive Scaffolds Based on Gelatin and PEDOT:PSS for Cardiac Regenerative Medicine
Franco FURLANI, National Research Council of Italy - Institute of Science and Technology for Ceramics (ISTEC-CNR), Faenza (RA), Italy

10:45 | O3 Conductive biomaterials - Nanocellulose Composite Wound Dressings with Integrated pH Sensing Capabilities for Detection of Wound Infections
Elisa ZATTARIN, Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

11:00 | FP01 Conductive biomaterials - Novel Pulsed Electrodeposition Method for Hybrid Conductive Soft Hydrogel based on PEDOT/Alginate for Versatile Drug Delivery
Arua DA SILVA, Implantable Bioelectronics Laboratory. Department of Automatic Control and Systems Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

11:05 | FP02 Conductive biomaterials - The interplay of collagen/bioactive glass nanoparticle coatings and electrical stimulation regimes distinctly enhanced osteogenic differentiation of human mesenchymal stem cells
Poh Soo LEE, Institute of General Electrical Engineering, University of Rostock, Rostock, Germany; Max Bergmann Centre of Biomaterials, Technische Universität Dresden, Dresden, Germany

11:10 | FP03 Conductive biomaterials - Electrohydrodynamics based functional nanofibers electrically stimulate neuron regeneration
Menglin CHEN, Department of Biological and Chemical Engineering, Aarhus University, Aarhus, Denmark

SCHEDULE – WEDNESDAY, 7 SEPTEMBER 2022

>> 09:45 - 11:15 | SYMP-10 - NANOFIBROUS MEMBRANES FOR BIOMEDICAL APPLICATIONS

Chairpersons: Cathy Ye & Nazely Diban Gómez
Location: Room E

09:45 | KL Nanofibrous materials - Design and Fabrication of Multifunctional Micro-/Nanofiber Meshes for Healthcare
Andreas LENDLEIN , Institute of Chemistry & Institute of Biochemistry and Biology, University of Potsdam , Potsdam, Germany

10:15 | O1 Nanofibrous materials - Core-shell electrospun nanofibers incorporated with Silver Nanoparticles for inhibition of microorganisms
EDVANI CURTI MUNIZ, State University of Maringá, Maringa -Brazil and Federal University of Piaui, Teresina-Brazil

10:30 | O2 Nanofibrous materials - Engineering of dual-stimuli responsive nanofibrous magnetic membranes for localized cancer treatment
Paula SOARES, i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, Caparica, Portugal

10:45 | O3 Nanofibrous materials - An Improved Wet-electrospun Technology for Fabrication of High Porous PCL Matrix for Cartilage Tissue Engineering
Haoyu WANG, Institute of Orthopaedic & Musculoskeletal Science, UCL, London, UK

11:00 | FP01 Nanofibrous materials - Electrospun PNIPAAm-based fibers for pH- and thermo-responsive localized drug release
Adriana GONÇALVES, CENIMAT | i3N, Department of Materials Science, NOVA SST, Caparica, Portugal

11:05 | FP02 Nanofibrous materials - Topography-induced modulation of cell behaviour by alumina ceramic textiles
Deepanjalee DUTTA, Institute for Biophysics, University of Bremen, Bremen, Germany

11:10 | FP03 Nanofibrous materials - 3D membrane of electrospun fibers for cell culture
Bénédicte FROMAGER, Institut Européen des Membranes, Univ Montpellier, ENSCM, CNRS, Montpellier, FRANCE

>> 09:45 - 11:15 | SYMP-11 - TISSUE ENGINEERING INSPIRED TISSUE AND ORGAN MODELS: RECENT TECHNOLOGICAL ADVANCES AND ROAD TO THE BEDSIDE AND MARKET

Chairpersons: Gianluca Ciardelli & Alessandra Roncaglioni
Location: Room A

09:45 | KL Tissue & Organ engineering - The role of physiologically based kinetic modeling in interpreting the results of in vitro cell-based toxicity assays
Ronette GEHRING, Institute of Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

10:15 | O1 Tissue & Organ engineering - Porous Polysaccharide-Based Scaffolds Promote Hepatocytes Autoassembly into Functional Spheroids and Improve Survival After Acute Liver Injury in Mice
Teresa SIMON-YARZA, Université Paris Cité, INSERM U1148, Paris, France

10:30 | O2 Tissue & Organ engineering - Fibrin microgels for dermal papilla cell encapsulation as an alternative to spheroid cultures
Cristina QUÍLEZ LÓPEZ, Department of Bioengineering and Aerospace engineering, Universidad Carlos III de Madrid, Leganés, Spain

10:45 | O3 Tissue & Organ engineering - Hybrid scaffolds to create long-term in vitro co-culture model for targeted treatment of ovarian cancer
Elly DE VLIEGHERE, Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Belgium and Cancer Research Institute Ghent (CRIG), Ghent University, Belgium

11:00 | FP01 Tissue & Organ engineering - Innovative luminescent porous 3D-printed scaffolds based on 13-93B20 bioactive glass and persistent luminescent particles for bone bioengineering.
Amel HOUAOUI, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

11:05 | FP02 Tissue & Organ engineering - Organo-mineral 3D-printed scaffolds for bone regeneration
Baptiste CHARBONNIER, RMeS Lab - INSERM U 1229 - Nantes University, Nantes, France

>> 9:45 - 11:15 | SYMP-12 - POLYMER-BASED NANOPARTICLES FOR THERANOSTICS ? FROM FORMULATION TO SCALE-UP
Chairpersons: Alberto Rainer & Fillipo Rossi
Location: Room B

9:45 | KL Nanoparticles & Theranostic - Engineering Conformable Polymeric microMESH for the Delivery of Combination Therapies against Gliomas
Paolo DECUZZI, Laboratory of Nanotechnology for Precision Medicine, Genova, Italy

10:15 | O1 Nanoparticles & Theranostic - Extracellular Hyperthermia for the treatment of advanced cutaneous melanoma
Beatriz SIMÕES, i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, Caparica, Portugal

10:30 | O2 Nanoparticles & Theranostic - Multifunctional polydopamine nanoparticles as a platform for treating colorectal cancer
Matteo BATTAGLINI, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera, Italy

10:45 | O3 Nanoparticles & Theranostic - Ultrasound responsive polymer microbubbles for a targeted treatment of thrombotic diseases
Louise FOURNIER, INSERM U1148, LVTS, Université Paris Cité, Bichat Hospital, Paris, France

11:00 | FP01 Nanoparticles & Theranostic - Cytotoxicity of nitric oxide releasing Pluronic F-127 hydrogel containing silica nanoparticles loaded with cisplatin towards breast cancer cell
Amedea BAROZZI SEABRA, Center for Natural and Human Sciences, Federal University of ABC, Santo André, Brazil

11:05 | FP02 Nanoparticles & Theranostic - Peptide Mediation of Nanoparticles To Cross The Blood-Brain Barrier – A Platform For Brain Drug Delivery
Catarina I. P. CHAPARRO, CENIMAT/i3N (Centro de Investigação em Materiais), NOVA School of Science and Technology (FCT-NOVA), Almada, Portugal; Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

>> 9:45 - 11:15 | SYMP-13 - BIOHYBRID CELL-LADEN POLYMER MESHES FOR TISSUE REGENERATION
Chairpersons: Paul Wieringa & Yannis Missirlis
Locations: Room F

9:45 | KL1 Biohybrid materials - Electrowriting of functional biomaterials
Miguel CASTILHO, Dept. of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands; Inst. for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands ; Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands, The Netherlands

10:15 | O1 Biohybrid materials - Yarn of Human Amniotic Membrane Can Be Woven into a Vascular Graft with Clinically-Relevant Mechanical Properties.
Nicolas L'HEUREUX, Univ. Bordeaux, INSERM, Laboratory for the Bioengineering of Tissues - BioTis, UMR1026, Bordeaux, France

10:30 | O2 Biohybrid materials - Organoids generation in human decellularized extracellular matrix
Anastasia PAPOZ, Univ. Grenoble Alpes, CEA, Inserm, IRIG, Biomics, Grenoble, France

10:45 | O3 Biohybrid materials - Ice-Templated Collagen-Elastin Scaffolds As Potential Substrates For Lung Alveolar Organoids
Gengyao WEI, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

11:00 | FP01 Biohybrid materials - Quantitative analysis of distribution paxillin and vinculin in osteoblasts and fibroblasts binding to electrospun PMMA fibers based on super-resolution fluorescent images.
Krzysztof BERNIAK, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow, Poland

11:05 | FP02 Biohybrid materials - Development Of A Cardiac Bio-prosthesis
Jean-Philippe JEHL, Institut Jean Lamour UMR 7198 CNRS / Université de Lorraine, Nancy, France

11:10 | FP03 Biohybrid materials - Poly(L-lactic acid) and Ceramic Composite Structures for Fully Resorbable Cranial Implants
Ana GRZESZCZAK, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden

11:30 - 12:30 | General Assembly ESB
Location: Room A

12:30 - 13:45 | Lunch Break & Meeting Industrials and Professors

12:30 - 13:45 | Lunch Post-graduate Lunch Symposia: Publish with Acta Biomaterialia – Meet the Editor

13:45 - 14:30 | PL5 - Plenary Lecture
Pr. Marja A. Boermeester, Amsterdam University Medical Centers, Netherlands
Chairpersons: Peter Dubruel & Jonathan Massera
Location: Room A

14:45 - 16:15 | FSBE debate: "The house believes that in the next decade it will be possible to print fully functional tissues and organs"
Rui L. Reis, 3B´s Research Group, University of Minho, Portugal, Chair of the Steering Committee of the Fellows in Biomaterials Science and Engineering (FBSE)
Location: Room A

- Introduction of the debate rules and to the motion – Rui L. Reis, University of Minho, Portugal
- For the proposition team
  - Elizabeth Tanner, Queen Mary & Westfield College, UK
  - Michael Gelinsky, Technical University of Dresden, Germany
- For the opposition team
  - Maria Chatzinikolaidou, University of Crete, Greece
  - Abhay Pandit, National University of Ireland, Galway, Ireland

--- 14:45 - 16:15 | Parallel Sessions ---

>> 14:45 - 16:15 | PSOP-13 - TISSUE MODELS
Chairpersons: Audrey Ferrand & Khoon Lim
Location: Room B

14:45 | KL Tissue models - Writing 3D In Vitro Models of Human Tendon Within a Biomimetic Fibrillar Support Platform
Rui DOMINGUES, 3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Braga/Guimarães, Portugal

15:15 | O1 Tissue models - Design of 3D bioengineered cardiac tissue models for the evaluation of chemical cardiotoxicity
Gianluca CIARDELLI, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

15:30 | O2 Tissue models - A Humanized In Vitro Model of Innervated Skin for Transdermal Analgesic Testing
Paul WIERINGA, Complex Tissue Regeneration Department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

15:45 | O3 Tissue models - An in vitro model as a drug testing platform for glaucoma
Hannah LAMONT, Institute of Clinical Sciences, University of Birmingham, Edgbaston, Birmingham, UK; School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, UK

16:00 | FP01 Tissue models - Microphysiological systems for the study of neurodegenerative diseases in vitro
Eleonora DE VITIS, CNR NANOTEC – Institute of Nanotechnology, Lecce, Italy; Università Del Salento, Dipartimento di Matematica e Fisica E. de Giorgi, Lecce, Italy

16:05 | FP02 Tissue models - Decellularized Fibrilar Matrix for Engineering Organotypic Tumor-stroma 3D Biomodels
Vitor GASPAR, CICECO-Aveiro Institute of Materials, Aveiro University, Aveiro, Portugal

16:10 | FP03 Tissue models - Evaluating the use of synthetic self-assembling peptides to 3D bioprint in vitro cartilage tissue models
Patricia SANTOS BEATO, Biochemical Engineering, University College London (UCL), London, UK

>> 14:45 - 16:15 | PSOP-14 - PROTEIN SURFACE INTERACTIONS

Chairpersons: Elzebieta Pamula & Julien Gautrot
Location: Room C

14:45 | KL Protein surface interactions - Competitive Binding and Molecular Crowding Regulate the Cytoplasmic Interactome of Non-Viral Polymeric Gene Delivery Vectors
Julien GAUTROT, Queen Mary, University of London

15:15 | O1 Protein surface interactions - Correlation between protein adsorption onto Cu-doped sol-gel coatings and their antibacterial potential
Julio SUAY, Department of Industrial Systems and Design Engineering, Universitat Jaume I, Castellón, Spain

15:30 | O2 Protein surface interactions - Elastomeric, Bioadhesive and pH-responsive Copolymer Hydrogels Based on Poly(glycerol sebacate) and Poly(ethylene glycol)
Mina ALEEMARDANI, Biomaterials and Tissue Engineering Group, Department of Materials Science and Engineering, Kroto Research Institute, The University of Sheffield, Sheffield, UK

15:45 | O3 Protein surface interactions - "Stealth graft polymers for therapeutic mRNA vaccines"
Coral GARCIA FERNANDEZ, Group d'Enginyeria de Materials (GEMAT), Intitut Químic de Sarrià, Universidad Ramon Llull, Barcelona, Spain

16:00 | FP01 Protein surface interactions - Injectable hydrogels for microRNA delivery to promote direct cell reprogramming in cardiac regeneration
Elena MARCELLO, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

16:05 | FP02 Protein surface interactions - A protein-based multiplex assay to screen osteogenic properties of calcium phosphate biomaterials with inorganic additives
Maria EISCHEN-LOGES, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

16:10 | FP03 Protein surface interactions - Milk-Derived Extracellular Vesicles for siRNA Delivery into Intestine Mimicking Caco-2 Cells
Josepha ROERIG, Pharmaceutical Technology, Medical Faculty, Leipzig University, Germany

16:15 - 16:45 | Coffee Break & Posters

--- 16:45 - 17:45 | Parallel Sessions ---

>> 16:45 - 17:45 | PSOP-15 - HYDROGELS II
Chairpersons: Catarina Custodio & Jos Olijve
Location: Room B

16:45 | O1 Hydrogels II - Click Chemistry Crosslinked Hydrogel Sealant for Repairing Corneal Perforations
Patrick SHAKARI, Department of Chemistry - Ångström, Macromolecular Chemistry, Uppsala University, Uppsala, Sweden

17:00 | O2 Hydrogels II - Influence of hyaluronic acid within biomimetic pulmonary niche on the mesenchymal stem cells differentiation toward mature Type II pneuumocytes
Francesca DELLA SALA, Institute of Polymers, Composite and Biomaterials, National Research Council of Italy, Viale J.F. Kennedy 54, 80125 Naples, Italy

17:15 | O3 Hydrogels II - An innovative phyllosilicate-based hydrogel for skin decontamination against chemical warfare agents
Kardelen DURMAZ, IBCP, LBTI, CNRS UMR 5305, Lyon, France

17:30 | O4 Hydrogels II - Hyaluronan-O-Carboxymethyl Chitosan-based Hydrogels as Delivery Platforms of Osteogenic Factors and Mesenchymal Stem Cells
Daniel FERNÁNDEZ-VILLA, 1 Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC), Spain; 2 Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

>> 16:45 - 17:45 | PSOP-16 - ADDITIVE MANUFACTURING

Chairpersons: Nicolas Dunne & Vera Tiodorovic
Location: Room F

16:45 | O1 Additive manufacturing - Ceramics Dynamic Molding: a new technology of additive manufacturing for large-size Cranio-Maxillo-Facial orthopedic implants
Ambra PATERLINI, 3Deus Dynamics, Villeurbanne, France

17:00 | O2 Additive manufacturing - Drop-On-Demand Bioprinting From Micro To Macro Scale Using The Acoustic Droplet Ejection Principle
Stefan JENTSCH, Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany

17:15 | O3 Additive manufacturing - Engineering of a Bioartificial Filtration Unit of the Kidney using a Natural Polymer
Syed Mohammad Daniel SYED MOHAMED, Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

17:30 | O4 Additive manufacturing - Giving Life to Materials: Tuning bacterial behavior in Pluronic based bioinks for living therapeutic applications
Shardul BHUSARI, Leibniz Institute for New Materials (INM), Saarbrücken, Germany, Chemistry Depa rtment, Saarland University, Saarbrücken, Germany

>> 16:45 - 17:45 | PSOP-17 - CELL TISSUE BIOMATERIAL INTERACTIONS I

Chairpersons: Marie-Christine Durrieu & Lorenzo Moroni
Location: Room C

16:45 | O1 Cell & Tissue interactions I - A Tissue Engineering Model of Non-Syndromic Craniosynostosis for Identifying Potential Therapeutic Targets that Accelerate Bone Repair
Mariangela MEYER, (1) Tissue Engineering Research Group (TERG), Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin 2, Ireland. (2) Advanced Materials Bio-Engineering Research Centre (AMBER), RCSI and Trinity College Dublin (TCD), Dublin 2, Ireland

17:00 | O2 Cell & Tissue interactions I - High-Throughput Screening to Elucidate Biomaterial-Associated Infection
Lisa TROMP, Department of Biomedical Engineering, W.J. Kolff Institute for Biomedical Engineering and Materials Science, University of Groningen/University Medical Center Groningen, Groningen, The Netherlands

17:15 | O3 Cell & Tissue interactions I - Transcriptomics Analysis During Early Healing of Rabbit Calvarial Defects; Influence of Scaffold's Microarchitecture
Julien GUERRERO, University of Zurich, Center of Dental Medicine, Oral Biotechnology & Bioengineering, Zürich, Switzerland

17:30 | O4 Cell & Tissue interactions - Learning from Cell-Material Interactions to Improve MSC Therapeutic Potential
Nathan LAGNEAU, Nantes Université, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

>> 16:45 - 17:45 | PSOP-18 - DRUG DELIVERY I
Chairpersons: Sei Kwang Hahn & Catherine Picart
Location: Room E

16:45 | O1 Drug delivery I - Nanoparticles drug delivery systems to counteract inflammation on microfluidic-based neurovascular bone unit
Estrela NETO, INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

17:00 | O2 Drug delivery I - Regioselectively Oxidized Hyaluronate for Enhanced Cisplatin Delivery
Jan VICHA, Centre of Polymer Systems, Tomas Bata University in Zlín, Zlín, Czech Republic

17:15 | O3 Drug Delivery I - Drug-eluting metals by cold sintering processing: high strength and prolonged drug release
Aliya SHARIPOVA, Department of Material Science and Engineering, Technion – Israel Institute of Technology, Haifa, Israel

17:30 | O4 Drug delivery I - Stimulation of Neovascularisation by Controlled Delivery of VEGF using an Implantable Hydrogel Loaded Soft Robotic Drug Delivery System
Eimear WALLACE, 1. Anatomy and Regenerative Medicine Institute (REMEDI), School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland, 2. Aurum Laboratories, Explora-Biotech, Rome, Italy

>> 16:45 - 17:45 | PSOP-19 - POLYMER I
Chairpersons: Hélène Van den Berghe & Andreas Lendlein
Location: Room A

16:45 | O1 Polymer I - Mesenchymal Stem Cells Sense the Toughness of Interfaces
Julien GAUTROT, School of Engineering and Materials Science, Queen Mary, University of London, London, United Kingdom

17:00 | O2 Polymer I - Effect of WS2NT on PLLA Microstructure During Bioresorbable Stent Manufacturing Process
Lison ROCHER, School of Mechanical and Aerospace Engineering, Queen's University Belfast, UK

17:15 | O3 Polymer I - The Degradation and Encrustation Behaviours of Poly Glycerol Sebacate Citrate in Artificial Urine
Lu ZHANG, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

17:30 | O4 Polymer I - Role of Macromolecular Characteristics on the Morphology of Thermosensitive Chitosan Hydrogels

Pierre MARQUAILLE, Molecular, Macromolecular Chemistry and Materials, ESPCI Paris, CNRS, PSL University, France// Équipe de Recherche sur les Relations Matrice Extracellulaire-Cellule, CY Cergy Paris Université, France

17:45 - 18:15 | End of the sessions

18:15 - 19:00 | Bus departure for the Gala Dinner Château Giscours - Only with Ticket

23:00 - 23:59 | Return to the Congress Center or in Town

SCHEDULE – THURSDAY, 8 SEPTEMBER 2022

8:00 - 9:00 | Registration

--- 9:00 - 10:30 | Parallel Sessions ---

>> 9:00 - 10:30 | PSOP-20 - BIOMIMETIC AND BIOINSPIRED MATERIALS I

Chairpersons: Rui Reis & Christèle Combes
Location: Room A

9:00 | KL Biomimetics I - Engineered spider silk hybrid proteins with transforming growth factor b3 modulates stem cell response and anticipate new prospects for tendon repair
Albina FRANCO, 3B's Research Group –I3BS Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Braga/Guimarães, Portugal

9:30 | O1 Biomimetics I - Automated fabrication of biomimetic platforms to present bone morphogenetic proteins
Elisa MIGLIORINI, Univ. Grenoble Alpes, CEA, INSERM, U1292 Biosanté, CNRS EMR 5000 BRM, Grenoble, France

9:45 | O2 Biomimetics I - Combination of Proteolytic Sequences, VEGF-mimetic Peptide and Laminin-derived Peptide for the Spatiotemporal Direction of Angiogenesis and Neurogenesis in Elastin-Like Recombinamer Scaffolds
Fernando GONZÁLEZ-PÉREZ, G.I.R. BIOFORGE, Universidad de Valladolid CIBER-BBN, Valladolid, Spain

10:00 | O3 Biomimetics I - Four-axis fabrication of a biocompatible vascular graft mimicking mechanical vessel properties
Tim TEN BRINK, Department of Complex Tissue Regeneration (CTR)/MERLN Institute for Technology Inspired regenerative Medicine, Maastricht University, Maastricht, The Netherlands

10:15 | FP01 Biomimetics I - Characterization and Modeling of Functional Gradients for Enabling Tough Biomimetic Devices
Mauricio CRUZ SALDIVAR, Department of Biomechanical Engineering, Faculty of 3mE, Delft University of Technology, Delft, The Netherlands

10:20 | FP02 Biomimetics I - Development of tough 3D-printed biomimetic calcium phosphate scaffolds for bone regeneration
Maria-Pau GINEBRA, Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain; Barcelona Research Centre for Multiscale Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain

10:25 | FP03 Biomimetics I - Polyurethane-based Freeze-dried Systems Combined with Chondroitin Sulphate and Caseinophosphopeptides for Tendon Tissue Engineering
Eleonora BIANCHI, Department of Drug Sciences, University of Pavia, Pavia, Italy

SCHEDULE – THURSDAY, 8 SEPTEMBER 2022

>> 9:00 - 10:30 | PSOP-21 - BIOMATERIALS FOR CANCER

Chairpersons: Catherine Picart & Jens Puschhoff
Location: Room E

9:00 | KL Biomaterials for cancer - Applications of organoids in bioengineering
Jens PUSCHHOF, Epithelium Microenvironment Interaction Laboratory, Division Microbiome and Cancer, German Cancer Research Center, Heidelberg, Germany

9:30 | O1 Biomaterials for cancer - Designing a Peptide Hydrogel for Early Detection of Cancer
Niall MAHON, Department of Materials, Manchester Institute of Biotechnology, Manchester, UK

9:45 | O2 Biomaterials for Cancer - Patient-derived nanocarriers for precise hyperthermia against glioblastoma multiforme cells
Daniele DE PASQUALE, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), Italy

10:00 | O3 Biomaterials for cancer - Reactive Oxygen/Nitrogen Species-Scavenging Hydrogels with Therapeutic Potentials in Triple-Negative Breast Cancer
Amir ALSHARABASY, CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland, Galway (NUIG), Ireland

10:15 | FP01 Biomaterials for cancer - Multifunctional biomimetic pancreatic cancer cell membrane-camouflaged vitamin E-based prodrug micelles
Miguel PEREIRA DA SILVA, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

10:20 | FP02 Biomaterials for cancer - A 3D Co-Culture Spheroid Model to Assess the Response of Bone Metastases to Anticancer Drugs
Ceri-Anne SUURMOND, Department of Dentistry – Regenerative Biomaterials, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

10:25 | FP03 Biomaterials for cancer - Polymeric Nanoparticles Targeting Glycans in Gastric Cancer Cells Under Live Flow Conditions
Francisca DINIZ, i3S- Institute for Research and Innovation in Health, University of Porto, Portugal; ICBAS-Institute of Biomedical Sciences of Abel Salazar, University of Porto, Portugal

>> 9:00 - 10:30 | ESB SISTERS SOCIETIES - SUSTAINABILITY OF BIOMATERIALS

Chairpersons: Maria Grazia Raucci & Marc Bohner
Location: Room B

9:00 | KL1 Sustainability of biomaterials - Sustainability of Additive Manufacturing Technologies to create Smart Scaffolds for Regenerative Medicine
Lorenzo MORONI, Complex Tissue Regeneration Department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

9:20 | KL2 Sustainability of biomaterials - Sustainable Biomaterials of bacterial origin and their use in Biomedical Applications
Ipsita ROY, University of Sheffield, Sheffield, United Kingdom

9:40 | KL3 Sustainability of biomaterials - Are bio-based and circular solutions more sustainable? A scientific perspective to assess environmental, economic and social sustainability of bio-circular options.
Massimo PERUCCA, Project HUB-360, Turin, Italy

10:00 | O1 Sustainability of biomaterials - Sustainable highly elastic hydrogels made from naturally-derived materials for biomedical applications
Guy DECANTE, 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Barco GMR, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

10:15 | O2 Sustainability of biomaterials - Physiological polyphosphate, a smart nano-/bio-material for tissue regeneration and a prophylactic drug against SARS-CoV-2 infection
Werner MÜLLER, University Medical Center of the Johannes Gutenberg University Mainz, Germany

>> 9:00 - 10:30 | SYMP-14 - PUSHING FORWARD BIOPRINTING TECHNOLOGIES FOR IN VITRO MODELS AND TISSUE ENGINEERING APPLICATIONS
Chairpersons: Alessandro Polini & Franscesca Gervaso
Location: Room C

9:00 | KL Bioprinting technologies - Advances in Light-based Bioprinting: Layerwise and Layerless Volumetric Technologies for Organoid Culture and Tissue Engineering
Riccardo LEVATO, Living Matter Engineering and Biofabrication group, Regenerative Medicine Center Utrecht; Department of Clinical Sciences, Faculty of Veterinary Medicine; Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands

9:30 | O1 Bioprinting technologies - Development of Advanced Binary Cell-laden Hydrogels Consisting of Guanosine and Guanosine 5-Monophosphate for the 3D bioprinting of scaffolds for soft tissue defects
Maria MERINO-GÓMEZ, Bioengineering Institute of Technology, International University of Catalonia, Sant Cugat del Vallès, Spain

9:45 | O2 Bioprinting technologies - Fabrication of vascularized 3D mammary gland model using hybrid volumetric bioprinting and photoablation
Dominic RUETSCHE, Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland

10:00 | O3 Bioprinting technologies - Bone Mimetic Composite Biomaterial-Ink combining Hyaluronan, Collagen and Calcium Phosphate Particles for the Delivery of Chemically Modified RNA for Treatment of Bone Defects
Daphne VAN DER HEIDE, AO Research Institute Davos, Davos, Switzerland; Department of Health Science and Technology, ETH Zürich, Zürich, Switzerland

10:15 | FP01 Bioprinting technologies - Multi-Material Approach For The Replacement Of The Temporomandibular Joint
Joanna BABILOTTE, Complex Tissue Regeneration department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

10:20 | FP02 Bioprinting technologies - ECM mimicking hydrogel scaffolds for liver tissue engineering
Nathan CARPENTIER, Polymer Chemistry and Biomaterials group, Ghent University, Ghent, Belgium

10:25 | FP03 Bioprinting technologies - 3D printed, anisotropic, and porous dense collagen hydrogels to model cardiac extracellular matrix
Marie CAMMAN, Sorbonne Université, CNRS, UMR 7574, Laboratoire de Chimie de la Matière Condensée de Paris, Paris, France/Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, Inserm ERL U1164, Biological Adaptation and Ageing, Paris, France

>> 9:00 - 10:30 | SYMP-15 - DYNAMIC MATERIALS FOR BIOENGINEERING AND DIGITAL SURGERY

Chairpersons: Matthew B. Baker & Sandra Van Vlierberghe
Location: Room H

9:00 | KL Dynamic materials - Dynamic covalent hydrogels for advanced biomaterials design
Mark W. TIBBITT, Macromolecular Engineering Laboratory, Department of Mechanical and Process Engineering, ETH Zurich, Zurich, Switzerland

9:30 | O1 Dynamic materials - Patient-derived lipid-based magnetic nanovectors: a step forward towards personalized nanomedicine
Carlotta PUCCI, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), Italy

9:45 | O2 Dynamic materials - Molecular constants of reversible Schiff base formation: How to design dynamic hydrogels from the bottom up
Francis MORGAN, Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

10:00 | O3 Dynamic materials - Design of thermo-responsive polypeptide bioconjugates for the treatment of glioblastoma by PDT/RDT
Leslie E. DUBRANA, Laboratoire de Chimie des Polymères Organique, (LCPO), UMR 5629, Université de Bordeaux, CNRS, Bordeaux INP, Pessac, France

10:15 | O4 Dynamic materials - Mixed Reality for Surgery: A New Tool For Medical Devices
Marc PIUZZI, Lynx Medical, Paris, France

SCHEDULE – THURSDAY, 8 SEPTEMBER 2022

>> 9:00 - 10:30 | SYMP-16 - ADVANCES IN THE DESIGN OF MULTIFUNCTIONAL AND CELL-INSTRUCTIVE HYDROGELS FOR TISSUE ENGINEERING

Chairpersons: Catarina Custódio & Julieta Paez
Location: Room F

9:00 | KL Multifunctional & Cell-instructive hydrogels - Design of hydrogels using natural macromolecules with improved structural and functional properties
João F. MANO, University of Aveiro, Aveiro, Portugal

9:30 | O1 Multifunctional & Cell-instructive hydrogels - Instructing Engineered Living Tissues from Within using Biophysically and Biochemically Tunable Microbuilding Blocks
Niels WILLEMEN, Dept. of Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University Twente, Enschede, The Netherlands

9:45 | O2 Multifunctional & Cell-instructive hydrogel - A composite elastin derivative-based matrix for bone tissue engineering.
Nadia MAHMOUDI, BioIngenierie Tissulaire (BioTis), Inserm U1026, University of Bordeaux, Bordeaux, France

10:00 | O3 Multifunctional & Cell-instructive hydrogels - Optimized allyl-modified gelatin hydrogel for 3D cell culture of primary human umbilical vein endothelial cells and dermal fibroblasts
Alessandro CIANCIOSI, Department of Functional Materials in Medicine and Dentistry at the Institute of Functional Materials and Biofabrication (IFB), University of Würzburg and KeyLab Polymers for Medicine of the Bavarian Polymer Institute (BPI), Würzburg, Germany

10:15 | FP01 Multifunctional & Cell-instructive hydrogels - Development of a 3D polysaccharide porous membrane for the modelling of the outer blood retina barrier
Chloé DUJARDIN, Université Paris Cité, INSERM U1148, Paris, France

10:20 | FP02 Multifunctional & Cell-instructive hydrogels - Tuning the Physical Properties of Collagen/Hyaluronan Hydrogels to favor Mesenchymal Stem Cells Differentiation into NP Cells: A Step forwards Intervertebral Disc Regeneration
Christophe HELARY, Sorbonne University, CNRS, UMR 7574 – Chemistry of Condensed Matter Laboratory, Paris, France

10:25 | FP03 Multifunctional & Cell-instructive hydrogels - Critical aspects of Ti-based bulk metallic glasses for dental implant manufacturing
Laurabelle GAUTIER, 1 MATEIS UMR CNRS 5510, INSA Lyon, Université Claude Bernard Lyon 1, Villeurbanne, France

10:30 - 11:00 | Coffee Break & Posters

--- 11:00 - 12:00 | Parallel Sessions ---

>> 11:00 - 12:00 | PSOP-22 - BIOMIMETIC AND BIOINSPIRED MATERIALS II

Chairpersons: Rui Reis & Christèle Combes
Location: Room A

11:00 | O1 Biomimetics II - Development of a biomimetic stem cell scaffold system for spinal cord applications
Cian O'CONNOR, Tissue Engineering Research Group, Dept of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland & Advanced Materials & Bioengineering Research Centre, Trinity College Dublin, Dublin, Ireland

11:15 | O2 Biomimetics II - Microfabrication of Collagen Particles as Biomaterial Building Blocks for Bottom-Up Bone Tissue Engineering
Esra GÜBEN KAÇMAZ, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

11:30 | O3 Biomimetics II - Beyond RGD; nanoclusters of syndecan- and integrin-binding ligands synergistically enhances cell/material interactions
Daniel HEATH, Department of Biomedical Engineering, University of Melbourne, Parkville, Australia

11:45 | O4 Biomimetics II - Designing injectable and functional graphene oxide (GO) – self-assembling peptide hybrid hydrogels for biomedical applications
Alberto SAIANI, Department of Materials, University of Manchester, Manchester, UK; Manchester Institute of Biotechnology, University of Manchester, Manchester, UK

>> 11:00 - 12:00 | PSOP-23 - CELL AND BACTERIA SURFACE INTERACTIONS

Chairpersons: Emmanuel Pauthe & Maria Asplund
Location: Room H

11:00 | O1 Cell & Bacteria interactions - From laboratory scale to commercialization – Conception and evaluation for a virucidal surgical mask during the COVID-19 pandemic (Cidaltex®)
Nicolas BLANCHEMAIN, Univ. Lille, INSERM, CHU Lille, U1008 – Advanced Drug Delivery Systems, Lille, France

11:15 | O2 Cell & Bacteria interactions - Metallic Glass systems for biomedical applications
Ziba NAJMI, Center for Translational Research on Autoimmune and Allergic Diseases CAAD, Università del Piemonte Orientale UPO

11:30 | O3 Cell & Bacteria interactions - Controlling blood-material interaction and osseointegration potential by tuning the composition of amorphous metals
Markus ROTTMAR, Laboratory for Biointerfaces, Empa, Swiss Federal Laboratories for Materials Science and Technology, St Galen, Switzerland

11:45 | O4 Cell & Bacteria interactions - Poly-L-Lysine and Human Plasmatic Fibronectin Films as Bifunctional Coatings to Reduce Bacterial Adhesion and Enhance Tissue Integration
Anamar MIRANDA, Equipe de Recherche sur les Relations Matrice Extracellulaire Cellules, Institut des Matériaux, CY Cergy-Paris Université, Cergy-Pontoise, France

>> 11:00 - 12:00 | PSOP-24 - HYDROGELS III
Chairpersons: Elisabeth Engel & Francis Max Yavitt
Location: Room B

11:00 | O1 Hydrogels III - Enzyme-controlled, nutritive hydrogel for mesenchymal stromal cell survival and paracrine functions
Pauline WOSINSKI, Université de Paris, B3OA CNRS 7052 INSERM U1271, Paris, France & Ecole Nationale Vétérinaire d'Alfort, B3OA, Maisons-Alfort, France

11:15 | O2 Hydrogels III - Self Assembly Peptide Based Double Network Hydrogel
Zixuan LIU, Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK; Department of Materials, The University of Manchester, Manchester, UK

11:30 | O3 Hydrogels III - Tuning viscoelasticity in colloidal nanoparticle hydrogels towards enhanced cell activity
Pascal BERTSCH, Radboud University Medical Center, Regenerative Biomaterials, Nijmegen, The Netherlands

11:45 | O4 Hydrogels III - A multifunctional and programmable micro-platform for localized tumor invasion studies
Pouyan BOUKANY, Department of Chemical Engineering, Delft university of technology, Delft, The Netherlands

>> 11:00 - 12:00 | PSOP-25 - CELL TISSUE BIOMATERIAL INTERACTIONS II
Chairpersons: Karine Anselme & Riccardo Levato
Location: Room F

11:00 | O1 Cell & Tissue interactions II - Single Cell Artificial Niches (SCANs) to study mechanotransduction guiding stem cell fate
Castro JOHNBOSCO, Leijten Lab, Dept. of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands

11:15 | O2 Cell & Tissue interactions II - The Role of Vascularization in Adhesion to Biological Tissues
Estelle PALIERSE, Chimie Moléculaire, Macromoléculaire et Matériaux, ESPCI Paris, PSL University, Paris, France

11:30 | O3 Cell & Tissue interactions II - 3D-bioprinted bionic pancreas as an innovative method of treating and preventing diabetes - how far we are from clinical application?
Michał WSZOŁA, Polbionica Ltd; Foundation of Research and Science Development; CM Medispace, Warsaw, Poland

>> 11:00 - 12:00 | PSOP-26 - Drug Delivery II

Chairpersons: Hugo Oliveira & Frédéric Velard
Location: Room E

11:00 | O1 Drug delivery II - Immunotoxicity caused by near-infrared light-responsive nanoparticles on the integrity of the blood-brain barrier
Akhilesh RAI, Faculty of Medicine, University of Coimbra, Portugal

11:15 | O2 Drug delivery II - An eye-drop formulation of Fas-mediated apoptosis inhibitor attenuates age-related macular degeneration
Sang-Kyung LEE, Department of Bioengineering and BK21 FOUR Education and Research Group for Biopharmaceutical Innovation Leader, Hanyang University, Seoul

11:30 | O3 Drug Delivery II - Glucose Promotes Transplanted Human Mesenchymal Stromal Cell Paracrine Function Pertinent To Angiogenesis
Pauline WOSINSKI, Université de Paris, CNRS, INSERM, B3OA, Paris, France & Ecole Nationale Vétérinaire d'Alfort, B3OA, Maisons-Alfort, France

11:45 | O4 Drug delivery II - A Biomaterial Drug-Eluting Composite for Reducing Post-Surgical Inflammation and Fibrosis in Glaucoma
Alan HIBBITTS, 1. Tissue Engineering Research Group, Dept of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

>> 11:00 - 12:00 | PSOP-27 - Polymer II

Chairpersons: David Eglin & Joanna Babilotte
Location: Room C

11:00 | O1 Polymer II - Optimization and characterization of Gellan Gum/Alginate microspheres produced via coaxial airflow
Henrique CARRÊLO, Departamento de Ciência dos Materiais, CENIMAT/I3N, NOVA/FCT, Almada, Portugal

11:15 | O2 Polymer II - Increasing birefringence in 3D printed PLLA single layers
Luke MALONE, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

11:30 | O3 Polymer II - Biodegradable PLA-PEU-PLA membranes: A new solution for the reduction of post-operative peritendinous adhesions
Hélène VAN DEN BERGHE, Polymers for Health and Biomaterials/IBMM/Univ Montpellier, France

11:45 | O4 Polymer II - Designing Photo-responsive Double Network Hydrogels for Tissue Engineering
Ana Agustina ALDANA, Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands

12:15 - 12:45 | Closing Ceremony
Location: Room A

12:45 - 13:00 | End of the Conference

13:00 - 14:00 | Lunch box

ORAL SESSIONS | BOOK OF ABSTRACTS PER SESSION

>> YSF & YOUNG RESEARCHERS BIOMAT - OPENING WORKSHOP

Chairpersons: YSF and Young Researchers BIOMAT
Location: Room A

14:05 | Tips for successful science communication
Elodie CHABROL, Freelance science communicator and director of Pint of science international & France

14:50 | Wonder & synergies at the service of knowledge
Renaud POURPRE, Freelance science communicator / facilitator, France

15:35 | From lab to market: a giant leap
Paul DELTROT, Readily3D SA, EPFL Innovation Park, Lausanne, Switzerland

Tips for successful science communication

Elodie Chabrol
freelance science communicator and director of Pint of science international & France
* contact@elodiechabrol.com

Have you ever felt stuck when trying to explain your research to non-scientists? Or even to scientists but outside of your field?
During this workshop, I'll give you easy-to-apply tips to help you share your research with any kind of people and make your family dinners much easier as well as your science communication presentations!

Wonder & synergies at the service of knowledge

Renaud Pourpre
Freelance science communicator / facilitator
* renaudpourpre@gmail.com

Can wonder and synergy help your science go further? Let's dive into the Cell Worlds project, founded by Renaud Pourpre and Terence Saulnier: an unprecedented journey into unknown worlds: us. Cell Worlds is an Art & Science project that aims to raise public awareness of the beauty, fragility and complexity of cellular worlds through wonder and emotion. Cell Worlds' mission is to bring microscopy images and their stories to places where they have never been: far from the laboratories and hard drives of scientists, closer to the general public. A project born from multiple synergies with scientists, and which helps us think about models of scientific communication

From lab to market: a giant leap

Paul Delrot
Readily3D SA, EPFL Innovation Park, Building A, 1015 Lausanne, Switzerland
* paul@readily3d.com

Based on our journey at Readily3D, from proof-of-concept experiment on a lab bench to a standalone certified product, we present some aspects of product development and product management, notably minimum viable product development and customer-driven product development.

>> 10:30 - 12:30 | PSOP-01 - SCAFFOLDS

Chairpersons: Marc Bohner & Cecilia Persson
Location: Room C

10:30 | KL Scaffold - 3D sensing scaffold for extracellular pH mapping at single cell level in tumor models

Valentina ONESTO, Institute of Nanotechnology, National Research Council (CNR-Nanotec), Italy

11:00 | O1 Scaffold - Cell-Derived Extracellular Matrices for Establishment of 3D in vitro Tumor Models for Cancer Research

Gülsün BAGCI, 1Institute for Bioengineering of Catalonia (IBEC), The Barcelona Institute of Science and Technology, Barcelona, Spain. 2 CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain.

11:15 | O2 Scaffold - Composite Scaffolds Enriched with Nano-hydroxyapatite and Strontium-nano-hydroxyapatite Promote Osteogenic Differentiation

Maria CHATZINIKOLAIDOU, Foundation for Research and Technology Hellas and University of Crete, Heraklion, Greece

11:30 | O3 Scaffold - Engineered Porous Microshuttles for Drug, Nanomaterials and Cell Delivery

K. SONG, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

11:45 | O4 Scaffold - Nanoneedles for Targeted siRNA Transfection of the Human Corneal Endothelium

Ciro CHIAPPINI, Centre for Craniofacial and Regenerative Biology, King's College London, London, United Kingdom; London Centre for Nanotechnology, King's College London, London, United Kingdom

12:00 | FP01 Scaffold - Fabrication of 6-BromoIndirubin-3'-Oxime Incorporated Guanosine Diphosphate Crosslinked Chitosan Scaffolds to Promote Osteogenic Differentiation in Myoblastic C2C12 Cells

Celine J. AGNES, Department of Biomedical Engineering, McGill University, Montreal, Quebec, Canada

12:05 | FP02 Scaffold - Hybrid Core-Shell Scaffolds for Bone Regeneration and a 3D Printing Approach to Tune their Properties

Chiara PASINI, Department of Mechanical and Industrial Engineering, University of Brescia, Brescia, Italy

12:10 | FP03 Scaffold - Deposition of a polymer-based honeycomb-like membrane on 3D printed bioactive glass scaffold and decellularized bone matrix as scaffolds for bone tissue engineering

Michel BOISSIERE, Laboratoire ERRMECe, CY Cergy Paris Université, Neuville sur Oise, France

3D sensing scaffold for extracellular pH mapping at single cell level in tumor models

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INTRODUCTION

The tumor microenvironment is characterized by an elevated hydrogen ion concentration, which is the result of increased cellular metabolic demand and altered perfusion, e.g., oxygen availability or acidic metabolic waste products.[1] The acidity of the tumour microenvironment, which is spatially and temporally heterogeneous,[2] affects cancer initiation and progression, but also the efficacy of anti-cancer drugs treatments.[3] Therefore, monitoring the local pH metabolic fluctuations is critical in understanding the basic biology of the tumour, and can also be used as a valid metabolic readout for cancer diagnosis and treatment. Importantly, the complexity of the tumour microenvironment, which includes cell-cell interactions and extracellular matrix composition, coupled with the fast diffusion and mobility of extracellular protons, makes the extracellular pH mapping extremely challenging.[4] Here, we developed a method to embed pH microsensors into 3D alginate hydrogel, to monitor extracellular pH metabolic variations.

EXPERIMENTAL METHODS

The *in vitro* 3D pH sensing system was obtained through a multistep procedure:

(i) Synthesis and characterization of silica-based fluorescein isothiocyanate (FITC)/ rhodamine B isothiocyanate (RBITC) ratiometric pH sensors.

(ii) By using a home-made microdroplet encapsulation apparatus composed by high voltage generator, syringe pump and collecting dish, pH sensors were integrated within alginate (3% (w/v)) spheres in the presence of human pancreatic stromal cells (PSC) and human pancreatic cancer cells (Aspc1), either alone or in combination.

(iii) 4D (x,y,z,t) confocal laser scanning microscopy (CLSM): z-stack images of the whole hydrogel were acquired at regular interval of 1 hour for 10 hours.

(iv) Quantification over time and space of single cell extracellular pH metabolic variations by a customized algorithm for 4D image processing and analysis.

RESULTS AND DISCUSSION

Tumour and stromal pancreatic cells were microencapsulated in alginate hydrogels containing pH sensors, either alone or in combination, and their extracellular pH metabolic variations were monitored through CLSM. 3D colormaps were generated for each cell giving a topographical visualization of dynamic changes of pH sensors around the cells over time and space**.** Within the same time point, the sensors surrounding each cell showed different pH. These evidences reflect the heterogeneous distribution of proton pools in the extracellular environment, which can be visualized, for the first time, by the 3D pH sensing scaffold we have generated.

CONCLUSION

Here, we report the generation and characterization of spherical 3D sensing systems to monitor cell mutual metabolic interplay in a 3D complex microenvironment. Dysregulated pH causing intracellular alkalization and extracellular acidosis is a hallmark of cancer metabolism, and it is associated with tumour development, progression and treatment resistance. Therefore, such sensing 3D platform represents a valid and powerful tool for pH sensing with unprecedented spatial and temporal resolution and appropriate *in vitro* 3D cancer models, to decipher the role of pH tumour heterogeneity and assess the response to anticancer treatments, key aspects in precision medicine. The composition and the properties of the resulting 3D sensing platform can be tailored by using polymers with different functions (e.g., collagen, chitosan, Matrigel) as well as by integrating ratiometric particles sensitive to different key biological analytes (i.e., oxygen, potassium, calcium). The presented system fully preserves both the growth of multiple cell types and the sensitivity of optical sensors to local pH changes. Importantly, cells can be retrieved from alginate hydrogels by a simple de-gelling process that does not require disaggregation of multi-cellular structures. Finally, the functional pH sensing scaffolds can be successfully fabricated on a large variety of supports making them highly compatible for high-throughput drug screening applications and analysis of cell behaviours in 3D *in vitro* tumour models.

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Cell-Derived Extracellular Matrices for Establishment of 3D in vitro Tumor Models for Cancer Research

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INTRODUCTION

Three-dimensional (3D) *in vitro* tumor models are important for recreating the complexity of the tumor microenvironment (TME) and testing new anti-cancer drugs. Conventional 2D *in vitro* models and animal models are not capable of mimic the human tumor biology. 2D models lack of complex intracellular interactions occurring *in vivo* whereas animal models have different metabolic pathways, drug response and toxicity. 3D tumor models can solve these limitations as they can recreate cell-cell and cell-extracellular matrix (ECM) interactions, production of ECM, hypoxia, gradients of pH, O_2 and nutrients of human tumors. ECM is the main component of the TME and is involved in cancer progression[1]. Cell-derived matrices (CDMs) can mimic the complexity of the tumor ECM[2]. ECMs are natural biomaterials, which are composed of proteoglycans, glycoproteins, and fibrous proteins. They play role in cancer progression, metastasis, and drug resistance due to their protein composition and biomechanics. Combination of CDMs with hydrogels or their bioinks provides obtaining more accurate 3D tumor models for personalized therapy[3].

Our aim is to fabricate CDMs from fibroblast or bone marrow human mesenchymal stem cells (BM-hMSCs) in the presence of macromolecular crowders, that can be used to obtain more physiologically relevant cancer models not only for personalized medicine but also for testing anti-cancer drugs.

EXPERIMENTAL METHODS

In this study, we cultured different cell density of human dermal fibroblasts (hDFs) with microparticles (MPs) to produce *in vitro* 3D tumor models. Polylactic acid (PLA) MPs were produced by jet break-up method based on two different polymer concentration and dispensing rate such as 35DN30 and 40DN10 MPs. Their size distribution was calculated by ImageJ and their porosity was imaged under SEM. Then they were functionalized with fibronectin or Collagen-I. Afterwards, hDFs were seeded on the MPs with a spinner flask for 8h and leaved in culture for 10-15 days to produce CDM. Before 3D experiments, the optimal conditions to obtain CDM were optimized using conventional 2D cell culture. hDFs were treated with TGFβ-1 to differentiate fibroblasts into myofibroblasts and ascorbic acid to induce collagen synthesis. In addition, the effect of Ficoll 70/400, a macromolecular crowder, was assayed. The cellular viability and cell seeding in the MPs were evaluated. The ECM production was evaluated by BCA, hydroxyproline, SEM and immunofluorescence. Values were normalized by the total DNA.

RESULTS AND DISCUSSION

Ficoll, TGFβ-1 and ascorbic acid combination increased total protein and total collagen production in 2D culture for 10 days in contrast to the control group. Moreover, the ratio of α-SMA positive cells was higher when hDF cells which were treated with TGFβ-1 for 7 days, suggesting their differentiation into myofibroblasts. Moreover, size distribution of PLA MPs was calculated between 70 and 100µm in diameter. Porous PLA MPs was observed under SEM. After 15th day incubation in culture, we obtained more compact microtissues (MTs) under stereo microscopy in the presence of Ficoll 37.5/25mg/ml both for hDFs and BM-hMSCs. In addition, Ficoll increased total protein, total collagen production in MTs in contrast to control group.

CONCLUSION

CDMs are promising biomaterials for establishing 3D in vitro tumor models for cancer research, testing anti-cancer drugs and for personalized medicine in the coming years.

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Composite Scaffolds Enriched with Nano-hydroxyapatite and Strontium-nano-hydroxyapatite Promote Osteogenic Differentiation

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INTRODUCTION

Osteoporosis is a bone pathology caused by an imbalance in bone remodeling due to excessive osteoclast-mediated bone resorption and decreased action of osteoblasts. To sustain homeostasis in osteoporotic bone, it is essential to increase the action of osteoblasts, while in parallel decrease the action of osteoclasts. Bone tissue engineering (BTE) is an attractive strategy to treat long bone fractures using three dimensional (3D) constructs as bone graft substitutes mimicking the 3D porous environment of native bone [1, 2] with the capacity to induce osteogenesis. Among different 3D printing techniques, fused deposition modeling (FDM) is one of the most commonly used fabrication methods in the BTE field, since it can process materials with mechanical properties close to those of the natural tissue [3-4]. In this study, polymeric blends of poly-L-lactic acid (PLLA), polycaprolactone (PCL) and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) (90/5/5 %wt) (blend, in brief), blend+2.5%wt of nano-hydroxyapatite (nano-HA) and blend+2.5%wt of strontium-substituted-nano-HA (Sr-nano-HA) were fabricated into 3D scaffolds with specific geometry and evaluated for their osteogenic potential.

EXPERIMENTAL METHODS

Cylindrical scaffolds of 5 mm in diameter, 1 mm in height and 50% internal porosity were fabricated using FDM printing for each of the three filament compositions.

MC3T3-E1 pre-osteoblastic cells ($7x10^4$ cells/scaffold), were seeded onto blend and composite blend scaffolds consisting of PLLA/PCL/PHBV (90/5/5) and cultured in alpha-MEM including osteogenic medium for 21 days. Cell viability, proliferation and morphology were monitored via a reduction-based cell viability assay and scanning electron microscopy (SEM). Measurement of alkaline phosphatase (ALP) activity and calcium secretion were conducted to determine the effect of the polymeric scaffolds on the osteogenic responses of the pre-osteoblasts. Produced collagen was quantified by Sirius Red staining.

RESULTS AND DISCUSSION

The cell viability assessment displays that all scaffold compositions have excellent biocompatibility, allowing cells to proliferate. This finding has been confirmed by SEM images, revealing well-spread cells depicting a physiological morphology from the earliest time of observation for all scaffold compositions with increasing cell proliferation. After 14 days in culture, the ALP activity is almost two-fold higher compared to day 7 on the blend+2.5% nano-HA and blend+2.5% Sr-nano-HA. The ALP activity in the blend scaffolds shows similar levels at all time points, demonstrating that the substituted blend scaffolds promote the osteogenic differentiation on day 14. The results on the produced calcium by the pre-osteoblastic cells are in accordance with the ALP activity data. Both substituted blends indicate a significantly higher calcium concentration compared to the pure blend at all experimental time periods. Secreted collagen levels are similar in all scaffold compositions indicating a significant increase on day 21.

CONCLUSION

Polymeric blend scaffolds of 90/5/5 %wt PLLA/PCL/PHBV enriched with nano-HA or Sr-nano-HA were fabricated by FDM and assessed for their bone regeneration potential. All types of composite scaffolds indicated an excellent biocompatibility and significantly promoted the osteoblastic cell maturation compared to the pure polymeric blend control.

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Engineered Porous Microshuttles for Drug, Nanomaterials and Cell Delivery

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INTRODUCTION

Microparticles have been used in bottom-up engineering of 3D tissues to engineer the cellular assembly, mimic tissue's extracellular matrix and provide soluble and insoluble cues for cell instruction.[1-3] On the other hand, porous microparticles have played the role of carriers in biological systems, providing a high specific surface area.[4] Here, we fabricated a library of porous poly(lactic-co-glycolic acid) (PLGA) microparticles with various pore sizes and structures, and have taken the first steps to incorporate different cargos into them. We aim to ultimately integrate these microparticles in 3D hybrid cell-biomaterial modular assemblies, combining the vital roles of tissue building blocks and microcarriers of drugs, nanomaterials or cells.

EXPERIMENTAL METHODS

Varying amounts of a mixture of PLGA and poly(vinyl pyrrolidone) (PVP) dissolved in dichloromethane (DCM) were injected into deionized water (DIW) containing 0.5% poly(vinyl alcohol) (PVA) as emulsifier under high-speed homogenization at 8000–13000 rpm with oil/water ratios of 1%, 1.5%, 2%, 2.5%, 3% and 4% v/v and homogenized for 1 min. After overnight evaporation of DCM in air under stirring, the O/W emulsions were freeze-dried twice with resuspension in DIW and centrifugation steps in between, before collecting the microparticles. The pore size and structure were fine-tuned first, by varying the weight ratios of PLGA/PVP (i.e. 70/30, 60/40, 50/50, 40/60) and second, by etching the microparticles in sodium chloride (NaOH)-ethanol aqueous solution for varying time points. The microparticles were characterized with Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM). The microparticles' pore diameter was measured in SEM images using ImageJ. Selected groups of microparticles were incubated with a 1g/L Rhodamine B (RhB, model drug) aqueous solution overnight, followed by washing steps and drying under vaccum. Samples were observed with a fluorescent microscope and analyzed by Image J.

RESULTS AND DISCUSSION

Porous PLGA microparticles were fabricated via a one-step oil-in-water (O/W) emulsion with PVP as porogen, eliminating the complicated processing and risk of porogen residue associated with the use of other common porogens.[5] FTIR spectra of microparticles illustrated no organic solvent or PVP residues. Microparticles with larger pore sizes were obtained by increasing the oil/water ratio from 1% to 2.5% v/v. However, smaller surface pores were obtained with higher oil/water ratios, possibly due to a rapid removal of PVP from the DCM droplets into the continuous phase before PLGA solidification, as was seen earlier.[6] Five kinds of microparticles with distinct pore sizes, from non-porous to porous with an average pore size of 11.13±3.25 µm, and pore structures were selected for further study (Figure 1A-B). Fluorescent microscopy visualized the presence and distribution of RhB in the RhB-loaded microparticles (Figure 1C-D). Further analysis will be done to determine the RhB loading efficacy and release profile in microparticles with different pore sizes.

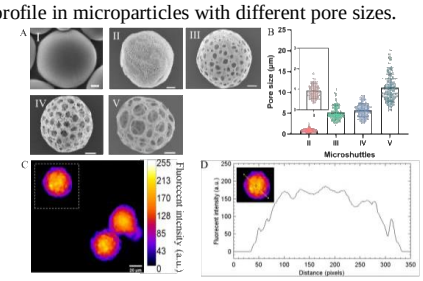

Figure 1. A) SEM images (scale bar: 10 µm) and B) pore size and distribution of I) 100/0-4%-1, II) 80/20-1%-1, III) 70/30-2%-1, IV) 70/30-2%-2 and V) 60/40-2%-2 PLGA microparticles. The labels indicate: PLGA/PVP ratio-oil/water ratio-etching time. C) Fluorescent microscopy image of RhB-loaded microparticles V (scale bar: 20 µm) and D) the corresponding profile of fluorescence intensity in one microparticle over the dash line.

CONCLUSION

We successfully developed a library of porous PLGA microparticles with different pore sizes and pore structures. A subset of the microparticles was loaded with RhB to investigate the potential of the microparticles for drug delivery, which will be further analyzed. We also plan to investigate the potential use of the microparticles as carriers of (nano)materials and cells.

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Nanoneedles for Targeted siRNA Transfection of the Human Corneal Endothelium

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Nanoneedles can target nucleic acid transfection to primary cells at tissue interfaces with high efficiency and minimal perturbation. The corneal endothelium is an ideal target for nanoneedle-mediated RNAi aimed at enhancing its proliferative capacity, necessary for tissue regeneration. Here we show that nanoneedles can deliver p16-targeting siRNA to human corneal endothelial cells *in vitro*, resulting in p16^{INK} silencing and increased cell proliferation. Furthermore, nanoneedle transfection of the human corneal endothelium tissue *ex vivo* silences p16^{INK} in transfected cells.

INTRODUCTION

In recent years vertical arrays of nanostructures have emerged as an efficient platform to interface with cells in order to deliver drugs, genes and nanoparticles, to sense their intracellular conditions and to manipulate cell behavior through biophysical stimuli[1]. In particular, porous silicon nanoneedles have emerged as the first platform to interface efficiently *in vivo*, to mediate localized in-situ gene therapy[2] and molecular diagnostics.

The corneal endothelium is an appealing target for topical nucleic acid therapy using nanoneedles[3]. Preserved corneal endothelial cells (CECs) function and monolayer integrity are essential to regulating the hydration state of the cornea and prevent loss of vision. Lost CECs cannot be replaced as the cells are in a senescent state. RNAi therapy targeting p16 and p21 genes shows promise in transiently restoring the proliferative ability of CECs. Nanoinjection is an attractive approach for RNAi therapy to the corneal endothelium (CE). Here we demonstrate that nanoinjection can mediate RNAi therapy silencing p16 targeted to CECs in human corneas.

EXPERIMENTAL METHODS

Porous silicon nanoneedles were fabricated according to established protocols[2]. siRNA was deposited on the nanoneedles for 30 minutes. CECs were isolated from human corneas and cultured following the dual media approach[4]. Nanoneedles were interfaced with CECs by centrifugation at 300RCF for 3 minutes. Following 30 minutes of incubation, the nanoneedles were removed and the cell cultured for 48 hours until prepared for imaging or qPCR.

RESULTS AND DISCUSSION

The nanoneedles interface tightly with corneal endothelial cells without inducing apoptosis, evidenced by lack of activation of Caspase 3/7. Nanoneedles deliver fluorescently-labelled siGLO to 27.6% of the CEC, resulting in 84% silencing of p16, when normalised for delivery efficiency. The silencing leads to a reduced number of p16 expressing-CECs from 60% to 40%. Such reduced expression increases the fraction of ki67-positive cells form around 20% to around 30%.

When nanoneedles interface with the human corneal endothelium they tightly interact with all the cells, with nanoneedles observed overlapping with the cytosolic space by confocal microscopy (Figure 2a). Similarly to cells in culture, nanoneedle interfacing does not induce apoptosis in the corneal endothelium tissue *ex vivo*. The co-transfection using siGLO and p16 siRNA indicates that transfected cells significantly downregulate their p16 expression, with a signal reduction in fluorescence intensity of 1 order of magnitude (Figure 2b).

CONCLUSION

This work shows the potential of nanoneedles for topical RNAi therapeutics targeted at the corneal endothelium. siRNA nanoinjection can transfect and induce mRNA silencing for the p16 therapeutic target in the endothelial cells of human corneas without appreciable toxicity when treated *ex vivo*. These findings highlight the suitability of nanoinjection for advanced therapies in CED.

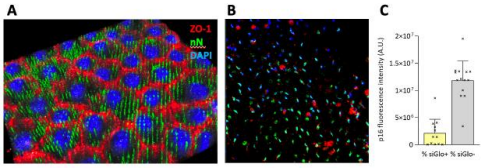

Figure 1 Nanoneedle RNAi therapeutics in human corneal endothelium. (A) Nanoneedles interfaced with the human corneal endothelium ex vivo. ZO-1 in red, nN in green and DAPI in blue. (B) siRNA transfection and p16 expression following nanoneedle-mediated delivery. siGLO in RED, DAPI in blue and p16 in green. (C) Quantification of p16 expression following nanoneedle-mediated transfection. Yellow bar nN transfected corneas, grey bar, untransfected control.

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Fabrication of 6-BromoIndirubin-3'-Oxime Incorporated Guanosine Diphosphate Crosslinked Chitosan Scaffolds to Promote Osteogenic Differentiation in Myoblastic C2C12 Cells

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INTRODUCTION: Critical size bone defects derive from traumas, infections, or tumor resections where large pieces of bone need to be removed therefore not allowing the bone to heal itself adequately[1]. Thus, healing critical size defects remains a major unmet clinical challenge. To encourage healing within these defects, researchers work on designing biological substitutes which mimic endogenous bone healing within the defect site. Our laboratory has previously developed one of these biological substitutes, the guanosine diphosphate (GDP) crosslinked chitosan scaffold, which gels in 1.6 seconds allowing for localization at injection site[1,2]. The objective of this research is to fabricate a new formulation of this scaffold incorporating 6-Bromoindirubin-3'-Oxime (BIO) and pyrophosphatase (PPTase). The addition of BIO is expected to promote osteogenic differentiation through mimicking of the Wnt signaling pathway[3] and PPTase is expected to promote mineralization since it allows GDP to act as a reservoir for phosphate ions[1]. Our hypothesis is that this combination will promote osteogenic differentiation and mineralization of cells within our scaffold. Therefore, an additional goal of this research is to assess the beneficial effects of these encapsulants both together and separately on myoblastic C2C12 cells.

METHODS: To fabricate the BIO incorporated scaffold, two BIO doses (10, 100 μM) were tested, and incorporation was confirmed using NMR. Material characterization experiments were conducted including gelation time measurements, rheological studies, and MicroCT/SEM for structural architecture. In addition, a Live/Dead assay was conducted on pre-osteoblastic MC3T3 cells to confirm cellular viability in this formulation. For BIO/PPTase encapsulation experiments, BIO dosages were decreased to 1 and 10 μM and myoblastic C2C12 cells were encapsulated. Preliminary tests were run to ensure cellular compatibility including AlamarBlue for metabolic activity, and Hoescht 33342 and Phalloidin staining for distribution. The effects of BIO and pyrophosphatase on osteogenic differentiation were assessed both separately and jointly using an ALP production assay and qPCR gene expression studies for osteogenic markers such as RUNX2, OSX, ALPL, COL1A1, and OCN.

RESULTS/DISCUSSION: The fabrication of the new BIO incorporated scaffold was successful with solid-state NMR spectra showing a novel concentration-dependent peak at 41 ppm, which could be attributed to an interaction between chitosan and BIO. The addition of BIO still allowed for sponges to form within 1.6 seconds while maintaining the viscoelastic properties after crosslinking. BIO incorporated scaffolds demonstrated highly porous networks with well-connected pores throughout, similar to the internal structure of control sponges. MC3T3 cell viability was not affected by

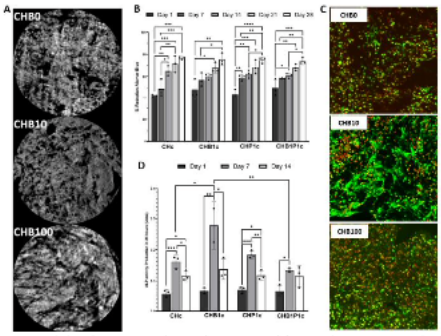

Figure 1. (A) MicroCT sections of sponges with BIO (0, 10, 100 uM). (B) Cellular metabolic activity over 28 days for BIO or PPTase jointly or separately. (C) Live/Dead staining of encapsulated MC3T3 cells after 1 day. (D) ALP production of C2C12 cells cultured indirectly over 14 days.

BIO addition as all scaffolds allowed for adequate viability over 28 days. No significant difference in metabolic activity was observed in C2C12 cells when BIO and PPTase were added either separately or jointly with values increasing over 28 days. Staining showed homogeneous cell distribution regardless of BIO or PPTase incorporation with a decrease in density over time. Regarding effects on differentiation, ALP production was found to be upregulated at day 7 in all groups with a significant increase in quantity in the BIO 1 uM alone group compared to control sponge and the synergistic group. No significant difference was shown in osteogenic gene expression between groups of increasing BIO dosages, but the synergistic group showed increased gene expression of RUNX2 at day 3 compared to all other groups.

CONCLUSION: Results from this study illustrate the successful formulation of the BIO incorporated sponge with minimal effects to material properties at relevant concentrations. Testing of C2C12 cell differentiation demonstrated a BIO dose-dependent beneficial effect in terms of ALP production but interestingly not in the synergistic group. Studies are currently being conducted to further assess the differentiation and BIO's mechanism of action in sponges through western blot. These experiments will be repeated as well using adipose derived stem cells.

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Hybrid Core-Shell Scaffolds for Bone Regeneration and a 3D Printing Approach to Tune their Properties

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INTRODUCTION

The quest for scaffolds with enhanced bioactive response has seen hydrogels emerging as key biomaterials for tissue engineering, due to their biocompatibility, biodegradability, hydrophilicity, and excellent mimicry of the natural extracellular matrix.[1] However, their mechanical properties are typically inadequate to temporarily substitute mineralized tissues such as bone. To solve this issue, hybrid structures were proposed, incorporating in the hydrogel a stiffer biopolymer (*e.g.* polylactic acid, PLA, or poly(ε-caprolactone), PCL) in the shape of non-woven fibers or 3D-printed lattices.[2]

In this work, we developed and characterized bioresorbable hybrid scaffolds composed of a bioactive gelatin-chitosan hydrogel (Hy) shell and a stiff PLA-based core. The hydrogel was specifically chosen because it was found to support cell proliferation and osteogenic/chondrogenic differentiation in previous studies.[3] For the core, two types of structure were prepared: i) a highly interconnected porous structure made of a PLA-PCL blend, obtained by a novel technique consisting in the introduction and subsequent leaching of superabsorbent particles, SAP;[4] ii) a PLA lattice structure realized by additive manufacturing. This latter system, thanks to the design freedom enabled by 3D printing, allowed to explore the possibility to tune the mechanical properties of the scaffold by changing the core/shell ratio.

EXPERIMENTAL METHODS

The PLA-PCL core was prepared by melt-blending PLA (55%), PCL (14%) and, in a second step, crosslinked sodium polyacrylate SAP (31%), by means of a discontinuous mixer. The obtained material was compression molded as plates, from which bar-shaped specimens (8 x 4 x 3 mm^3) were machined and later immersed in distilled water for 7 days to promote SAP swelling and leaching. 3D-printed core specimens (10 x 10 x 10 mm^3) were prepared as lattice structures with cubic cells by fused deposition modelling of a PLA filament. Both types of core specimens were immersed at 40°C in a hydrogel forming solution (74% gelatin, 8% chitosan and 18% poly(ethylene glycol) diglycidyl ether as crosslinking agent), freeze-dried and post-cured.

The obtained materials were subjected to physicochemical analyses (infrared spectroscopy; thermogravimetric analysis, TGA; optical and scanning electron microscopy, SEM), and a mechanical characterization under compression (electromechanical dynamometer). Core-shell specimens were immersed in distilled water for 24 h before mechanical tests.

RESULTS AND DISCUSSION

SAP particle leaching created PLA-PCL core specimens with 60 v% interconnected porosity, which allowed successful grafting of a 2-3 wt.% hydrogel shell on their surface, as confirmed by infrared spectra and TGA. The highly interconnected porous structure (Fig. 1a, left) and the stiffness (Fig. 1b, compressive apparent modulus, E_{app}) of PLA-PCL-Hy revealed its potential for biomimicking the spongy bone tissue and, therefore, for its regeneration. Furthermore, this type of scaffold is an excellent substrate for proliferation and osteogenic differentiation of mesenchymal stems cells (MSCs), thanks to the bioactive properties of the hydrogel shell.

The possibility to modulate the properties of the scaffolds was investigated by changing their core/shell ratio. With this aim, their core was 3D-printed with holes of various sizes. Specifically, their core void fraction, CVF, was systematically varied between 45% and 90%, leading to scaffolds with broad ranges of hydrogel content (5÷45%) and water uptake (50÷300%), both increasing with CVF. The typical appearance of their structure is displayed in Fig. 1a, right (insert), while the corresponding SEM image shows the interconnected porosity of the hydrogel shell, developed during the freeze-drying process[3]. Interestingly, their stiffness can be significantly tuned (E_{app} = 50÷600 MPa, Fig. 1b) by changing the CVF, and even scaffolds with CVF > 60% were stiffer than PLA-PCL-Hy, while containing more bioactive hydrogel.

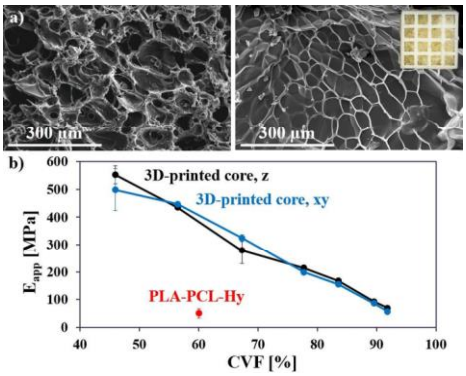

Fig. 1. a) Porous structure of PLA-PCL-Hy (left) and of the hydrogel (right) grafted on a 3D-printed specimen (insert). b) Compressive apparent modulus (E_{app}) *vs.* core void volume fraction (CVF) (z: 3D printing build direction; xy: transverse direction).

CONCLUSION

The core-shell design proved to be an effective strategy to obtain bioresorbable scaffolds that provide both a temporary mechanical support and an adequate environment for MSC proliferation. Moreover, it is a versatile approach, especially when combined with additive manufacturing, since the core and shell materials and their relative proportions can be easily modified to modulate scaffold properties such as stiffness, strength, bioactivity, and biodegradability.

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Deposition of a polymer-based honeycomb-like membrane on 3D printed bioactive glass scaffold and decellularized bone matrix as scaffolds for bone tissue engineering

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INTRODUCTION

Materials used to regenerate bone tissue must present several essential properties *i.e.* biocompatibility, osteoconductivity/osteoinductivity, while promoting angiogenesis[1]. One major challenge often encountered when using these materials (naturel or synthetic) is the invasion of the implantation site by fibrous tissue before complete bone regeneration. The implant site invasion is due to the faster proliferation rate of the cells involved in the wound healing process (*e.g.*, fibroblasts) compared to bone cells proliferation rate[2] leading to incomplete bone regeneration[3]. To prevent this fibrous tissue invasion, barrier membranes have been used to cover the defects and avoid this adverse effect. However, with the currently available products, membranes degrades faster than the bone regeneration occurs which does not solve the problem of fibrous tissue ingrowth[4]. To overcome this second challenge, researchers have turned their eyes on the bone regeneration itself, with the aim to make it faster to close the gap between the membrane degradation and the bone regeneration rate. One strategy to accelerate bone regeneration is the use of bone graft in combination with the barrier membrane[5]. In such cases, the barrier membrane and the graft are two distinct materials that are not in direct contact which requires a two-step procedure that can be challenging for surgeons. In this study, combining bioactive glass (BaG) and/or cortical bone (CB) with a polymer-based honeycomb membrane, a new type of biphasic scaffold is proposed to avoid the two-step procedure.

EXPERIMENTAL METHODS

The two phases affixed are 1) the organic phase formed of poly-L-co-D, L-lactic acid (PLDLA), known to be biodegradable and biocompatible, shaped with a honeycomb-like structure through the Breath Figure Method (BFM), and 2) an inorganic phase. The inorganic substrates used are a) the 13-93B20, an experimental BaG composition containing boron, already reported in our previous work[6] and b) a decellularized xenogenic CB matrix provided by BIOBank©. The BaG used here has been specially designed and 3D printed to allow the deposition of the membrane through the BFM. Our BaGs were used pre-immersed (conditioned) in TRIS buffer or bare prior to membrane deposition.

RESULTS AND DISCUSSION

Our study demonstrated that PLDLA honeycomb-like membrane was successfully deposited onto CB and 3D printed BaG scaffold through BFM. Materials were incubated in TRIS to study their degradation and bioactivity. After 28 days of immersion in TRIS, no membrane detached from their substrate regardless of their nature, exhibiting the strong link of the membrane/substrate assembly. Furthermore, on the BaG based scaffolds, Ca/P precipitation was evidenced by SEM (Figure 1). However, such finding was not visible on the materials prepared from CB. ICP EOS analysis evidenced the important SiO_2, Ca^{2+} and PO_4^{2-} release from BaG based scaffolds compared to bone. This explains that bone scaffolds do not precipitate an apatite layer compared to BaG scaffolds. Indeed, BaGs degrade upon immersion, releasing biologically interesting ions, resulting in apatite precipitation.

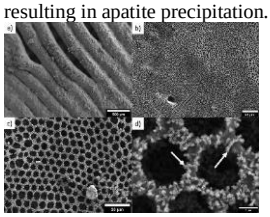

Figure 1. SEM images of a) BaG scaffold 3D printed without treatment (scale bar 500 µm), b) BaG scaffold untreated after membrane deposition (scale bar 50 µm), c) and d) BaG scaffold untreated after membrane deposition and immersion in TRIS for 28 days (scale bar 20 µm and 2µm respectively), white arrows show Ca/P precipitates.

CONCLUSION

The next step of this study will focus on the membrane/substrate assembly-cell interactions using osteogenic progenitor cells, MC3T3 and HGF to elucidate the effect of such assembly on cells involved in bone regeneration processes.
Our hope is to prove a surface dependent cell proliferation and growth showing that osteogenic progenitor cells develop mostly on the BaG/bone surfaces while fibroblastic cells grow better on the PLDLA membrane. This would support the fact that having a biphasic material allowing a one-step procedure and a separation of the underlying bone (or graft) from the outside (or fibrotic tissue) would be beneficial for a proper bone regeneration and an easier procedure for surgeons.

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ACKNOWLEDGMENTS

The authors would like to thank the Chair RETIS and Jane and Aatos Eerko Foundation for providing financial support to this project.

>> 10:30 - 12:30 | PSOP-02 - Wound Healing

Chairpersons: Emilie Attiogbe & Arn Mignon
Location: Room B

10:30 | KL1 Wound healing - Dense Collagen/PLGA Composite Hydrogels Generated by In Situ Nanoprecipitation as Novel Medicated Wound Dressings: In Vitro and In Vivo Evaluation.
Christophe HELARY, Sorbonne University, CNRS, UMR 7574 – Chemistry of Condensed Matter Laboratory, Paris, France

11:00 | KL2 Wound healing - The comparability of in vitro and in vivo experiments for degradable Mg-implants: basis for a digital twin of implant degradation
Regine WILLUMEIT-RÖMER, Institute for Metallic Biomaterials, Helmholtz-Zentrum Hereon, Max-Planck-Str. 1, 21502 Geesthacht, Germany

11:30 | O1 Wound healing - Wound Dressings Based on Gellan Gum, Alginate and Lipid Nanoparticles Loaded with Antibacterial Peptide Nisin
Elzbieta PAMULA, Faculty of Materials Science and Ceramics, AGH University of Science and Technology, Kraków, Poland

11:45 | O2 Wound healing - Augmented siloxane-based rapid action foams for hemostatic treatment
Pritha SARKAR, Department of Materials Science and Engineering, University of Central Florida, United States

12:00 | O3 Wound healing - Effect of local administration zoledronic acid and PRP with bone allograft on bone defect healing
Elyarbek TASHMETOV, Surgical diseases department, Karaganda medical university, Karaganda, Kazakhstan

12:15 | FP01 Wound healing - Three Fungal Exopolysaccharides as New Biomaterials for Wound Healing
Masoud HAMIDI, Université libre de Bruxelles (ULB), École polytechnique de Bruxelles – 3BIO-BioMatter, Brussels, Belgium

12:20 | FP02 Wound healing - Multifunctional hydrogel-based patch for targeting inflammation and regeneration in chronic intestinal wounds
Marco ARAÚJO, i3S - Instituto de Investigação e Inovação em Saúde, University of Porto, Porto, Portugal

12:25 | FP03 Wound healing - Development of Intelligent Hydrogel-Based Burn Wound Dressings
Manon MINSART, Polymer Chemistry & Biomaterials Research Group, CMaC, Ghent University, Ghent, Belgium

Dense Collagen/PLGA Composite Hydrogels Generated by In Situ Nanoprecipitation as Novel Medicated Wound Dressings: In Vitro and In Vivo Evaluation.

Xiaolin Wang [1], Olivier Ronsin [2], Nicolette Farman [3], Tristan Baumberger [2], Frédéric Jaisser [3], Thibaud Coradin[1], Christophe Hélary[1] *

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INTRODUCTION

Cutaneous chronic wounds are characterized by the absence of healing after six weeks. When current treatments based on wound compression are not efficient, the application of wound dressings is required. To date, no biomaterial is suitable for treating the different types of wounds. Nowadays, research orientation is towards medicated wound dressings incorporating therapeutic molecules within biomaterials in order to favor skin repair. Collagen based hydrogels are broadly used in tissue engineering as they are biocompatible and biodegradable. Unfortunately, these biomaterials are poor drug delivery systems, thereby limiting their utilization to prevent infection or modulate inflammation in chronic wounds. Hence, associating collagen with PLGA constitutes a promising method to encapsulate lipophilic molecules such as anti-inflammatory drugs. In this study, dense collagen/PLGA composite hydrogels have been developed to improve hydrogel physical properties and to deliver dexamethasone or spironolactone in a controlled manner to modulate inflammation in the wound bed. For this purpose, an original technique of PLGA nanoprecipitation have been tested. To evaluate composite hydrogels as novel medicated wound dressing, hydrogel stability, mechanical properties, drug loading and release kinetic have been analyzed. Then, the in vivo performance of composite hydrogels has been evaluated in a pig model of impaired wound.

EXPERIMENTAL METHODS

Dense collagen hydrogels at 40 $mg.mL^{-1}$ were dehydrated using tetrahydrofuran and incubated overnight in solutions containing PLGA and anti-inflammatory drugs (spironolactone or dexamethasone). Poly Lactic-co-Glycolic Acid (PLGA) with different chain lengths (from 7 to 60 kDa) were used. Then, the PLGA nanoprecipitation within collagen was triggered by incubation in PBS. The quantity of PLGA immobilized within hydrogels and the swelling properties were assessed. Then, the ultrastructure of composites was analyzed by scanning electron microscopy. Their mechanical properties were evaluated by rheology and traction test. Last, the drug release kinetic from composites was studied over 28 days and their cytotoxicity evaluated on fibroblasts and keratinocytes using a live/dead assay. The pig model of impaired wound healing was carried out by a cutaneous pre-treatment with clobetasol for 10 days followed by a 6 mm skin punch to create a full thickness wound. Then, composite hydrogels loaded with spironolactone were applied into the wound bed. Their effect on wound closure and re-epithelialization was evaluated using histological sections.

RESULTS AND DISCUSSION

The nanoprecipitation enabled the immobilization of a large amount of PLGA regardless of the polymer chain length (50 % of the total mass). The presence of PLGA negatively impacted the swelling properties but all composite hydrogels exhibited a high degree of hydration (over 80%). When PLGA 28 kDa or 60 kDa were used, the collagen hydrogel stiffness increased but its deformability dropped. On the opposite, PLGA 7 kDa did not altered the hydrogel deformability and doubled the storage modulus. The ultrastructure analysis revealed the presence of polydispersed nano/microparticles at the surface of collagen fibrils. These results suggest a strong interaction between PLGA and the collagen network. Compared to pure collagen hydrogels, the drug loading in composite hydrogels was 5 times higher in all composite hydrogels. The release kinetic of spironolactone and dexamethasone from collagen/ PLGA 7 kDa hydrogels was quasi constant over the first two weeks and total after 28 days. Unlike pure collagen hydrogels, no burst release was observed. Increasing the chain length negatively impacted the drug delivery as only 20% of the initial dose was released at day 28 for PLGA 60 kDa. Cell viability experiments showed the absence of cytotoxic effect of composites hydrogels on fibroblasts and keratinocytes regardless of the PLGA type used. The in vivo experiment in pig revealed a high performance of collagen/PLGA 7 kDa composite hydrogels on wound healing. Spironolactone loaded composite hydrogels improved wound closure by 50% and permitted a complete re-epithelialization after 6 days

CONCLUSION

Taken together, these results show that dense collagen/PLGA 7kDa composite hydrogels are promising medicated wound dressings for the treatment of chronic wounds as they deliver constant doses of drugs favoring skin repair and possess high physical properties.

ACKNOWLEDGMENTS

The authors would like to thank ANR (Grant N°ANR-14-CE16-0010) for providing financial support to this project.

The comparability of in vitro and in vivo experiments for degradable Mg-implants: basis for a digital twin of implant degradation

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INTRODUCTION

Magnesium (Mg) implants have found their way into the clinics and thousands of patients have already successfully been treated. Still, we have not yet reached a full understanding of all processes which occur during the degradation of the material and tissue regeneration, which makes the prediction of the behavior for new material systems difficult. We can identify important parameters and trends from *in vitro* experiments but a gap remains to give a reliable prediction for the *in vivo* behaviour[1]. The combination of high resolution *in vitro*, *ex vivo* and *in situ* data obtained by e.g. synchrotron microtomography[2, 3] and histology can be the basis to create a digital twin of the implant-tissue system which might help to overcome this problem.

EXPERIMENTAL METHODS

Two Mg alloys with 5 and 10 weight % (wt. %) Gd (Mg-5Gd, Mg-10Gd) were prepared and machined into screws. They were studied *in vitro* (e.g. degradation rate by weight loss over 8 weeks and *in situ* synchtrotron tomography, structure and composition of the degradation layer by XPS). High resolution synchrotron microCT and nanoCT data was used to develop and calibrate a degradation model. In parallel the screws were implanted into rat tibia for 4, 8 and 12 weeks. After explantation the specimens were examined by synchrotron microCT, histology and µXRF to determine among others the degradation rate and e.g. bone-to-implant-contact (BIC). To this end, an automated convolutional neural network to segment the microCT data of the degraded Mg-based material was developed[4].

RESULTS AND DISCUSSION

The *in vivo* degradation rates were found to be higher than *in vitro* rates, by a factor 2-3. Fig 1 shows exemplary 3D renderings of screws before and after 8 weeks of degradation. Synchrotron microCT enables the visualization of both the degrading metal, the forming degradation layer and the bone, showing that the implant shape stays intact with the bone growing well around it. While current computational models of *in vitro* degradation are able to capture the degradation behaviour well, they need to be extended further to include strong localized degradation as observed in the *in vivo* case (Fig 1)[5,6]. *(In situ)* micro and nanoCT experiments help to reveal the mechanisms of the corrosion that need to be taken into account in the computational model to this end, including the potential for galvanic corrosion posed by intermetallic particles.

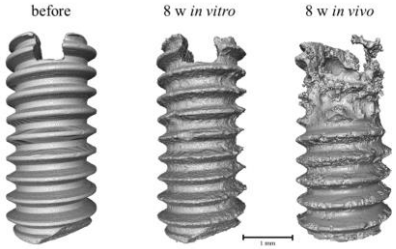

Fig 1: 3D renderings of Mg-10Gd screws before degradation and after 8 weeks of *in vitro* and *in vivo* degradation respectively. Scale bar is 1 mm.

CONCLUSION

The careful analysis of Mg degradation *in vitro* and *in vivo* gives a valuable basis for the development of a digital twin which helps to predict implant behavior.

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Wound Dressings Based on Gellan Gum, Alginate and Lipid Nanoparticles Loaded with Antibacterial Peptide Nisin

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INTRODUCTION

Wound healing is a complicated, multi-step process involving inflammation, proliferation, migration and remodeling of a new tissue [1], which may be compromised by the presence of pathogenic bacteria at the wound site and subsequent development of bacterial infection [2]. Topical application of antimicrobials, e.g. in a form of a controlled drug release dressing, is commonly utilized to eradicate bacteria and facilitate wound healing [2].

The aim of this study was to encapsulate antibacterial peptide – nisin (NSN) in lipid nanoparticles (NP) and to use the developed NP as antibacterial agents in composite hydrogel-based wound dressing.

EXPERIMENTAL METHODS

Stearic acid-based lipid nanoparticles (empty – NP, and loaded with NSN – NP_NSN) were fabricated using double emulsification/solvent evaporation method. The morphology and size of NP were characterized by atomic force microscopy and dynamic light scattering. O-phtalalaldhyde based amine detection method was used for evaluation of NSN encapsulation efficacy. The cytotoxicity of NP was tested in contact with L929 fibroblasts using resazurin reduction assay.

As prepared NP were suspended in gellan gum (GG) or sodium alginate (Alg)-based hydrogels to form an antibacterial wound dressing. GG and GG/Alg samples containing pure NSN (the same drug dose as in the NP) were used as control. The morphology in dry and hydrated state, swelling capacity and NSN release from the developed dressings were evaluated. Antimicrobial efficacy of the samples was tested using Kirby-Bauer method in contact with *Staphylococcus pyogenes.* Final *in vitro* evaluation was done in contact with L929 fibroblasts in terms of sample cytotoxicity, influence on cell migration (wound closure assay) and cell adhesion to materials surface.

RESULTS AND DISCUSSION

NSN-loaded lipid nanoparticles (NP_NSN) were spherical in shape (Fig. 1A) with average particle size of around 300 nm (Fig. 1B). Encapsulation efficacy was above 80%, resulting in NSN loading equal to 5.2 ± 0.3%. NP were cytocompatible with L929 fibroblasts for up to 500 µg/ml.

GG and GG/Alg hydrogels containing either free NSN (GG+NSN and GG/Alg+NSN) or NP_NSN (GG+NP_NSN and GG/Alg+NP_NSN) were highly porous (Fig. 1C) with high swelling capacity (swelling ratio above 2000%). Encapsulation of NSN within lipid nanoparticles significantly slowed down NSN release from GG-based samples for up to 24 h (as compared to GG+NSN). The most effective antimicrobial activity against Gram-positive *S. pyogenes* was observed for GG+NP_NSN, while in GG/Alg it was decreased by interactions between NSN and Alg leading to NSN retention within hydrogel matrix (Fig. 1D). All materials, except GG/Alg+NP_NSN, were cytocompatible with L929 fibroblasts and did not cause significant delay in wound healing.

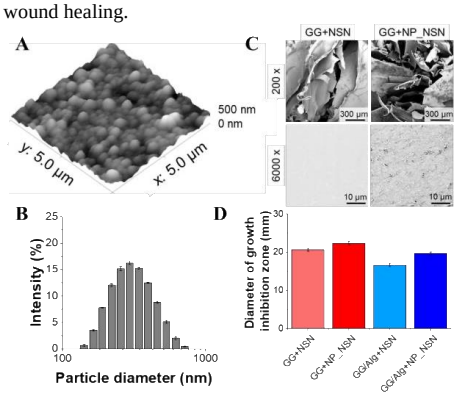

Fig. 1 (A) NP_NSN morphology evaluated by AFM (A), NP_NSN size distribution (B), gross morphology of GG-based samples (C) and antimicrobial efficacy of GG and GG/Alg-based samples containing NSN or NP_NSN (D).

CONCLUSION

The study showed that wound dressings based on polysaccharide hydrogel GG (i.e. GG+NSN and GG+NP_NSN) were able to uptake significant volume of fluids and were able to release over 70% of its NSN cargo in a sustainable manner. The materials were not cytotoxic for L929 fibroblasts, neither impaired cell migration nor wound healing. Prolonged release of active NSN and thus increased antimicrobial efficacy were ensured by inclusion of NSN-loaded lipid nanoparticles within GG matrix in GG+NP_NSN samples. The developed materials are promising for wound healing application and the treatment of bacterial infections in the wounds.

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Augmented siloxane-based rapid action foams for hemostatic treatment

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Statement of Purpose: Hemorrhage remains the main cause of preventable death on the battlefield. This underscores the need of developing appropriate hemostatic treatments that can effectively stanch blood loss while remaining easily applicable at the point of care. Traditional methods such as gauzes and tourniquets have gained inadequate success, and as such the fabrication of novel hemostatic materials has advanced tremendously in the last decade[1]. Although there has been considerable success in recent years in engineering novel hemostats[2], developing an effective hemostatic material that is biocompatible, fast-acting with hassle-free application and removal, yet being a cost-effective viable option remains a challenge. In our current study, we report a self-conforming, rapid action siloxane-based hemostatic bandage system that is capable of treating large amounts of blood loss. The objective is to provide the patient with a means to rapidly arrest bleeding from trauma-related wounds in a manner superior to those currently available, within few minutes.

Experimental Methods: The formulation of our bandage system comprises of a two-part system: Part A and Part B. Part A consists of a homogenous mixture of siloxane A, a platinum-based curing catalyst, calculated volume of inorganic oxide, a non-ionic organic surfactant. Similarly, Part B consists of a homogenous mixture of siloxane B, calculated volume of H_2O_2 in water, silica, and surfactant. The two components are reacted *in situ* to form a self-conforming flexible sponge-like foam that conforms on and around the wound, thereby acting as an artificial blockage by creating an autogenous pressure on the site to arrest bleeding. A mixing and delivery system combines the two parts in a turbulator-dispersed dual-syringe device. When Part B interacts with Part A, decomposition of H_2O_2 to form O_2 gas and H_2O ($H_2O_2 \rightarrow O_2 + H_2O$) is catalysed, and the liberation of oxygen foams up the siloxane matrix to form the sponge-like foam in less than an minute. A variety of inorganic oxides have been attempted with, over a range of concentrations in order to determine the best composition so as to optimize both expansion and temperature, to avert possible risks of embolism. The resultant foam was then characterized for SEM image analysis. (**Fig 1**).

Results and Discussion: Several inorganic oxides were tested and characterized in order to develop foams with optimal mechanical properties to arrest bleeding. The selection of these oxides was also based on their inherent biological benefits in promoting the coagulation cascade and imparting antimicrobial properties. Using contact-angle goniometry, we have determined the foam has a hydrophobic surface. This is a desirable feature in wound-healing systems as it can minimize the interaction of the foam with the blood stream. Compression tests have shown the mechanical properties of the spongy polymeric matrix to vary depending on the types of oxides used, thereby rendering such polymer-based foams versatile hemostats for usage in different scales of injury (**Fig 2a**). Further, from the viscoelastic tests, the liquid-to-foam point has been identified from the storage and loss modulus curves (**Fig 2c**). The adhesive properties of the foams render them optimal for wound dressing applications.

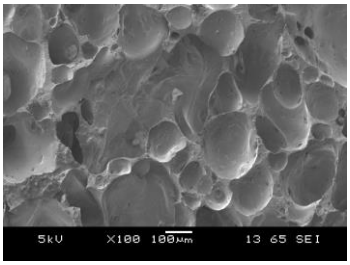

Fig. 1: *SEM image (100x) of closed-cell expanded foam.*

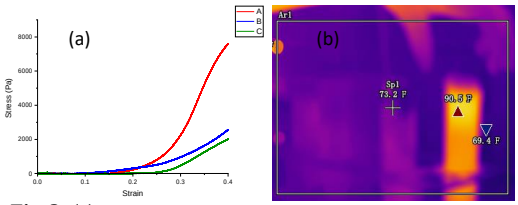

Fig. 2: (a) *Compression test data comparison with different oxide based foams.* **(b)** *Thermal imaging and temperature control.*

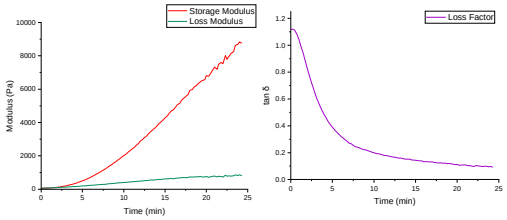

Fig. 2(c): *Characteristic viscoelastic behaviour of foam.*

By tuning the relative concentrations of the respective oxides and H_2O_2, we have succeeded in also controlling the temperature of the reaction. From the thermal analysis data we have observed a maximum temperature of 35°C/95°F **(Fig 2b)** accompanied by a six-fold expansion (volume) in a matter of seconds. Support data will include detailed characterization of the polymers and the hemostatic sponge formed upon reaction. Studies on mechanical durability, surface adhesion, hydrophobicity, SEM image analysis, spatiotemporal analysis, along with additional in-vitro assays will also be presented.

Conclusion: This unique strategy to develop a hemostatic dressing that contains oxygen-generating materials for supplying topical oxygen is a convenient approach for a biocompatible, hydrophobic hemostatic bandage system that may find utility in treating different types of skin wounds, including pressure sores, ulcers, and recalcitrant open wounds. It can also be used under inclement weather conditions, which is often the case at the fields of operation.

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Effect of Local Administration Zoledronic Acid and PRP with Bone Allograft on Bone Defect Healing

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INTRODUCTION

In orthopedic surgery, autologous bone grafting is commonly used to treat bone defects [1,2]. However, the insufficient amount of obtained autologous bone and the additional trauma caused by autologous bone grafting limit its use in clinical practice [3,4]. The present study aimed to evaluate the effect of platelet-rich plasma (PRP) and zoledronic acid (ZOL)-impregnated bone allograft, prepared according to the Marburg bone bank system, on reparative bone tissue regeneration in bone defects.

MATERIALS AND METHODS

Seventy-two healthy rabbits (24 rabbits in each group) were used for this study. Bone defects (6-mm diameter) were created in the femur. The human femoral head prepared according to the Marburg bone bank system was used as a bone allograft. Platelet-rich plasma was obtained by 2 steps centrifugations. In the experimental groups, in 1 group - the defects were filled with bone allograft combined with autologous PRP, in 2 group – bone allograft combined with ZOL (5μg), in 3 group (control) – with allograft. The 8 animals from each group were sacrified after 14, 30, and 60 days. Evaluations consisted of X-ray plain radiography, histology at 14-, 30- and 60-days post-surgery. Comparisons between two groups were performed with Chi-Squared Test with Yates Continuity Correction, multiple comparisons were performed with Pearson's Chi-Squared Test. Statistical processing of research results was analyzed using IBM SPSS Statistics 20.0. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

According to histomorphometric analysis, the new bone formation inside the bone allograft was significantly greater in those impregnated with both PRP and ZOL than in those from the control group at 30 and 60 days after implantations ($p<0,05$). The regenerate differs from the adjacent bone in the chaotic arrangement of the bone trabeculae and their different maturity. In the regenerate, blood vessels of various sizes are found between the bone trabeculae. Allograft fragments with signs of resorption were present in the medullary canal. There were no signs of an inflammatory reaction in the medullary canal. The radiological findings were in accordance with the histomorphometric results.

CONCLUSION

The local co-administration of both PRP and ZOL on heat-treated allograft promoted and maintained newly formed bone structure in the bone defect.

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Three Fungal Exopolysaccharides as New Biomaterials for Wound Healing

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INTRODUCTION

Exopolysaccharides (EPSs) are high-value functional biomaterials mainly produced by bacteria and fungi, with nutraceutical, therapeutic and industrial potentials.[1] Fungal EPSs can be produced within a few days, using simple recovery approaches and by utilizing industrial waste as feedstock.[2, 3]

In this study, EPSs from three fungal strains, *Papiliotrema terrestris* PT22AV, *Rhodosporidium babjevae* IBRC-M 30088 and *Sclerotium glucanicum* DSM 2159 were extracted and their physicochemical properties (structural, morphological, monosaccharide composition and thermal properties) were characterized. Also, *in vitro* biological activities as well as wound healing potential of the EPSs was evaluated.

EXPERIMENTAL METHODS

Microorganism cultivation

P. terrestris PT22AV, a yeast isolated from olives in Molise (Italy), *R. babjevae* and *S. glucanicum* were cultured in agar media. After preparation of appropriate inoculum for each strain, batch cultures consisting of 250 mL Erlenmeyer flasks containing 100 mL medium were used for microbial growth and production of EPSs.

EPS extraction and purification

After incubation, the EPS-containing supernatants were recovered via centrifugation and the EPSs were precipitated by adding cold ethanol, purified by dialysis, and lyophilized.

Morphological and physicochemical characterization of the extracted EPSs

Scanning electron microscopy (SEM) observations were performed for microstructure determination of the EPSs from *P. terrestris and S. glucanicum.* Also, FT-IR analyses were done for functional group determination of the EPSs.SEM and FT-IR studies had been done previously for *R. babjevae.* Molecular weight (M_w) determination [through steric exclusion chromatography (SEC)-HPLC1)] and monosaccharide composition determination (GC-MS), were performed for the EPSs from *P. terrestris.* For the other two strains, *Mw* and monosaccharide composition is already available.

In vitro biological activity

The antioxidant activity of the EPSs was assessed by measuring their DPPH radical scavenging activity. Also, their compatibility with human red blood cells (RBCs) and human fibroblasts (ATCC: CCL-186) and macrophages (U937, ATCC: CRL-1593.2) cell lines was assessed.

In vivo wound healing studies

The wound healing effects of the three EPSs were evaluated *in vivo* using adult male Wistar rats based on wound closure percentage (WC)% and histopathological assessments.

Statistical analysis

Minitab ®20.4 software was used to conduct statistical analysis of the WC% association with various time intervals and treatment groups. GraphPad Prism 9.0.0 (GraphPad Software, LLC, USA) was used for *in vitro* data analysis. Since data of WC% and cell viability followed a non-parametric distribution, the two-way ANOVA test with Dunnett's multiple comparisons was used. Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

The EPSs obtained from *P. terrestris* had an average molecular weight of 202 kDa. Mannose and glucose with 97% and 3% molar percentages, respectively, constituted the EPSs which was then considered as mannan polysaccharides. SEM showed that this EPSs have porous surface structure with grain-like elongated structural units. The antioxidant activity of the EPS at different concentrations (0.1-5 mg/mL) showed a positive correlation between the EPS concentration and the DPPH radical antioxidant activity. The increase of EPSs concentration from 0.1 mg/mL to 5 mg/mL, improved the scavenging activity from 20 % to 37 %.

Scleroglucan (the EPSs from *S. glucanicum*) was also shown to have a solid surface characterized by irregular shapes. IR spectroscopy indicated the presence of hydroxyl, carbonyl, and carboxyl functional groups like the commercial scleroglucan. DPPH scavenging activity of scleroglucan was 33.03 ± 4.31% at a concentration of 5 mg/mL.

All the three EPSs showed compatibility with the human fibroblast and macrophage cell lines (at concentrations of 100-1000 µg/mL), and with RBCs (1-10 mg/mL). The animal studies showed a dose-dependent wound healing capacity of three EPSs with significantly higher WC% values at 10 mg/mL after 14 days. The effects of the three EPSs on WC% were not significantly different at the highest concentration (10 mg/mL).

CONCLUSIONS

These findings demonstrate that the EPSs from *P. terrestris*, *R. babjevae* and *S. glucanicum* are promising biopolymers for wound healing acceleration.

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Multifunctional hydrogel-based patch for targeting inflammation and regeneration in chronic intestinal wounds

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INTRODUCTION

Inflammatory bowel disease (IBD), comprising Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the gastrointestinal tract of unknown aetiology. IBD exhibits a common chronic wound environment, characterized by overproduction of pro-inflammatory cytokines, reactive oxygen species (ROS) and matrix metalloproteinases (MMP), which leads to loss of epithelial cells and degradation of the extracellular matrix (ECM)[1]. Current therapies focus on reducing symptoms by delivering anti-inflammatory drugs, or by using biologics combined with immunomodulatory agents. Epithelial protection and restoration of the impaired intestinal barrier has recently emerged as a key goal for IBD therapy[2]. We hypothesized that novel biomaterial-based platforms for IBD should address both inflammation and mucosal healing, restoring intestinal barrier function and preventing disease relapse. This work presents a bioinspired multifunctional alginate-melanin 3D scaffold for potential application in multi-targeted IBD therapy.

EXPERIMENTAL METHODS

Multifunctional photopolymerized hydrogel scaffolds were obtained from a pre-gel solution containing modified ultrapure sodium alginate, Irgacure 2959, melanin nanoparticles (mNP) and a bisthiolated MMP-sensitive peptide. The pre-gel solution was irradiated using a BlueWave® 200 curing spot lamp, inducing polymer crosslinking, MMP-cleavable peptide incorporation and entrapment of the mNP. The hydrogels were freeze-dried giving 3D spongy-like scaffolds. Their in-vitro anti-inflammatory potential was evaluated by analysing the conditioned media from Lipopolysaccharide (LPS)-stimulated macrophages, and the pro-regenerative ability assessed in the presence of human intestinal fibroblasts (HIF) and Caco-2 cells. Friedman's test followed by corrected Dunn's test were performed for the *in vitro* radical scavenging assay (***p<0.001).

RESULTS AND DISCUSSION

The mNP-loaded scaffolds were able to neutralize the extracellular ROS initially present in the media of macrophages stimulated with LPS, to levels similar to non-stimulated control, demonstrating better performance than the scaffolds containing only the MMP-cleavable peptide or than free mNP (**Figure 1A**). The results highlight a synergistic effect from the components of the **mNP0.3Pep4FD** scaffolds, as well as the advantage of entrapping mNP in a hydrogel matrix, preserving their radical-scavenging ability. The same scaffold maintained this ability for at least 3 consecutive cycles, suggesting that once implanted, it may present sustained radical-scavenging ability during fares of inflammation, which may be key to prevent disease relapse[3]. Cells were able to adhere to scaffolds, spreading and forming cell-cell interactions. HIFs accumulated within macropores, where they deposited ECM. Caco-2 epithelial cells were also able to adhere and grow and to migrate into the macropores (**Figure 1B**).

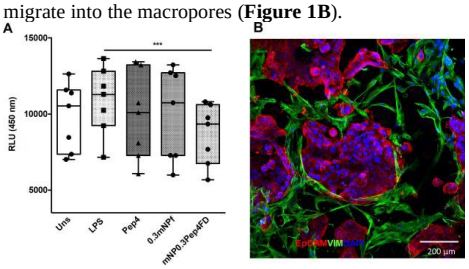

Figure 1. A: Ability of free mNP (**mNP0.3f**), and freeze-dried scaffolds containing 4 mM MMP-cleavable peptide (**Pep4**), and combined with mNP (**mNP0.3Pep4FD**) to capture extracellular ROS present in conditioned media from macrophages alone (**Uns**) or in the presence of LPS for 1h (**LPS**). B: Co-culture of HIF and Caco-2 cells within **mNP0.3Pep4FD** scaffolds (top view). EpCAM (red) marks Caco-2 cells and VIM (green) marks HIFs, DNA is in blue. Reproduced from Ref. 3 with permission from the Royal Society of Chemistry.

CONCLUSION

The scaffolds demonstrated ability to normalize an inflammatory-like environment and to support efficient colonization by intestinal cells. This novel platform may change the paradigm on the design of new biomaterial-based therapeutic strategies for IBD.

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ACKNOWLEDGMENTS

The work was performed under the framework of IBEROS (0245_IBEROS_1_E) - funded by POCTEP 2014-2020 and FEDER, and ANGIONICHE - funded by FCT. MBG acknowledges the BiotechHealth Programme and FCT for the PhD fellowship (PD/BD/135489/2018).

Development of Intelligent Hydrogel-Based Burn Wound Dressings

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INTRODUCTION

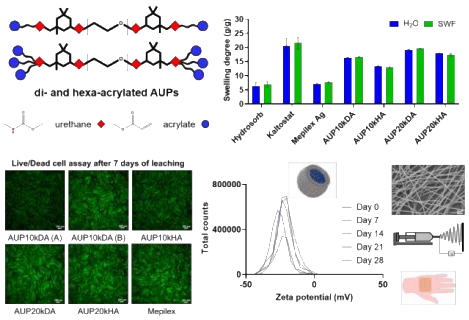
Hydrogels elegantly combine various of the desired properties of an ideal burn wound dressing, particularly as they can both absorb and release moisture, thus ensuring an optimal moisture control of the wound healing environment. Furthermore, electrospun fiber mats, especially those composed of hydrogel precursors, could provide an excellent environment to promote wound healing as these nanofibers can mimic the native extracellular matrix. Their high surface area enables efficient fluid absorption and enhanced drug delivery[1,2]. In this regard, acrylate-endcapped urethane-based precursors (AUP) were selected for the design of an intelligent burn wound dressing. The backbone that was selected in this work was poly(ethylene glycol), due to its biocompatibility and its highly hydrophilic nature, which is beneficial to enable a high exudate uptake. The AUP materials have been shown to possess excellent biocompatibility, as well as solid-state photo-crosslinkability at room temperature, which is crucial to produce mechanically stable, crosslinked nanofibers after electrospinning. Additionally, they are suitable for waterborne electrospinning, thus eliminating the use of toxic solvents[3]. As a final part of our dressing, an intelligent, biocompatible delivery system was envisioned for the triggered release of agents such as antimicrobials (e.g. tannic acid). To this end, liposomes were selected based on their intrinsic capacity to be lysed under the action of toxins (e.g α- and δ-toxins for *S. aureus*) which are secreted by pathogenic bacteria, after reaching the critical colonization threshold[4]. The main research objective of this project is thus the development of a burn wound dressing with an intelligent drug delivery system.

EXPERIMENTAL METHODS

AUPs with varying backbone molar masses (10 – 20 kg/mol) as well as endcap chemistries were synthesized using an improved protocol, yielding di- and hexa-acrylated polymers (DA and HA, respectively). The precursors were subsequently processed into both hydrogel sheets and electrospun mats, crosslinked via UV-A irradiation and benchmarked against some of the most used commercial wound dressings (e.g. Kaltostat), mainly in terms of swelling, mechanical properties and biocompatibility. The fiber morphology was assessed via scanning electron microscopy (SEM). *In vitro* indirect cell tests were performed using human foreskin fibroblasts (HFF). The liposomes were prepared according to the thin-film hydration method, extruded and characterized using dynamic light scattering (DLS).

RESULTS AND DISCUSSION

AUPs were successfully synthesized as indicated by ^{1}H-NMR and could be effectively processed into crosslinked hydrogel sheets and electrospun fibers. The AUP sheets showed high gel fractions (> 90%) together with strong swelling degrees in water and simulated wound fluid (12.7 – 19.6 g/g), as well as tunable mechanical properties (e.g. Young's moduli of 26 – 61 kPa, swollen state). The AUPs had significantly ($p < 0.05$) higher swelling degrees than most of the tested commercial dressings, while also being mechanically resistant. SEM analysis indicated homogeneous fiber morphologies with diameters ranging between 0.7 and 1.4 µm. The phospholipid liposomes encapsulating tannic acid proved to be stable for at least 28 days with an average zeta potential of -23.4 (± 4.5) mV and an average size of 191 (± 52) nm. Furthermore, they showed enhanced stability compared to unloaded liposomes and could be successfully incorporated in both hydrogel sheets and electrospun fibers without altering the mechanical properties ($p < 0.05$). As a proof of concept, liposome lysis was induced using Triton X-100. The di- and hexa-acrylated AUP materials showed good *in vitro* biocompatibility (viabilities > 90 %), which was evidenced by indirect MTS and live/dead cell assays.



CONCLUSION

It can be safely concluded that AUP materials hold great promise as novel candidate burn wound dressings and are able to compete with several commercial wound dressings. Ongoing research focuses on the combination of liposomes with different bio-active agents using co-axial electrospinning.

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ACKNOWLEDGMENTS

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>> 10:30 - 12:30 | SYMP-01 - New bioinks for complex 3D printed tissue substitutes: from native functionality to disease modelling

Chairpersons: Alexandra P Marques & Christophe Marquette
Location: Room A

10:30 | KL1 Bioinks - 3D Bioprinting: in vitro model, implantable bioink and intraoperative methods

Christophe MARQUETTE, 3d.FAB, Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Villeurbanne, France

11:00 | KL2 Bioinks - Extending the capabilities of bioprinting with the Dynamic Formulation Module (multi-material patterning, mixing and core-shell) in a single dispensing process

Mauro PETRETTA, REGENHU SA, Villaz-St-Pierre, Switzerland

11:30 | O1 Bioinks - Novel Approach for Guiding Stem Cell Organization and Tissue Growth by Multi-head 3D Bioprinting to Mimic Human Corneal Stroma

Paula PUISTOLA, Eye Group, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

11:45 | O2 Bioinks - Development of a novel hybrid bioink containing living microfactories

Maryam GHASEMZADEH-HASANKOLAEI, CICECO–Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal

12:00 | O3 Bioinks - Drop-on-demand micropatterning of the novel amphiphilic peptide I3K on regenerated silk fibroin substrates to guide and promote adhesion and proliferation of neuronal cells

Ana JIMENEZ-FRANCO, Department of Chemical and Biological Engineering, University of Sheffield, Sheffield, UK

12:15 | FP01 Bioinks - A Collagen-Glycosaminoglycan Scaffold Coating Enhances the Bio-Lubrication of a 3D-Printed Framework for Cartilage repair

Austyn MATHESON, Tissue Engineering Research Group, Dept. of Anat.& R.M., Royal College of Surgeons in Ireland, Dublin, Ireland; Trinity Centre for Biomedical Engineering, Trinity College Dublin, Ireland Advanced Materials & Bioengineering Research Centre, RCSI and TCD, Dublin, Ireland Biomedical Engineering, School of Dental Medicine, UConn Health, USA

12:20 | FP02 Bioinks - Micellar nitric oxide-releasing hydrogels for DLP-based 3D printing

Marcelo GANZAROLLI DE OLIVEIRA, Institute of Chemistry, University of Campinas, UNICAMP, Campinas, SP, Brazil

12:25 | FP03 Bioinks - Clickable Dynamic Bioinks

Vianney DELPLACE, Université de Nantes, Oniris, CHU Nantes, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

3D Bioprinting: in vitro model, implantable bioink and intraoperative methods

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INTRODUCTION

Producing *in vitro* large pieces of tissues or organs is already within reach of few bioprinting laboratories. Advances in 3D printing, 3D bioprinting, but also complex tissue engineering and 3D tissue maturation are some of the key technologies that will make this happen.

Nevertheless, nothing will happen without informed strategies for biomaterial formulation.

EXPERIMENTAL METHODS

Here we will present a bioinspired approach for hydrogel formulations [1,2] and their use as bioinks for regenerative medicine. In this approach, the lesson learned from the human tissue properties such as water content, biomolecules and mechanical properties, were used to design and validate proliferative and regenerative bioinks.

RESULTS AND DISCUSSION

Three examples of clinical applications of the bioinspired hydrogel will be use to illustrate the approach:

- A multilayered skin model, using the specially developed bioink together with bioprinting hybrid strategies.
- An intraoperative bioprinting approach for the reconstruction of severe burn skin using bioink robotic deposition [3,4].
- A breast cancer reconstruction method using non-cellularized 3D printed hydrogels of tuned properties.

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ACKNOWLEDGMENTS

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Extending the capabilities of bioprinting with the Dynamic Formulation Module (multi-material patterning, mixing and core-shell) in a single dispensing process

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REGENHU, a pioneer in 3D bioprinting, will introduce the new Dynamic Formulation Module (DFM), with examples of how it extends the possibilities of bioprinting, including adapting the chemical formulation of base materials in time and space, in a single process.

The DFM creates 3D bio-constructs with the right material concentration at the right place, homogenous component mixes, spatial tuning of mechanical properties, and advanced strand composition.

The DFM brings the capability to create complex material gradients, tailor cell concentrations, modify matrix stiffness and build bioactive transport systems.

The DFM also ensures optimization of gelification strategies using chemical crosslinking, reducing toxicity (crosslinking photo-initiator by-products) and cell stress (over-exposure to non-ideal cell environment).

Moreover, the core-shell coaxial printing ability of the DFM enables the creation of complex tubular cellular structures, ideal for vascularization.

In detail, this new all-in-one platform provides functions that enhance the biological relevance of bioprinted constructs:

- **4-to-1 Mixing**
  - Mixing of up to 4 individual components to custom ratios
  - Mixing of components that are hard to mix or have large viscosity differences
  -
- **4-to-1 Patterning**
  - 4 independent inputs
  - Pattern between individual components
  -
- **2-to-1 Core-Shell Bundle / Co-axial**
  - 2 independent inputs
  - Component A in shell, component B in core

Discover how the DFM, alone and in combination with other REGENHU bioprinting technologies, can increase the successful outcomes of our users research projects.

Novel Approach for Guiding Stem Cell Organization and Tissue Growth by Multi-head 3D Bioprinting to Mimic Human Corneal Stroma

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INTRODUCTION

Globally, over 12 million people suffer from corneal blindness. Currently, the only treatment for corneal blindness is corneal transplants from cadavers, however, there is a severe shortage of the transplants.[1] The corneal stroma has a fundamental role in the corneal transparency and mechanical strength. The foundation of these crucial features lies in the highly organized collagen fibers of the stroma aligned perpendicular to each other.[2] The conventional 2D tissue engineering methods fail to produce native-like corneal structures. 3D bioprinting offers a solution to this issue by enabling layer-by-layer assembly of cells and biomaterials into a 3D structure[3]. However, the native-like microstructure of the corneal stroma cannot be achieved by bioprinting only one bioink, and therefore, multi-head bioprinting is required. Multi-head bioprinting of a cell-laden bioink and a thermoplastic polymer has been a widely studied method to guide cell growth. The challenges of using thermoplastic polymers include high extrusion temperature, insufficient transparency and poor resemblance to native soft tissues. In addition, most of the studies in 3D bioprinting focus on the printability instead of biocompatibility and tissue formation within the 3D structure. Here, human adipose tissue -derived stem cells and tissue adhesive hydrogel bioinks with two different stiffnesses were used to bioprint composite structures in order to guide stem cell orientation and tissue growth, as well as to improve the mechanical stability of the bioprinted structures.

EXPERIMENTAL METHODS

Bioprinting was done by extrusion-based bioprinter, and the stiffnesses of the bioinks were adjusted by modifying the concentration of the crosslinking components. Transmittance and viscosity measurements were used to characterize the transparency and the shear-thinning properties of the bioinks, respectively. Printability and stability of the bioinks were evaluated by assessing their shape fidelity after printing and during incubation in cell culture medium. Self-healing between the two bioinks were studied by preparing gel disks from each bioink, combining the disk halves and testing their adhesion after 24 h. Four different structure types were printed to compare their mechanical properties: 1. cell-free structure made of only the soft bioink, 2. cell-free structure made of only the stiff bioink, 3. cell-free composite with soft and stiff bioinks, and 4. composite with cell-laden soft bioink and cell-free stiff bioink. Mechanical properties were studied by performing amplitude and frequency sweeps with a rotational rheometer at three timepoints during two weeks of culture. Finally, the ability of the multifilament approach to guide cellular growth in the bioprinted structures was studied by printing composite structures using either stiff or soft bioink as the cell-free bioink. Cell viability and proliferation were studied from the structures with LIVE/DEAD staining and PrestoBlue, respectively. Immunofluorescence staining with phalloidin, DAPI and connexin 43 was used to study cell orientation and growth.

RESULTS AND DISCUSSION

Both bioinks were highly transparent and demonstrated excellent transparency (70-90% at 400-700 nm) according to the corneal transparency classification[4]. The bioinks were shear-thinning, and the stiffer bioink had 10 times higher viscosity. The printability and the filament shape fidelity of the bioinks were excellent. In addition, the filament thickness did not show significant decrease during culture. The adhesion between the bioinks was strong enough to hold the two gel halves together when pulling them apart. The storage modulus of the cell-free composite structure was between the modulus of the soft and stiff single-bioink structures. The cell-laden composite structure had significantly higher storage modulus especially after two weeks, which indicates the importance of cell growth for the mechanical strength. The cell viability in both composite structures was 99%, and the cell proliferation increased during two weeks of culture. Stiff, cell-free bioink was necessary for creating and maintaining cell organization in the composite. No cell organization along filaments was observed in the composite structures when cell-free, soft bioink was used for cell guidance. Moreover, the stiff bioink significantly reduced shrinkage of the printed structures during culture as well as increased the handling of the structures.

CONCLUSION

This was the first study where multi-head bioprinting of hydrogel bioinks was used to successfully orientate stem cell growth within the corneal stroma -mimicking structures. In addition, the study provides valuable information about the effects of cell growth and tissue formation on the stability and mechanical properties of the bioprinted structures. The approach presented here can be used to fabricate organized 3D constructs which mimic the microstructure of native corneal stroma.

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Development of a novel hybrid bioink containing living microfactories

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INTRODUCTION

Bioprinting has emerged as a promising technology that enables the fabrication of 3D tissue constructs via deposition of a bioink composed of cells and biomaterials. However, the dense hydrogel networks that develop after the gelation of bioink often limit cell mobility and hinder the diffusion of essential factors, hence restricting cell viability and infiltration[1]. To address this limitation, we designed a natural-based bioink enriched with living microfactories which provides a porous liquified network, thus enhancing nutrient delivery and cell growth. These biofactories consist of liquefied pockets encapsulating cells and surface-modified microparticles. Such a liquified system has been shown to be an ideal cell encapsulation strategy for tissue engineering purposes[2]. The novelty of the system lies in the combination of the liquefied cell encapsulation strategy with an external hydrogel to confer bioprintability to the system and develop clinically relevant tissue engineered constructs. On the other hand, lack of cell adhesion motifs is one of the main limitations in selecting various bioinks for bioprinting of anchorage-dependent cells. The proposed technology addresses this restriction by introducing surface-modified microparticles which act as adhesion sites for encapsulated cells.

EXPERIMENTAL METHODS

Three different formulations of bioinks were prepared. Native bioink defined as a cell-laden methacrylated hyaluronic acid (HAMA), hybrid bionk composed of a combination of HAMA and alginate microbeads encapsulating cells, and hybrid$^+$ bioink which includes HAMA and alginate microbeads encapsulating cells and microparticles. The electrospraying technique was employed to produce micro-sized alginate beads encapsulating human adipose-derived stem cells (hASCs) or hASCs and microparticles (Fig. 1, I). Then, alginate microbeads were combined with HAMA to produce hybrid bioinks (Fig. 1, II). Following bioprinting and crosslinking, a mild liquefaction was applied to the printed structure to liquify the alginate microbeads containing hASCs or hASCs and microparticles (Fig. 1, III-V). Afterwards, the printed structures cultured up to 7 days in basal medium (Fig. 1, VI).

RESULTS AND DISCUSSION

The viscoelasticity of alginate microbeads during the bioprinting, as well as the porous structure and liquid microenvironment created after liquefaction process, enhanced the viability of encapsulated cells in both hybrid bioinks, significantly in hybrid$^+$ bioink (Fig. 2 A and B).

Moreover, studying the mechanical properties of bioinks confirmed the presence of porous network in both hybrid bioinks compared to the native HAMA (Fig. 1 C). More importantly, biological assays including fluorescence staining of F-actin filaments demonstrated that the surface-modified microparticles offered a proper support for complex cellular interactions and structural organization in hybrid$^+$ bioink (Fig. 1 D).

CONCLUSION

Overall, the proposed system demonstrated a great promise in tissue engineering strategies due to its innate porosity, as well as providing cell attachment sites for encapsulated cells. Moreover, the liquefied environment provided would allow free cell movement. Hybrid$^+$ bioink promoted cell survival, along with corresponding *in vivo* like behavior of cells. Considering the fact that lack of cell attachment sites was a restricting factor in selecting various bioinks for bioprinting of anchorage-dependent cells, this novel strategy helps to bridge this gap by providing microparticles. The versatility of this technique aids engineering of desired microenvironments for encapsulated cells, while the designed bioink can be developed with ideal mechanical features without cellular bio-performance constrains. We envision to propose the developed technology in fabrication of diverse tissue constructs for various applications in tissue engineering.

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Drop-on-demand micropatterning of the novel amphiphilic peptide I_3K on regenerated silk fibroin substrates to guide and promote adhesion and proliferation of neuronal cells

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INTRODUCTION

Silk is a natural biomaterial that can be obtained from silkworms such as *Bombyx mori*. It is composed of two proteins called: sericin and fibroin[1]. After removing the silk sericin, the remaining silk fibroin is dissolved and dialyzed generating an aqueous solution known as regenerated silk fibroin (RSF). Although RSF can be used as a biomaterial in tissue engineering because of its high biocompatibility and biodegradability, it lacks the needed biological activity to support the adhesion and proliferation of cells[2,3]. In order to improve cell attachment and proliferation the novel amphiphilic peptide Ac-IIIK-NH_2 (I_3K) was coated onto the silk surfaces. I_3K is a self-assembling peptide that can form nanotubes in aqueous solution arranging itself so the positively charged hydrophilic lysine domain is facing outwards. Dispensing aqueous I_3K using a drop-on-demand (DOD) inkjet printer, high-resolution patterns can be obtained functionalizing only specific areas of the surface thus guiding neuronal cell proliferation. Moreover, the effect of I_3K on cell attachment and growth is studied using the PC-12 Adh cell line which can be differentiated into neuronal cells.

EXPERIMENTAL METHODS

1. Extraction and dissolution of regenerated silk fibroin

B. mori cocoons were degummed, rinsed and dried. The dried silk fibroin fibers were dissolved in Ajisawa's reagent, dialyzed against water for 2 days, centrifuged and stored at 4°C until further use.

2. Dissolution of synthetic peptide

The synthetic peptide I_3K was dissolved in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (pH 6.0) and left at least 5-7 days under ambient conditions to allow the peptide to self-assemble.

3. Spin coating of regenerated silk fibroin

RSF films are spin coated on cover slips (13 mm diameter). These are washed with 70% EtOH prior to use. Then, 100 µl of RSF solution (40 mg/mL) was deposited in the cover glass and spun followed by 100 µl of 95% EtOH.

4. Inkjet printing of peptide micropatterns

A custom-designed inkjet printer was used in this study. Jetting devices from MicroFab were used with 40 µm nozzle diameters. The pattern was designed using an XY plot on an excel spreadsheet. The I_3K solution with a final concentration of 1 mg/ml was used for printing on top of RSF coated cover glasses.

5. Culture of PC12 on patterned surfaces

Cell culture on RSF films were made using PC-12 Adh cells, 1000 cells were seeded on each pattern and incubated for 7 days. After 7 days, cells are fixed, permeabilized and stained with phalloidin-FITC and DAPI.

RESULTS AND DISCUSSION

The amphiphilic peptide I_3K was used to print the pattern onto the RSF surfaces via DOD inkjet printing. The pattern chosen was parallel lines with a width of 271 ± 14 µm (Figure 1A). To assess cellular adhesion of the PC12 cells, nuclei and F-actin microfilaments were stained (Figure 1B,1C, 1D).

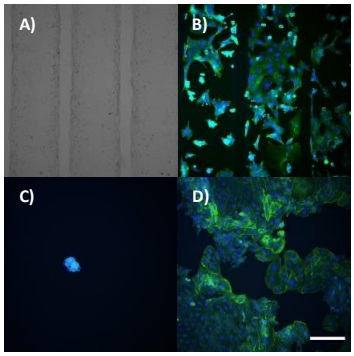

Figure 1. A) Bright-field image of I_3K line patterns on top of RSF surfaces via DOD inkjet printing. PC12 cells on B) three layers I3K lines. B) RSF surface. D) on control surface, TCP. Cell nuclei are labelled with DAPI (blue) and phalloidin-FITC (green) for F-actin filaments. Scale bar = 200 µm.

Cell attachment of pure RSF films was low and non-uniform (Figure 1C), on the other hand cells attached to the printed I_3K lines readily (Figure 1B). Cells grew on the TCP control homogenously distributed across the surface (Figure 1D) in contrast to the printed samples, where the cell attachment was focused on the printed patterns.

CONCLUSION

This study demonstrated that even though silk fibroin in its regenerated form, RSF, is biocompatible and exhibits no detectable cytotoxicity on PC12 cells, it lacks the biological activity to readily support cell adhesion. Moreover, the use of DOD inkjet printing allows for the precise deposition of I_3K to form high-resolution micropatterns. These can be used to guide and promote cell adhesion to specific locations when combined with an antifouling surface such as RSF.

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A Collagen-Glycosaminoglycan Scaffold Coating Enhances the Bio-Lubrication of a 3D-Printed Framework for Cartilage repair

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INTRODUCTION

The frictional properties of cartilage biomaterials are invariably ignored in preference to enhancing features for added load support. Indeed, recently in our lab, novel 3D printed polycaprolactone (PCL) frameworks have been incorporated within our original collagen-based scaffolds for such load bearing support[2]. These porous multi-layered collagen-glycosaminoglycan (GAG) scaffolds for osteochondral repair enable cell infiltration and layer-specific tissue formation *in vivo*[1], yet 3D printed supports may cause mechanical abrasion to opposed cartilage. Equivalent to many cartilage repair biomaterials, these 3D printed frameworks may initiate progressive degeneration of opposed cartilage[3], although, such damage mechanisms are difficult to determine as limited testing exists on the frictional qualities (bio-lubrication) of cartilage-scaffold interfaces. Therefore, the goal of this study was to characterize the frictional behavior of different cartilage–scaffolds across a range of conditions.

EXPERIMENTAL METHODS

Cartilage surfaces from intact bovine osteochondral cores were articulated against hydrated scaffolds or cartilage (controls) in phosphate-buffered saline (PBS) or bovine synovial fluid (bSF) lubricants. Scaffold test groups included PCL alone & PCL set in a freeze-dried collagen-GAG scaffold (PCL-Coll). **Friction test**[4,5]: scaffolds & cartilage were compressed by ε_N=20% and held by decreasing pre-hold time (12min, 120, and 12s) followed by 2 rotations in each direction at an effective velocity (v_{eff}) of 0.3mm/s. **Wear test & histology**[3]: ε_N=30% and v_{eff} =1mm/s for 100 cycles (50/direction, ~2m sliding distance). Cartilage was stained with Safranin-O to assess changes in tissue morphology. Statistical analysis: 1-way ANOVA was used to assess the coefficient of friction (COF: torque/load x radius). Data (n=4) mean ± S.E.M.

RESULTS AND DISCUSSION

An adapted cartilage-scaffold friction test as per **Fig. 1** was used for testing.

Figure 1: Friction test set-up.

The static friction decreased by pre-hold time and the PCL-Coll group was observed to have a lower COF vs. the PCL group but a higher COF than the cartilage controls (Fig. 2AB). After the wear test, PCL, grooves were visible on cartilage (Fig. 2C). Post wear testing, safranin-O staining revealed cartilage surface fibrillation on PCL-articulated cartilage (Fig. 2D).

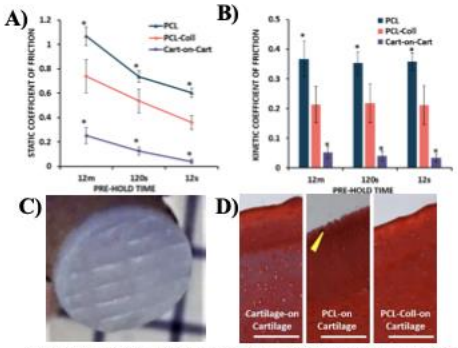

Figure 2: A) The static and B) kinetic COF of samples in PBS, C) Visible groves on cartilage after PCL wear-test, D) Damage post PCL wear-test (Safranin-O).

Increased resistance to articulation (torque) and lower load support was evident during cartilage-on-scaffold articulation and resulted in increased friction compared to cartilage-on-cartilage interfaces. The field to date has focused on improving the compressive properties of cartilage scaffolds, yet such improvements may result in 3D printed materials with reduced frictional qualities, and enhanced risk of cartilage wear. Indeed, beyond visible grooves after testing, microscopic images showed fibrillation to cartilage surfaces articulated against PCL, whereas collagen-coated scaffolds (PCL-Coll) did not.

CONCLUSION

Collagen-GAG coatings enhanced the bio-lubrication of PCL-frameworks and provided protection against cartilage wear. These data collectively point to the importance of frictional properties as design and validation parameters for cartilage biomaterials to understand and minimize potential adverse secondary effects (e.g., undue wear or damage to opposing tissues).

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Micellar Nitric Oxide-Releasing Hydrogels for DLP-Based 3D Printing

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INTRODUCTION

3D printing of hydrogels is increasingly being explored in the field of biomaterials, especially when considering the customized printing of hydrogel prostheses for cartilage tissue replacement[1]. In this case, in addition to biocompatibility, the hydrogels must possess appropriate elastic properties. Recently, we demonstrated the 3D printability of supramolecular hydrogels comprised of chemically cross-linked poly(acrylic acid) (PAA), interpenetrated by a micellar network of Pluronic F127 micelles, containing cellulose nanocrystals, capable of releasing nitric oxide (NO)[2]. The interest in NO release by prostheses is motivated by the potential beneficial actions that NO can provide, including improved and faster tissue integration, along with microbicidal and angiogenic actions[3,4]. In the present study, we developed a novel photocrosslinkable micellar PAA/F127 hydrogel for 3D printing via Digital Light Processing (DLP), though the. Incorporation of preformed polyvinyl alcohol (PVA) in the intermicellar space.

EXPERIMENTAL METHODS

3D-printable resins were comprised of 30 wt% acrylic acid, 0.26-0.32wt% N,N'-methylenebisacrylamide, 7.6-20wt% Pluronic F127, 1-2wt% poly(vinyl alcohol), 0.08wt% Irgacure 818 and 0.01wt% SUDAM I. Hydrogel constructs were 3D printed in a MoonRay D75 DLP printer (75 µm minimum pixel size/100 µm printing step size). S-nitrosoglutthione (GSNO) (20-130 nmol/g) was incorporated through absorption from solution. Compression tests were performed in a TA.XT Plus Texture Analyzer. Cryo-TEM micrographs were obtained in a transmission electron microscope TALOS F2000. GSNO charge and real-time NO release were measured by chemiluminescence in a NOA, Sievers 280i (GE Analytical Instruments). Numerical results are the average of triplicates.

RESULTS AND DISCUSSION

The AA/micellar F127/PVA resins showed good printability and fidelity to the computational model (Fig. 1a-d). Mechanical compression/decompression tests showed that the presence of 2 wt% PVA significantly reduces the hysteresis and Young's modulus of the PAA/F127 hydrogels. This effect can be attributed to the plasticizing action of the interpenetrated PVA chains, which is exerted through intermolecular hydrogen bonding interactions among PVA hydroxyls, terminal F127 hydroxyls at the crowns of the F127 micelles, and the protonated carboxyl groups of PAA. The presence of F127 micelles in two different micelle packings was confirmed by Cryo-TEM (Fig. 1 e-f). PAA/F127/PVA hydrogels charged with GSNO were shown to release NO spontaneously under hydration, at rates which can be modulated by the GSNO charge.

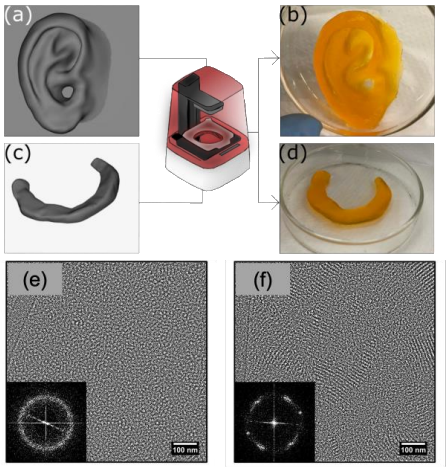

Fig 1. 3D models of ear (a) and knee meniscus(c) and the corresponding DLP-3D printed constructs (b) and (d) of PAA/F127/PVA hydrogel. Cryo-TEM micrographs of F127 7.6%/PVA hydrogel (e) and F127 22%/PVA hydrogel (f).

CONCLUSION

The incorporation of PVA improves the elastic behavior of 3D-printed PAA/F127 hydrogels, by reducing their plastic deformation in the compression process. PAA/F127/PVA hydrogels loaded with S-nitrosoglutathione (GSNO) release NO locally in a dose-response manner, upon hydration. PAA/F127/PVA/GSNO hydrogels have potential for the 3D-printing of customized cartilage replacement constructs, with enhanced biocompatibility and mechanical properties.

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Clickable Dynamic Bioinks

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INTRODUCTION

Bioprinting is a booming technology, with broad applications in tissue engineering and regenerative medicine.[1] It holds the promise of fast and straightforward access to any type of living tissues, with tailored composition and architecture. Yet, while bioprinters have quickly evolved, the field of printable biomaterials is much behind. The main challenge consists in designing cell-containing materials, i.e., bioinks, that can flow for printability, and yet possess the desired composition and mechanical properties after printing. To date, most advanced strategies have revolved around the use of printing baths[2-3] and photocrosslinking[4-5], with inherent limitations; and a simple and universal bioink strategy remains to be found.

EXPERIMENTAL METHODS

To address this challenge, we hypothesized that a dynamic covalent hydrogel, which is printable, can be chemically modified with reactive groups to allow the adjustment of a construct composition and mechanical properties after printing. More specifically, post-printing modifications can be performed by the simple addition to the culture medium of a diffusible molecule (e.g., covalent crosslinker, adhesive peptide) modified with a reactive group complementary to the one grafted onto the hydrogel network. The post-printing reaction has to meet the criteria of click chemistry (i.e., mild conditions, no byproducts, no purification), overall providing what we called « clickable dynamic bioinks ». Using hyaluronic acid (HA) as a polymer of interest, we first developed and characterized a new class of dynamic covalent bioinks based on boronate ester crosslinking. Then, taking advantage of the strain-promoted azide-alkyne cycloaddition (SPAAC) as a click reaction, we investigated the feasibility of various post-printing modifications (e.g., composition, stiffness, adhesion), assessed by fluorescent labelling, rheological and mechanical measurements, and/or cell behavior.

RESULTS AND DISCUSSION

For the first time, we demonstrated that boronate ester crosslinking can be used for the design of printable hydrogels, with appropriate shear-thinning, absence of swelling, long-term stability, and tunable viscoelastic properties (G' of 80 to 2500 Pa, at 1 Hz). We showed that these hydrogels are cytocompatible (>90% cell viability) with various cell types (i.e., fibroblasts, MSCs, chondrocytes), and that they can prevent cell sedimentation in a cartridge for days, circumventing a common issue in bioprinting. These new bioinks allowed us to design constructs of various shapes and volumes (tested up to 10 layers). Regarding post-printing modifications, we first showed that the construct composition can be easily modified with various molecules of interest (chondroitin sulfate, low molecular weight HA, gelatin). This technique also allowed to increase the construct rigidity (G' increased from 200 to 1200 Pa) upon the addition of a desired crosslinker, or trigger cell adhesion upon the addition of a reactive adhesive peptide (e.g., RGD-azide). Of major value, we finally showed that these post-printing modifications can be controlled in time and space.

CONCLUSION

Clickable dynamic bioinks constitute a simple and versatile bioprinting tool that combines printability and tailorable composition and mechanical properties in a most unique fashion. It paves the way toward 4D bioprinting, with virtually unlimited tissue engineering applications.

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>> 10:30 - 12:30 | SYMP-02 - Antioxidant nanomaterials for biomedical applications

Chairpersons: Gianni Ciofani & Matteo Battaglini
Location: Room H

10:30 | KL Antioxidant - Nanozymes in Biomedicine: Route towards Clinical Applications
Gianni CIOFANI, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), Italy

11:00 | O1 Antioxidant - Bionic tissue with antioxidant microreactors reduce oxidative stress on HepG2 cells
Stefan PENDLMAYR, (1) Interdisciplinary Nanoscience Center (iNANO), Aarhus University, Aarhus, Denmark. (2) Sino-Danish Center for Education and Research, University of Chinese Academy of Sciences, Beijing, China

11:15 | O2 Antioxidant - Engineering Biomimetic Epigenetically-Activated Extracellular Vesicles to Promote Bone Formation
Kenny MAN, School of Chemical Engineering, University of Birmingham, Birmingham, UK

11:30 | O3 Antioxidant - Establishment of Selenium-incorporated Mesoporous Silica Nanoparticles (MSNs) for Osteosarcoma Therapy
Lei HE, Department of Instructive Biomaterials Engineering (IBE), MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, the Netherlands

11:45 | O4 Antioxidant - ALTERNATIVE Horizon 2020 project for building an innovative platform to assess the cardiotoxicity of chemicals
Gianluca CIARDELLI, Politecnico di Torino, Torino, Italy

12:00 | FP01 Antioxidant - Designing a nanointelligent bioartificial pancreas to treat type I Diabetes
Joana MOREIRA MARQUES, i3S - Institute for Research & Innovation in Health, University of Porto, Porto, Portugal; Faculty of Pharmacy of the University of Porto, Porto, Portugal

12:05 | FP02 Antioxidant - Firefly-Bioinspired Hydrogels with Redox-responsiveness as Cell-encapsulating Injectable Matrices
Minye JIN, INM – Leibniz Institute for New Materials. Campus D2-2, Saarbrücken, Germany; Chemistry Department, Saarland University, Saarbrücken, Germany; University of Twente, Enschede, The Netherlands.

Nanozymes in Biomedicine: Route towards Clinical Applications

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Administration of redox-active nanoparticles is becoming a popular strategy to improve the biological effects of traditional molecular antioxidants [1]. Here, we will summarize properties, mechanisms of action, and activity of nanomaterials that show an intrinsic redox activity, in particular focusing our attention on cerium oxide nanoparticles (Figure 1, on the left) as inorganic nanomaterial, and on polydopamine nanoparticles (Figure 1, on the right) as organic nanomaterial.

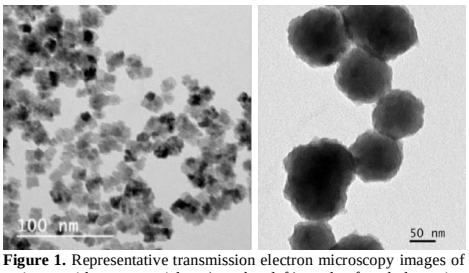

Figure 1. Representative transmission electron microscopy images of cerium oxide nanoparticles (on the left) and of polydopamine nanoparticles (on the right)

Cerium oxide nanoparticles (nanoceria) mimic both superoxide dismutase and catalase activities, and in the past decade many *in vitro* and *in vivo* studies demonstrated their strong biological antioxidant properties, reducing both basal and stress-induced reactive oxygen species (ROS), and acting as pro-survival agents.
Based on these excellent features, cerium oxide nanoparticles have become a promising pharmacological tool for the treatment of many diseases associated with oxidative stress, and our group extensively studied effects of nanoceria on many cellular model, including their protective actions on muscle cells exposed to microgravity conditions onboard of the International Space Station [2, 3].
In vivo radioprotective effects on stem cells and tissue regeneration have been moreover assessed, using low-dose irradiated planarians as model system. Nanoceria demonstrated to increase the number of stem cells and tissue regenerative capability, and to reduce cell death and DNA damage after low-dose irradiation, suggesting a protective role on stem cells [4].
Despite their intriguing ROS scavenger properties, cerium oxide nanoparticles are an inorganic and non-biodegradable material (or at least, their degradation rate is extremely slow), and this is motivation of concerns for their future application in the clinical practice. At this aim, recently our attention is being focused on organic materials presenting analogous ROS scavenging properties.
In this scenario, we have to consider polydopamine, a polymer deriving from the self-polymerization of the biomolecule dopamine. Polydopamine can be easily synthesized to obtain spherical nanoparticles, tunable in terms of size, loaded cargo, and surface functionalization. Polydopamine nanoparticles (PDNPs) have been increasingly attracting the attention of the research community due to their elevated versatility in the biomedicine field, for their excellent ability to encapsulate drugs, to convert near-infrared (NIR) radiation into heat, and to act as antioxidant agents [5].
In our research, we propose PDNPs as smart nanomaterials for ROS scavenging and as nanotransducers for cellular stimulation. We demonstrated the efficiency of this nanoplatform in protecting neuronal-like cells from oxidative stress and in promoting, when irradiated with near-infrared light, neuronal activation upon a localized increment of temperature [6]. Their suitability as therapeutic agent in neurodegenerative diseases has been moreover provided, and corroborated by an extensive proteomic analysis on cell derived from patients affected by Autosomal recessive spastic ataxia of Charlevoix-Saguenay.
In this talk, we will summarize our most recent results about biomedical application of PDNPs, with particular attention to their properties as "nanozymes" applied to the treatment of central nervous system disorders. Future activities, concerning also exploitation of PDNPs as smart antioxidants in space medicine, will be finally depicted.

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Bionic Tissue With Antioxidant Microreactors Reduce Oxidative Stress On HepG2 Cells

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INTRODUCTION

Natural and synthetic materials are used to explore how cells, organelles and enzymes can be mimicked within the field of bottom-up synthetic biology.[1] Micro/Nanoreactors that can perform encapsulated catalysis are popular examples for the former cases. However, the first examples of their integration with mammalian cells only start to emerge. For example, microreactors with matrix vesicles from osteoblast have shown increased bone mineralisation,[2] growth factor loaded microgels increased *in vivo* myocyte viability after stroke,[3] and multi-layer anti-oxidant assemblies could partly protect neuroblastoma cells from hydrogen peroxide (H_2O_2) and ammonia.[4] Recently we have demonstrated that microreactors loaded with catalase could support HepG2 cells in 3D cell aggregates when stressed with H_2O_2.[5] The artificial moieties could only provide support for up to 24 h because the encapsulated enzyme lost its activity.

Here, we use the superoxide dismutase (SOD)/catalase mimetic Eukarion (EUK) as the active unit in alginate-based microreactors to alleviate the oxidative stress in 3D cell aggregates.

EXPERIMENTAL METHODS

A water-soluble EUK derivative was synthesized and conjugated to alginate resulting in an active cross-linkable polymer. The microreactors were then made using an Encapsulator B-390 and droplet microfluidics resulting in ~74 and ~40 µm sized beads, respectively.

The catalase-like activity was measured by detecting the remaining H_2O_2 after 30 min incubation with the microreactors using the Amplex Ultra Red assay. The SOD-like activity was investigated using a SOD activity kit.

Active and in-active microreactors were coated with poly(L-lysine) (PLL) or cell membrane vesicles (CMVs) and co-cultured with HepG2 in low adhesive round-bottom well plates. After 3 days of culture, the tissue aggregates were exposed to H_2O_2 containing media for 48 h followed by a 72 h recovery period and subsequently another 24 h of H_2O_2 exposure. The viability of the tissue was followed over time and visualised by confocal laser scanning microscopy.

RESULTS AND DISCUSSION

The microreactors showed catalase-like and SOD-like activity in a concentration dependent manner. The incorporation of both sized microreactors with HepG2 cells was successful and resulted in a stable aggregate (Figure 1). CMVs were purified from HepG2 cells to be employed as a nature-like coating that imitates the natural cell surface. The CMV coating showed a comparable or even better integration with HepG2 cells than a pure PLL coating, thereby resulting in aggregates with better structural integrity.

The bionic tissue showed a concentration dependent toxicity to H_2O_2. Tissues with active microreactors showed increased viability compared to aggregates containing non-active microreactors. This result showed that the presence of the microreactors helped to lower the impact of oxidative stress on the HepG2 cells. Specifically, the HepG2 cells in the aggregates exposed to H_2O_2 for 48 h recovered to a comparable viability level as the untreated control after 72 h in fresh media.

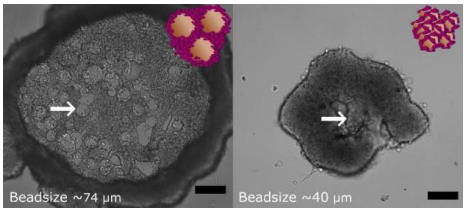

Figure 1: Brightfield microscopy images of aggregates consisting ofHepG2 cells and either ~74 µm (left) or ~40 µm (right) sized microreactors after 4 days of culture. Arrows indicate microreactor on both images. Scalebars are 200 µm.

CONCLUSION

We have created synthetic microreactors, which incorporated in a self-organised bionic tissue with HepG2 cells. This bionic tissue showed how the biological world could benefit from being combined with synthetic designs.

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ACKNOWLEDGMENTS

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Engineering Biomimetic Epigenetically-Activated Extracellular Vesicles to Promote Bone Formation

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INTRODUCTION

Extracellular vesicles (EVs) are emerging as promising instructive acellular tools to recapacitate the natural bone healing process, circumvent many limitations with the translation of cell-based therapies [1]. Although these cell-derived nanoparticles have shown promise, there is a tremendous need to enhance their therapeutic efficacy to promote clinical adoption. It has become increasingly apparent that epigenetic regulation plays a pivotal role in controlling cell fate [2]. Several studies have shown that the process of hyperacetylation and hypomethylation promotes the differentiation capacity of cells [3, 4].

Therefore, this study aimed to develop epigenetically-activated pro-osteogenic EVs as a novel acellular tool to promote bone formation.

EXPERIMENTAL METHODS

The effects of DNA methyltransferase inhibitor - 5-azacytidine (AZT) and histones deacetylase inhibitor - trichostatin A (TSA) on osteoblast viability, epigenetic functionality and osteogenic differentiation was evaluated. EVs were isolated from the conditioned medium of mineralising osteoblasts treated with AZT, TSA or AZT/TSA over a 2-week period. EV size and concentration were defined using nanoparticle tracking analysis, transmission electron microscopy and CD63 ELISA. Osteogenic differentiation of human mesenchymal stem cells (hMSCs) cultured with EVs-derived from untreated (MO-EVs), AZT treated (AZT-EVs), TSA treated (TSA-EVs) and AZT/TSA treated (AZT/TSA-EVs) osteoblasts was evaluated by qPCR, biochemistry and histological analysis.

Experiments were performed in triplicate. Statistical analysis was conducted using ANOVA multiple comparisons test with Tukey modification using IBM SPSS software (IBM Analytics, version 21). *p* values equal to or lower than 0.05 was considered as significant. * $p \leq 0.05$, ** $p \leq 0.01$ *** $p \leq 0.001$.

RESULTS AND DISCUSSION

AZT treatment significantly reduced osteoblast histone and DNA methylation levels ($P \leq 0.05$), whilst TSA treatment significantly enhanced histone acetylation levels ($P \leq 0.001$). Combined AZT/TSA treatment further augmented osteoblast epigenetic functionality and increased mineralisation capacity when compared to AZT and TSA treatments alone (Fig 1) ($P \leq 0.05$ - 0.001)

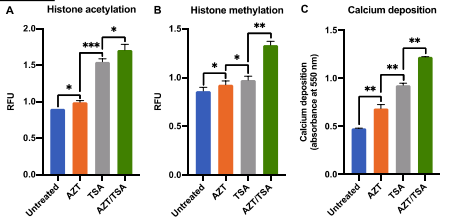

Figure 1. The effects of epigenetic modification on osteoblast A) histone acetylation, B) histone methylation and C) calcium deposition. Data expressed as mean ± SD (n=3). * $p \leq 0.05$, ** $p \leq 0.01$ *** $p \leq 0.001$.

AZT-EVs, TSA-EVs and AZT/TSA-EVs treatment significantly promoted hMSCs proliferation and migration when compared to untreated cells. Moreover, AZT/TSA-EVs substantially enhanced hMSCs transcriptionally permissiveness through inducing decreased methylation and enhanced acetylation levels compared to the AZT-EV treated, TSA-EV treated and untreated controls. Importantly, AZT/TSA-EVs treatment significantly accelerated hMSCs osteogenic differentiation and extracellular matrix mineralisation when compared to the AZT-EV treated, TSA-EV treated and untreated cells (Fig 2) ($P \leq 0.01$).

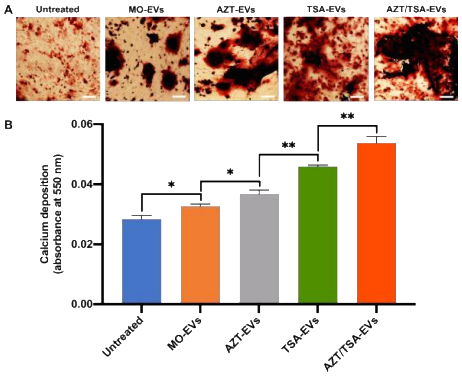

Figure 2. EV-induced hMSCs extracellular matrix mineralisation. A) Alizarin red staining and B) quantification following 3 weeks osteoinductive culture. Data expressed as mean ± SD (n=3). * $p \leq 0.05$, ** $p \leq 0.01$ *** $p \leq 0.001$. Scale bar = 100 µm.

CONCLUSION

Taken together, we have demonstrated the development of epigenetically-enhanced pro-osteogenic EVs as a novel acellular tool to promote hMSCs capacity for bone formation through transcriptional activation.

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Establishment of Selenium-incorporated Mesoporous Silica Nanoparticles (MSNs) for Osteosarcoma Therapy

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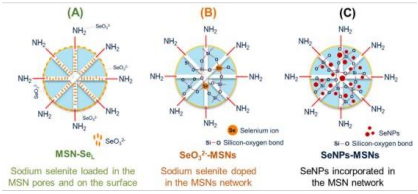
INTRODUCTION

Common treatments for osteosarcoma (OS), one of the most common malignant bone tumors, involve surgical resection combined with adjuvant chemo/radiotherapy. However, anti-OS treatment efficacy has gradually decreased due to side effects such as drug resistance[1]. Inorganic ions and compounds prepared from selenium (Se) have received attention as promising alternative therapeutic option for OS therapy. Therapeutic ions have many advantages for their use including their biosafety, stability, easy preparation, and low cost of development. Se, an essential micronutrient in biological tissue, can maintain metabolism and repair DNA as an anti-oxidant in low doses while at high dose Se is oxidative and can induce apoptosis by generating ROS[2]. ROS acts as a double-edge sword, boosting metabolism in low concentrations while inducing apoptosis in high concentrations[3]. Inducing ROS can be used to achieve cancer cell selectively since cancer cells regularly contain higher level of ROS compared to normal cells[3]. Thus, Se induced ROS to selectively induce apoptosis in OS cells is a promising therapy for OS treatment. However, direct Se delivery is complicated by limited targeted efficiency and uncontrollable accumulation. Nanoparticles such as mesoporous silica nanoparticles (MSNs) can be used for Se delivery to allow tissue targeting, controllable Se release and efficient cellular internalization. MSNs have been widely used as nanoparticle delivery system due to their good biocompability, tunable size and surface. On account of its mesoporous structure, MSNs can incorporate Se ions in several ways, namely: loaded in the mesopores and on the surface (MSN-Se_L), incorporated in the silica matrix (SeO_3^{2-}-MSNs), and incorporates as Se nanoparticles (SeNP-MSNs) (scheme 1)[4–5]. The aim of this study is to compare different Se-incorporation methods and its effectiveness against OS cancer cells in vitro.

EXPERIMENTAL METHODS



Scheme.1 Three Groups of Se-MSNs

All groups of synthesized Se-MSNs were characterized by DLS, TEM and FTIR. ICP-MS was used to investigate the total Se content in all groups of Se-MSNs. In vitro, MTS assay was used for cytotoxicity tests for all groups of Se-MSNs in both OS cell (Saos-2) and osteoblast (hFOB 1.19). Cell viability by MTS was detected by microplate reader. One-way analysis of variance (ANOVA) was carried out using SPSS 27.0 and GraphPad 9.0 with Tukey's multiple comparison tests for statistical analysis.

RESULTS AND DISCUSSION

Three groups of Se-MSNs were successfully synthesized with good homogeneity and similar size of around 80-100 nm. ICP-MS results showed that MSN-Se_L and SeO_3^{2-}-MSNs could incorporate around 5-6% of selenite ions. In contrast, SeNP-MSNs contained more than 95% of Se. Next, cell cytotoxicity assays were performed in OS cells and in osteoblast healthy cells (OB). In OS cells, all groups showed dose-dependent trends on inhibiting OS activities with IC_{50} values of 72, 42 and 56 µg/ml respective to groups MSN-Se_L, SeO_3^{2-}MSNs and SeNP-MSNs. The IC_{50} values also showed that groups SeO_3^{2-}MSNs and SeNP-MSNs displayed more promising anti-OS effects. In OB, all groups showed significant decreased cytotoxicity towards OS and OB cells, proving our MSN-Se can selectively inhibit OS activity with limited effect on healthy cells. SeO_3^{2-}-MSNs group showed the best selectivity among the three groups.

CONCLUSION

In this work, three new Se-incorporated MSNs have been developed. Specific Se amount in every groups and variables have also been detected, proving different forms of Se can be incorporated in MSNs. In vitro, selectivity of Se towards OS cells has also been verified. Thus, it can be concluded Se-MSNs can be a great candidate as novel anti-OS in OS therapy.

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ALTERNATIVE
Horizon 2020 project for building an innovative platform to assess the cardiotoxicity of chemicals

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Abstract: Horizon 2020 research project ALTERNATIVE develops an innovative platform and an integrated approach for testing and assessment of chemicals in terms of cardiotoxicity. The novel approach will strengthen the capacity of regulators and industry to prevent cardiotoxic co-exposures to industrial chemicals and pharmaceuticals in an effective way.

CONSORTIUM PARTNERS

The consortium includes 11 partners from 8 European countries with intersectoral character (3 academic partners, 4 public research institutes, 4 companies).
Politecnico di Torino (Italy) is in charge of the overall management and scientific coordination of the project and designs the in vitro 3D model of young and aged myocardial tissue. The model is used by **CNR Institute of Clinical Physiology, (Italy)** in the biological testing of chemical mixtures toxicity studies. The dynamic culture system is realized by **IVTech srl (Italy) and Elvesys (France)** which is also in charge of the sensorization. **Fundació Eurecat (Spain)** develops the electrodes for the cell stimulation and leads the omics analysis on the cells populating the model. **Istituto di Ricerche Farmacologiche Mario Negri IRCCS (Italy)** role is on developing computational models for the evaluation of heart chemical hazard by the use of AI and machine learning approaches to predict cardiac toxicity of chemical mixtures. **Utrecht University (The Netherlands)** builds physiologically-based computational models to bridge the gap between in vitro toxicity findings and in vivo risk for cardiotoxicity. **Sciensano (Belgium)** and **Medical University Innsbruck (Austria)** carry out regulatory issues based on their expertise in the field, while, **CST (Bulgaria)** develops the data service, including an on-cloud database, big data lake, system integration between different stakeholders and **Eurescom GmbH (Germany)** is in charge of the administrative project management support and impact creation.

FURTHER INFORMATION

Coordinator: Gianluca Ciardelli, Politecnico di Torino
Technical Manager: Federico Vozzi, CNR
Beneficiaries: 11 partners
Duration: 1 October 2021 – 30 September 2024 (36 months)
Type of Project: Horizon 2020 Research and Innovation Action (RIA)
EU contribution: € 5.5m

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Highly Versatile Carbon Platform for Mimicking and Evolutionary Understanding of Natural Enzymes

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INTRODUCTION

Self-assembling enzyme mimics offer an easy way to imitate activities of natural enzymes[1] but have not been thus far used to understand the effect of different amino acids on the catalytic activity and why they are evolutionarily preserved for specific catalytic roles. Here, we hypothesized that fullerenes functionalized with catalytically active amino acids, which form multiple active sites via the self-assembly process in the aqueous environment[2], can serve as an effective system to distinguish the catalytic activity differences resulting from single amino acid changes. Furthermore, since we used the carboxyl-imidazole couple found in quite different enzymes as the main catalytic unit, we also hypothesized that they can mimic different enzyme classes, like hydrolases and lyases.

EXPERIMENTAL METHODS

Amino acid (Histidine, Arginine, Serine, Tyrosine, Threonine, Glutamine) functionalization onto fullerene nano-cages was performed using a well-established procedure[2] and synthesized nanocatalysts were characterized by elemental analysis, ^{1}H-NMR, and HPLC techniques. Kinetic analyses were performed by using three different substrate analogues, 4-Nitrophenyl acetate (pNPA), 4-Nitrophenyl phosphate (pNPP), and Bis(4-nitrophenyl) phosphate (bis-pNPP), to test the nanocatalysts' general hydrolase, phosphomonoesterase, and phosphodiesterase-like catalytic activities, respectively. Carbonic anhydrase (CA)-like activity of the nanocatalysts was determined by blowing CO_2 into the assay solution and monitoring the pH over time. Lastly, alkaline phosphatase (ALP)-like activity of the nanocatalysts was analyzed *in vitro* by qRT-PCR and alizarin red mineralization assay.

RESULTS AND DISCUSSION

Fullerene-based nanocatalysts decorated with histidines and side amino acids showed significant bio-catalytic activity for the hydrolysis of various ester substrates and hydration of CO_2, indicating the system's versatility for different enzyme-catalyzed reactions (Figure 1). Among the designed nanocatalysts, histidine (H) and arginine (R) containing fullerenes, called HR, showed the highest catalytic efficiency for the hydrolysis of pNPA and pNPP, which is due to outstanding transition state stabilizer role of the arginine in enzyme active sites. For bis-pNPP, HY was the best nanocatalyst in terms of catalytic efficiency, since tyrosine functions in the coordination of substrates in the active site phosphodiesterases. As for the CA-like catalytic activity, HT was the most effective nanocatalyst, which is evident from a greater decrease at pH due to faster CO_2 hydration by HT molecules. This was because HT provides the most similar nano-environment to the active site of the α-CA enzyme. Additionally, all these catalytic activities increased in the presence of the Zn^{2+} ions that behave as Lewis acids to activate reaction sites and water molecules for the nucleophilic attack. These designed nanocatalysts also functioned in biological systems, showing ALP-like enzymatic activity. They caused increased ALP expression in the Saos-2 osteosarcoma cells because their ALP-like activity increased inorganic phosphate (P_i) concentration, which is a positive sign for the expression of ALP. Besides, they increased bio-mineralization of Saos-2 cells due to increased P_i concentration by their activity and increased ALP expression.

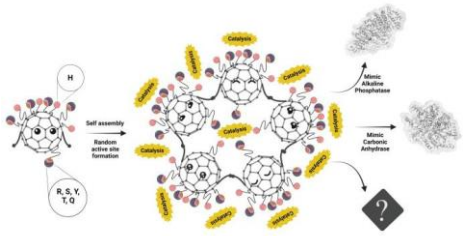

Figure 1. Schematic representation of fullerene nanocatalysts and their catalytic action upon self-assembly.

CONCLUSION

Since the fullerene-based nanocatalyst platform is highly modular by nature, it is not limited to the examples discussed in this research; it can be used to form different enzyme mimicry systems with varying combinations of catalytic units. Besides, because the effects of different functional groups on catalytic performance can be analyzed, it may be a new convenient approach to investigate site-directed mutagenesis. Although still early in its development, we believe that the proven catalytic activity of fullerene nanocatalysts, in combination with their highly modular nature, makes fullerene nanocatalysts a powerful platform for mimicking and evolutionary understanding of natural enzymes.

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Chemotherapeutic-releasing SiO_2 and ZnO nanoplatforms combined with nitric oxide donor for cancer cells sensitization

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INTRODUCTION

Currently, cancer treatments still face difficulties related to high biodistribution, non-specificity, and also the growing tumor resistance to conventional treatments. In this scenario, nanotechnology has gained space in the field of medicine by enabling the design of materials that provide efficient, targeted applications with minimal side effects.[1] Among several nanoparticles (NPs) with applications in this area, porous NPs, such as zinc oxides and silicon (ZnO and SiO_2) consist in a potential strategy for an efficient delivery of drugs with reduced side effects. A few years ago, the use of nanotechnology in cancer was based only on a targeted treatment using drug-loaded NPs. However, it has been increasing a simultaneous multi-drug delivery strategy, in order to sensitize tumor cells, especially cells resistant to current therapeutics. In this sense, a combination of chemotherapeutics with nitric oxide (NO) releasing molecules, consists of an innovative strategy that aims to sensitize tumor cells with low NO rates, potentializing the chemotherapy treatment. This work aimed to develop chemotherapeutic-loaded NPs and study the efficiency against prostate cancer cell line (PC3), combined or not with NO donors.

EXPERIMENTAL METHODS

SiO_2 NPs were synthesized by sol-gel method adapted from literature.[4] ZnO NPs were obtained by hydrothermal reaction (Wang et al., 2016).[5] SiO_2 and ZnO NPs were characterized by several techniques, such as dynamic light scattering (DLS), transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier Transform Infrared Spectrometry (FTIR), in order to confirm their obtainment. These NPs were carried with doxorubicin (DOX) and cisplatin (CIS). The antitumoral potential was evaluated against PC3 cell lines and MTT was performed to evaluate cell viability.

RESULTS AND DISCUSSION

SiO_2 and ZnO NPs were successfully synthesized by the proposed routes. SiO_2 NPs showed a hydrodynamic size of 198 ± 6.0 nm, polydispersity index (PDI) of 0.144 ± 0.04 and zeta potential of -27.37 ± 0.51 mV. ZnO NPs showed a hydrodynamic size of 272.17 ± 20.82 nm, PDI of 0.190 ± 0.04 and zeta potential of -20.57 ± 7.41 mV. Results were in accordance with TEM analysis (Fig. 1), in which we observed NPs with 158 ± 20 nm and 118 ± 43 nm with spherical and hexagonal morphologies, respectively. XRD and FTIR also confirmed the obtainment of the NPs. Moreover, both SiO_2 and ZnO NPs demonstrated 1-month stability in aqueous suspension.

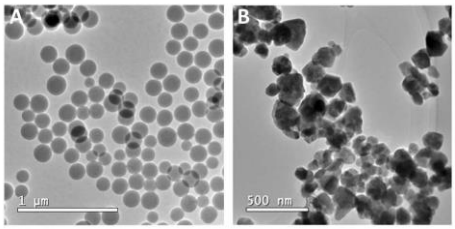

Fig 1. TEM micrographs of (a) SiO_2 NPs and (b) ZnO NPs.

SiO_2 NPs and ZnO NPs were carried with DOX and CIS, separately. The loading efficiency was from 17% to 58%, achieving concentrations from 69 to 586 µg of chemotherapeutic per mg of NP.

The cell viability of PC3 cells were significatively reduced ($p<0.05$) when treated with chemotherapeutic-loaded NPs compared to control cells. SiO_2 demonstrated higher biocompatibility, while ZnO NPs showed significant cytotoxicity even in lower concentration. Previous studies from our group indicate promising synergic effect between carried NPs and NO.

CONCLUSION

Both SiO_2 and ZnO NPs were successfully synthesized by the proposed routes. The chemotherapeutics were loaded and evidenced an outstanding loading efficiency when compared to chemotherapeutic-loaded NPs previously reported. Antitumoral potential was confirmed against PC3 cell line.

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Designing a NanoIntelligent bioartificial pancreas to treat type I Diabetes

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INTRODUCTION

Diabetes mellitus (DM) is one of the biggest health problems nowadays, being estimated that 537 million individuals suffer from DM worldwide[1]. Type 1 DM (T1DM) is a chronic auto-immune disease characterized by insulin insufficient secretion due to β-cells destruction[1]. Since T1DM is not preventable and most of the cases are diagnosed after extensive β-cells destruction[2], definitive cure consists in replacing the destroyed pancreas[3]. However, the number of available donors is limited, in addition to the need of a lifelong immunosuppressive therapy. In this work, we propose the development of an innovative biomimetic pancreas, comprising β- and α-cells differentiated from human induced pluripotent stem cells (hiPSCs) immobilized in a biofunctional matrix, embedding glucose-responsive nanoparticles (NPs) encapsulating a GLP-1 analogue. Glucose sensitiveness will be accomplished by the incorporation of glucose oxidase (GOx) in the system, which oxidizes glucose into gluconic acid, thus transiently decreasing the surrounding pH (-5).

EXPERIMENTAL METHODS

pH-sensitive NPs based on hybrid PLGA/ polymethacrylates matrices were produced by double emulsion technique to encapsulate GLP-1 analogue exenatide (EXN) or semaglutide (SMG). NPs were characterized regarding average size, size distribution and zeta potential by DLS. Association efficiency (AE) and drug loading (DL) were determined by HPLC. EXN and SMG *in vitro* release profiles were assessed at different pH (5.0 and 7.4), while secondary structure stability after release was assessed by circular dichroism. WLS-4D1 hiPSCs were differentiated into β- and α-cells[4,5] and the differentiation efficiency was assessed at different stages by flow cytometry through the expression of key cell markers. Differentiated β-cells functionality was assessed *in vitro* by glucose-stimulated insulin secretion (GSIS) assay. Undifferentiated hiPSCs were immobilized in alginate hydrogel and cell viability was evaluated by resazurin reduction assay.

RESULTS AND DISCUSSION

Different NP formulations showed a monodisperse population (PdI <0.170) with an average size ranging from 130 to 155 nm and zeta potential (ZP) around +25-40 mV for higher polymethacrylates ratios, decreasing up to -7 mV for higher PLGA proportions. EXN and SMG were encapsulated using different DL (between 5% and 15%). For EXN-loaded NPs, AE values ranged from 34% to 49%, while SMG-loaded NPs AE ranged from 50% to 65%, depending on polymers ratios. NPs showed a pH-dependent *in vitro* release profile, despite showing a burst release of around 40% at pH 7.4 and 60-70% at pH 5 after 5 minutes, depending on the peptide. Both EXN and SMG maintained their secondary structure after at least 48h of *in vitro* release, when compared to native structure. Regarding hiPSCs differentiation into β-cells, insulin-positive cells (INS^+) were successfully obtained (99%), despite showing low glucose-responsiveness in the GSIS assay, due to <1% co-expression of NKX6.1 and insulin. Concerning α-cells differentiation, monohormonal glucagon-positive cells (GLU^+) were obtained, as well as a population of polyhormonal GLU^+,INS^+ cells (3.8%). Undifferentiated hiPSCs remained viable 3 days after immobilization in alginate, thus supporting the suitability of the protocol developed for cell immobilization.

CONCLUSION

GLP-1 analog-loaded glucose-responsive NPs were successfully produced and encapsulated peptides retained their secondary structure. Glucagon- and insulin-producing differentiated cells were obtained, even though the last were not fully responsive to changes in glucose levels. Furthermore, the protocol developed for hiPSCs immobilization in alginate can be easily applied to the immobilization of differentiated cells. Currently, an *in vivo* study is being performed to assess the efficacy of the biofunctional system after transplantation in a diabetic mouse model.

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Firefly-Bioinspired Hydrogels with Redox-responsiveness as Cell-encapsulating Injectable Matrices

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INTRODUCTION

Cell-encapsulating hydrogels are biomaterials used as extracellular matrix mimics for basic study of cell function, high-throughput drug screening, and delivery of therapeutics. To facilitate their adaptability for diverse applications, there is a need for hydrogel crosslinking strategies that are smart, flexible, and user-friendly. Recently, firefly-bioinspired hydrogels for 3D cell culture based on the coupling reaction between cyano-benzothiazole (CBT) and cysteine (Cys) groups were reported[1]. These hydrogels showed good cyto-compatibility and high mechanical and biochemical tunability. However, this initial molecular design showed some limitations that complicated its formulation as injectable matrices. This is relevant to expand the use of these hydrogels into therapeutic delivery scenarios. Thus, the aim of this work is to develop firefly-inspired hydrogels further as injectable and stimuli-responsive materials. It is envisioned that the incorporation of redox-triggering property to the design of poly(ethylene glycol) (PEG) hydrogel networks is the key to solve the mentioned limitations (Fig. 1a). When macromers denoted as PEG-Cys(SR), bearing protected Cys groups, are mixed with PEG-CBT macromers; no gel formation occurs because the protecting group at the Cys blocks its reactivity. Upon the addition of a biocompatible reductant, the protecting group is cleaved, exposing a Cys group, and the crosslinking reaction is triggered on demand. In this work, the relevant molecular and environmental parameters, necessary to trigger the onset of gel formation and to achieve biomaterials with tailorable properties, are investigated.

EXPERIMENTAL METHODS

4-arm, 10 and 20 kDa PEG-CBT[1] and PEG-Cys(SR) (R=Et, tBu)[2] macromers were synthesized. Tris(2-carboxyethyl)-phosphine (TCEP), dithiothreitol (DTT) and glutathione (GSH) were used as biocompatible reactants. PEG (3-10 wt%) and reductant solutions (12-40 mM) were prepared in HEPES buffer, pH 7-8 at mild temperature (25-37ºC); and used for hydrogel preparation. The effect of molecular parameters (structure of the Cys protecting group, reductant type) and environmental parameters (pH and temperature), over gelation kinetics and final mechanical strength of the hydrogels was investigated by shear rheology, before and after swelling. Gel cytocompatibility was studied by encapsulating human mesenchymal stem cells (hMSCs) for 1 day and analysing cell viability via live/dead assay.

RESULTS AND DISCUSSION

Redox-triggerable hydrogels were prepared under physiological conditions, demonstrating controlled onset of the crosslinking reaction and efficient gelation kinetics. Rheological results revealed tunable gelation times, spanning from ca 15 s to ca 15 min, which is convenient for injectability purposes. Gelation rate depended on the choice of molecular and environmental parameters of the system. At the molecular level (Fig. 1b), a smaller protecting group at the Cys site and a stronger reductant, resulted in faster-curing gels. Environmental parameters such as higher pH and higher temperature, also lead to faster-curing materials. Gel mechanics was not affected upon variation of protecting group, reductant, or pH; whereas it was impacted by temperature. Moreover, hydrogels showed good cyto-compatibility, evidenced by high cell viability of encapsulated hMSCs (Fig. 1c). Finally, preliminary experiments, where a mixture of both PEG precursor solutions was injected into a reductant-containing bath enabled high control of the gelation onset, indicating that this platform is promising as injectable and extrusion-based bioprinting formulations.

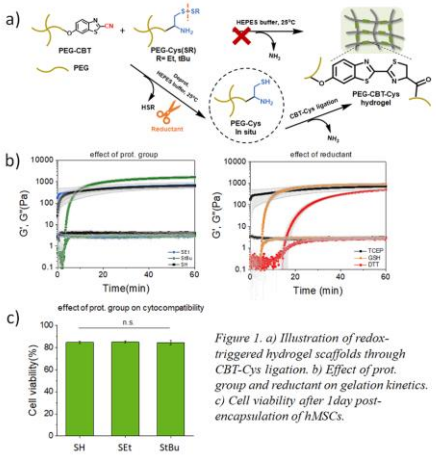

Figure 1. a) Illustration of redox-triggered hydrogel scaffolds through CBT-Cys ligation. b) Effect of prot. group and reductant on gelation kinetics. c) Cell viability after 1day post-encapsulation of hMSCs.

Firefly-bioinspired hydrogels with redox-triggerable properties were developed, thus expanding their application versatility as injectable biomaterials. The molecular and environmental parameters to control materials properties were elucidated. These matrices are expected to become valuable for tissue engineering.

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ACKNOWLEDGMENTS

The authors thank DFG's support (proj. no. 422041745).

>> 10:30 - 12:30 | SYMP-03 - Contactless bioassembly processes: new avenues in the biofabrication world

Chairpersons: Hugo Oliveira & Catherine Le Visage
Location: Room E

10:30 | KL Contactless - Contactless Bioassembly Processes For Controlling Tissue Organization
Tiziano SERRA, AO Research Institute, Davos Platz, Switzerland

11:00 | O1 Contactless - Novel Electro-Assisted Printing of Soft Hydrogels by Electrochemistry
Arua DA SILVA, Implantable Bioelectronics Laboratory. Department of Automatic Control and Systems Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

11:15 | O2 Contactless – Additive Manufacturing for the Development of Microneedle Arrays as Minimally Invasive Drug Delivery Systems
Nikoletta SARGIOTI, 1 School of Mechanical and Manufacturing Engineering, Dublin City University, Ireland. 2 School of Mechanical Materials Engineering, University College Dublin, Ireland

11:30 | O3 Contactless - Magnetic and Matrix-assisted 3D Bioprinting for Biomimetic Tendon Tissue Model
Syeda Mahwish BAKHT, 13B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Braga/Guimarães, Portugal

11:45 | O4 Contactless - A micro physiological system recapitulating inflammation in a microtissue
Martin FRAUENLOB, Institut Pasteur, Biomaterials and Microfluidics core facility, C2RT, Paris, France & Bioassays, Microsystems and Optical Engineering Unit, BIOASTER, Paris, France

12:00 | FP01 Contactless - In vitro and in vivo characterization of a novel tricalcium silicate-based ink for bone regeneration using Laser-Assisted Bioprinting
Nicolas TOUYA, INSERM U1026 BIOTIS

12:05 | FP02 Contactless - Physical and biological behaviour of flowable fiber reinforced composite compared to alternative bulk filling composites
Nina ATTIK, Université de Lyon — Université Claude Bernard Lyon 1, UMR CNRS 5615, Laboratoire des Multimatériaux et Interfaces, Lyon, France; Universitéde Lyon, Université Claude Bernard Lyon 1, Faculté d'Odontologie, Lyon, France

12:10 | FP03 Contactless - Hybrid HMSCs-Microcomposite Building Blocks for Bottom-Up Engineering of Bone Tissue
K. SONG, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

Contactless Bioassembly Processes For Controlling Tissue Organization

Tiziano Serra

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Morphogenesis, a complex process, ubiquitous in developmental biology, tissue engineering, and many pathologies, is based on self-patterning of ensembles of cells.

Such ensembles are gently orchestrated and perfectly arranged through chemical gradients, structural anisotropies, and hierarchical compositions.

Conventional tissue engineering approaches involve the development of physiologically relevant living microenvironments, by combining biomaterials, cells, and biochemical factors to direct the generation of functional tissues.

However, over the last few years, the application of extrinsic fields is opening exciting new perspectives to better control and reproduce the structural complexity of tissue organization toward the in vitro engineering of physiologically relevant constructs.

Within this recent trend, acoustic, magnetic, electric, hydrodynamic fluids, and optical fields have shown time-effective, gentle, and contactless strategies to organize cells, materials, and biochemical factors toward morphogenesis and morphologically relevant tissue generation.

First, I will introduce our research activities on the use of extrinsic fields (light, magnetic, electric) for contactless cell assembly and stimulation.

Then, I will present Sound Induced Morphogenesis (SIM) approach for patterning and locally enhancing cell density for vasculogenesis.

Finally, I will give an overview of our team activities, aiming to explore SIM as a holistic approach, from a size and time perspective, to control tissue organization toward clinical functionality.

Novel Electro-Assisted Printing of Soft Hydrogels by Electrochemistry

Aruã Clayton Da Silva[1], Junzhi Wang[1], Teuku Fawzul Akbar[2,3], Christoph Tondera[1,2,3]and Ivan R. Minev[1,2]

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INTRODUCTION

Soft hydrogels are an important class of materials applied for interfacing soft tissues due to their similar properties[1]. They can be modified promoting different functionalities, such as electronic/ionic conductivities, specific cell recognition (e.g. RDG peptides), etc.
Electrochemical reactions can be used to control very precisely the generation of intermediate species or promote protons generation/depletion over electrode interfaces by using water electrolysis. The intermediate specie or changes at the electrodes interface can trigger the crosslinking of different polymers obtaining a soft hydrogel[2].
Here we present a novel method of printing soft hydrogels by using electrochemical reactions. For this, we used chitosan, alginate and PEG-heparin matrices. Additionally, we demonstrated a way to control gelation either by ionically or covalently crosslinking systems.

EXPERIMENTAL METHODS

Herein we modified a 3D-printer nozzle with a wire of Ag/AgCl pseudoreference. The modification enables to use it as reference electrode (RE), the stainless steel of the needle as counter electrode (CE) and a conductive substrate as working electrode (WE), here we applied gold electrode and ITO-covered PET flexible substrates. The electrodes were connected to a portable potentiostat and the modified nozzle connected to the 3D-printer in the adapted setup (**Figure 1**).

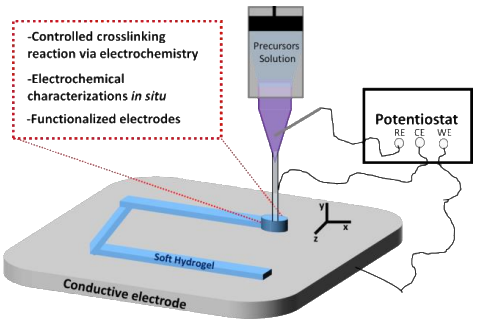

Figure 1. Schematic representation of the modified system coupling the portable potentiostat to 3D-printer.

RESULTS AND DISCUSSION

We investigated 3 different crosslinking reactions. All of them follows an electrochemical-chemical-chemical (ECC) mechanism. Chitosan hydrogel was covalently crosslinked by applying +1.8 V (*vs.* Ag/AgCl pseudoreference) over gold electrode (**Fig. 2a**). Additionally, another route of obtaining a precipitated chitosan gel was found by applying -2.0 V (*vs.* Ag/AgCl pseudoreference). Alginate hydrogel was ionically crosslinked by applying +3-5 V (*vs.* Ag/AgCl pseudoreference) using any conductive substrate (**Fig. 2b**). In addition, a hybrid hydrogel was investigated using PEDOT/Alginate showing that the PEDOT decreased the resistance and increased the capacitance of the hybrid material. Lastly, PEG-Heparin hydrogel was covalently crosslinked by applying -1.5 - -3.0 V (*vs.* Ag/AgCl pseudoreference) over any conductive substrate (**Fig. 2c**). All the proposed hydrogels were patterned in different shapes using the 3D-printer.

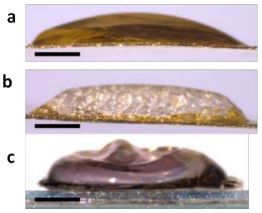

Figure 2. a Chitosan covalently crosslinked hydrogel over gold surface. **b** Alginate ionically crosslinked hydrogel over gold surface. **c** PEG-Heparin covalently crosslinked hydrogel over ITO-covered PET surface. Scale bar is 1 mm.

CONCLUSION

In the current work we described a novel method of obtaining soft hydrogels by using controlled electrochemical reactions to trigger the crosslinking of hydrogels.

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ACKNOWLEDGMENTS

The authors would like to thank funding from ERC Starting Grant: IntegraBrain (804005).

Additive Manufacturing for the Development of Microneedle Arrays as Minimally Invasive Drug Delivery Systems

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INTRODUCTION

Current drug delivery systems (e.g. topical creams, transdermal patches etc.) have associated limitations including pain and poor administration of small and large drug molecules [1]. A painless micro-sized device capable of achieving easy and efficient drug delivery, with no side effects is required [2]. Additive manufactured microneedles (MNs) have been considered as an optimal and cost-effective innovative platform. This research aims to develop a 3D–printed stainless steel (316L) MN array with optimal mechanical/biological properties for disease management and treatment. Initially, a parametric study was conducted using a Design of Experiment (DoE) approach to optimise and validate the printing parameters followed by experimental investigations of the functional properties of MN arrays (e.g., penetration and drug elution studies).

EXPERIMENTAL METHODS

SolidWorks (DS SolidWorks Corp., USA) software was used for the design of conical-shaped 6 x 6 MN arrays (height: 1,000 µm and base diameter: 250 µm) on a square patch (10 x 10 mm). A DoE (Design Expert 11, Stat-Ease, Inc., USA) study, varying the laser speed (500–700 mm/s), power (35–70 W), and trace width (0.09–0.12 mm), was conducted to determine and optimise the influence of printing parameters. The MN arrays were printed and subsequently electropolished [3]. Direct Metal Laser Melting (Concept Laser Mlab, GE Additive, Germany) process was used as per the DoE matrix. The geometric features (needle height, aspect ratio, and tip diameter) and mechanical properties were determined and compared (ANOVA analysis). The optimal 3D printed MNs were characterised in terms of their morphology and geometry (SEM and optical microscopy). To determine the penetration depth and force – testing was conducted using parafilm, synthetic (SynTissue) and porcine skin models at a displacement rate of 0.5 mm/min to max force of 20 N [4]. The ability of the MN arrays to absorb, store and release a drug was then determined using rhodamine B (RhoB).

RESULTS AND DISCUSSION

SEM analysis showed reductions in the needle height (30%) and base diameter (20%) for the final MN following e-polishing when compared to the initial CAD file (Fig.1a). SEM analysis indicated the presence of un-melted particles, which decreased in number after e-polishing (Fig.1b). DoE analysis and optimisation indicated that a laser power (65 W) and speed (650 mm/s) led to optimal geometrical and mechanical properties without significantly effecting the trace width (p-value: 0.139). T-test analysis was conducted to compare the predicted and experimental values (Fig. 1c), verifying the accuracy of DoE models. Geometrical features met the requirements for a painless micro-sized device. In particular, the electropolished MNs achieved a 0.4 mm insertion after a force of 7 N, leading to a successful penetration without tip failure (Fig. 1d). Dry forms of rhodamine B were successfully eluted from the MN array into porcine ear skin.

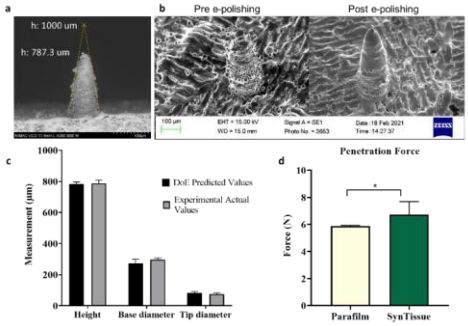

Figure 1: SEM image of (a) MN height and (b) unmelted particles pre- and post e-polishing, (c) comparison of predicted (DoE) and actual values and (d) max penetration force, * $p \leq 0.05$.

CONCLUSION

Development of optimised 3D printed MN arrays, with a height of 1,000 µm and aspect ratio of 4:1, that demonstrated successful penetration and drug elution was achieved. The printing parameters that achieved the optimal geometrical, mechanical and functional properties of the MN arrays were a laser power of 65 W, speed of 650 mm/s and trace width 0.11 mm. Future work will focus on developing the required post-processing steps to fabricate MN arrays that demonstrate the required biological properties to be an effective transdermal drug delivery system.

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ACKNOWLEDGMENTS

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Magnetic and Matrix-assisted 3D Bioprinting for Biomimetic Tendon Tissue Model

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INTRODUCTION

Tendon tissue comprise of well-defined aligned fiber bundles arranged in a hierarchical structure from the nano to the macro scale, which are responsible for its biomechanical performance and biological organization. For invitro tendon modeling the recreation of the hierarchical and anisotropically organized fibrous nature of tendon extracellular matrix (ECM) could provide tendon biomimetic cues induced via cell contact guidance resulting in proved impact over their alignment, differentiation, phenotype maintenance as well as matrix deposition. We hereby propose a concept of magnetically and matrix-assisted 3D bioprinting to fabricate high-resolution constructs with magnetic bioinks that remain liquid for long enough before gelation to allow the orientation of magnetic elements in situ (Figure 1. A), thus building 3D fibrillar patterns resembling the microstructure of tendon tissues. In addition, magnetic nanoparticles (MNPs) remote response enables their use as magnetomechanical actuators to control cellular/tissue behavior.

EXPERIMENTAL METHODS

Highly magnetized monodisperse iron oxide based MNPs were synthesized through thermal decomposition. By addition of MNPs incorporated in the precursor solutions, Magnetically-responsive polycaprolactone (PCL) short fibers (MRFs@PCL) were produced by electrospinning and the subsequent cryo-sectioning of the obtained meshes. Magnetically-responsive bioinks were prepared by mixing the MRFs@PCL with gelatin solutions and human adipose-derived stem cells (hASCs). The 3D extrusion bioprinting steps were performed under the presence of fairly uniform external magnetostatic fields produced by a two parallel magnets setup. Agarose and cellulose nanocrystals (CNCs)-based fluid gels (supplemented with transglutaminase for gelatin crosslinking) were tested as support baths.

RESULTS AND DISCUSSION

Zinc-doping demonstrated to be the most efficient approach to increase the magnetic power of superparamagnetic iron oxide-based MNPs. Zn-Fe_3O_4 MNPs were used to prepare MRFs@PCL with 20-100 µm of length. Weak magnetic fields align MRFs@PCL with 20-60 µm in length when the relative amount of MNPs is >%5 (Figure 1. B-D). The bioink formulation consisting of MRFs@PCL along with human adipose stem cells encapsulated in in gelatin solution enabled the fabrication of high-resolution 3D-printed constructs when using CNCs (Figure 1. E) as suspension baths, but not when with the respective granular agarose gels (Figure 1. F). Due to the the high magnetic power of the designed MNPs, comparative to the available magnetic nanoparticle-based systems extremely low particle´s concentrations and magnetic field strength were enough to induce the alignment of magnetic fibers during the layer-by-layer extrusion printing steps. The anisotropic microstructure of the biomimetic constructs induced elongated growth and phenotypic commitment of the encapsulated cell similar to tendons tissues.

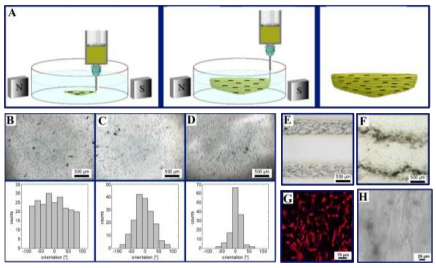

Figure 1. (A) Schematic illustration of magnetically and matrix assisted 3D bioprinting, Alignment of MRFs@PCL under (B) no magnetic field, (C) 8 mT,(D) 20 mT, resolution of 3D-printed constructs (E) agarose and (F) CNCs as suspension baths, (G,F) orientation of cytoskeleton day 7 of cell culture

CONCLUSION

Overall, the results establishes that our strategy allows fabrication of biomimetic magnetic constructs replicating the native tendons ECM. We demonstrated that our design strategy of MNPs allows synthesis of extremely magnetic particle allowing alignment of fibers with lower particle content and weaker magnatic field, hence minimizing the the toxic/safety risks associated with these factors. The CNC support bath is a key element to ensure in-situ magnetic alignment. The resulting anisotropic 3D fibrillar microstructure of the printed constructs revealed effective on directing encapsulated cell fate. The effects of remote magnetomechanical actuation on cellular constructs is currently under investigation.

ACKNOWLEDGMENTS

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A micro physiological system recapitulating inflammation in a microtissue

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INTRODUCTION

Recapitulating physiologically relevant tissue analogues calls for the development of systems capable of responding to inflammatory triggers that initiate a robust immune response. During inflammation, cytokines such as tumor necrosis factor and interleukins are released and activate remote lymphoid cells. In parallel, this cytokine release also affects cells from other surrounding tissues thus; the interface between circulating immune cells, endothelialized channel and tissue cells is of great interest to investigate inflammatory responses1.

The culture of circulating immune cells with conventional in-vitro methods is today very challenging and cells are apoptotic within hours[2]. Furthermore, static culture systems such as transwell studies, do not recreate the physiological environment sufficiently. The recent development of micro physiological systems (MPS) represents an alternative to conventional methods to overcome this bottleneck of immune cell maintenance and co-culture[3].

EXPERIMENTAL METHODS

This study aims at developing a novel MPS model that recapitulates the tissue-circulation flow interface with a prolonged PBMC survival. More specifically, we want to study the link between local microtissue inflammation and the subsequent PBMC activation. There, we developed a PDMS-glass microfluidic device that spatially organizes a co-culture consisting of primary human and endothelial cells mimicking the interface between blood flow and a microtissue (Fig.1A). An injectable biodegradable, a natural hydrogel, is introduced in-between both circulation and microtissue compartments to maintain tissue integrity. To simulate in vivo-like conditions, shear stress can be applied to circulating PBMCs in a physiological range up to 40 dyn/cm2.

RESULTS AND DISCUSSION

The evaluation of the microdevice demonstrates the production and maintenance of the differentiated tissue cells and a functional endothelial barrier (Fig.1B). A diffusion study showed the permeability of the hydrogel for FITC-Dextran traveling from the tissue to the circulation compartment, establishing a molecular gradient expected for growth factors of similar molecular weights (Fig.1C). Finally, the comparison of immune cell maintenance in the MPS versus in-vitro culture will reveal the practicality of this device to study tissue inflammation.

CONCLUSION

This study reports on the fabrication and characterization of a micro physiological system for the maintenance of human cells to study the inflammation of microtissues.

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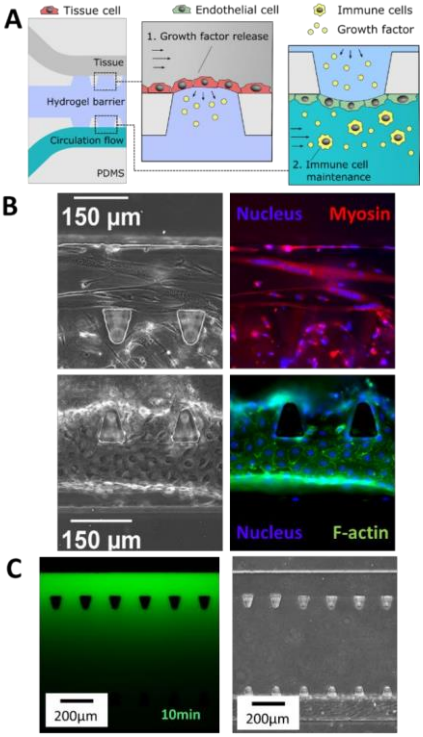

Figure 1: (A) Schematic drawing of the on-chip tissue and circulation compartments. (B) Images of on-chip tissue cell culture in bright field (upper left), stained for myosin and nuclei (upper right) and endothelial barrier in bright field (lower left), stained for F-actin and nuclei (lower right). (C) The diffusion of FITC Dextran after 10 min on-chip with endothelial barrier in the lower channel.

Physical and biological behaviour of flowable fiber reinforced composite compared to alternative bulk filling composites

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[3] Assistance Publique-Hôpitaux de Paris, Hôpital Rothschild, Service d'Odontologie, Université de Paris, Faculté dentaire, Paris, France
[4] Université de Lyon — Université Claude Bernard Lyon 1, CNRS, INSA Lyon, MatéIS, France
[5] Hospices Civils de Lyon, Service d'Odontologie, Lyon, France

INTRODUCTION

Many developments have been undertaken to overcome dental composite shortcomings, most notably the development of bulk-fill composites that can be inserted and polymerized in large cavities. They are considered to have higher physical and mechanical properties allowing them to endure high stresses due the masticatory forces. Fiber reinforcement of dental composite is another improvement that was introduced to enhance dental composite restorations toughness and fracture resistance[1]. On the other hand, potential toxicity and polymerization shrinkage stress of dental composites continue to shade some concern on their use. Clinically, bulk composites are mainly indicated for deep and large cavities, highlighting the importance for surveying their biological behaviour. The same holds true regarding the polymerization shrinkage stress. It is known that a higher configuration factor (C-factor) is generally associated with a higher polymerization stress[2]. Hence, stress occurrence during polymerization is an important parameter to investigate. The aim of this study was to assess the mechanical and biological behaviour of a flowable bulk-fill composite with fibers compared to bulk filling composites.

MATERIALS AND METHODS

EverX Flow™ (EXF, GC Corporation), Filtek™ Bulk Fill Posterior Restorative (FBF, 3 M) - one conventional bulk-fill composite, and SDR® flow+ (SDR, Dentsply) - one flowable bulk composite without fibers, were characterized. Composite samples light-cured with a LED device were evaluated in terms of flexural strength, flexural modulus (ISO 4049), fracture toughness (ISO 20795-1), and Vickers hardness. Polymerization stress was evaluated using a test setup that was intended to magnify the stress produced in an enclosed cavity. While, polymerization volumetric shrinkage s was investigated by Archimedes' principle according to ISO 17304:2013. *In vitro* biological assessment was achieved using primary human gingival fibroblast cells (HGF). Alamar Blue assay at 1, 3, and 5 days of contact to the 3 tested composite extracts was used to assess the metabolic activity (ISO 10993) and confocal imaging to evaluate cell morphology. Data were submitted to One-Way analysis of variance (ANOVA) and independent *t*-test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

FBF showed statistically higher flexural modulus and Vickers hardness than EXF and SDR. However, EXF showed statistically higher K_{IC} than FBF and SDR. EXF had the statistically highest shrinkage stress values and FBF the lowest. Archimedes volumetric shrinkage showed significantly lower values for FBF as compared to the other two composites. At day 1, slight cytotoxic effect was observed for the three composites. At day 5, an enhancement of metabolic activity was observed in cells treated with EXF extracts. The higher fracture toughness results of fiber reinforced composite, means that it has a lower propensity to undergo crack initiation during loading. Which could predict the longevity of the restoration in the oral cavity[3]. The lower stress developed during SDR polymerization compared to EXF may be associated with its lower modulus. Generally, composites containing fibers have been shown to undergo higher stresses during polymerization than conventional composites[4]. It has been assumed that this effect of fibers is due to a modification of polymerization kinetics of the polymeric matrix[4]. The results obtained in the current study shows, that the tested fiber reinforced composite EXF had less deleterious effect than FBF and SDR on primary gingival cells viability mostly at day 3. This trend remains at day 5 with an enhancement of the metabolic activity in the presence of EXF (Figure 1).

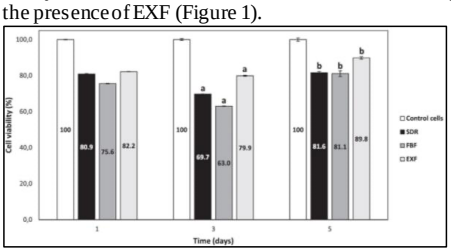

Figure 1: Metabolic activity of HGF subjected to the composite extracts after 1, 3 and 5 days. Lower case letters identify groups with statistical differences ($p < 0.05$): a compared with control cells and b compared with control cells in comparison with day 3.

Despite the limitations of the current *in vitro* study, the HGF metabolic activity enhancement at 5 days indicated a less cytotoxic effect. This result could suggest EXF use in large and deep cavities in contact with pulp tissue. Moreover, EXF had a significantly higher fracture toughness validating its potential use as a restorative material in stress bearing areas.

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ACKNOWLEDGMENTS

The dental companies GC, 3 M and Dentsply are gratefully acknowledged for the generous donation of the tested dental composites. The SEM and CLSM studies were supported respectively by the Centre Technologique des Microstructures (CTμ) and Centre d'Imagerie Quantitative Lyon-Est (CIQLE).

Hybrid HMSCs-Microcomposite Building Blocks for Bottom-Up Engineering of Bone Tissue

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INTRODUCTION

Cell-rich and cell-laden hydrogel-based three-dimensional (3D) assemblies, largely employed in bottom-up tissue engineering (TE) strategies [1-2], may not provide the stiffness and osteogenic-inducing properties required for load-bearing bone TE applications. Therefore, we suggest the use of bioinspired organic-inorganic composite microparticles stiffer than hydrogels as matrix-mimicking building blocks to produce bottom-up modular assemblies for bone TE. To that end, we fabricated a series of poly(lactic acid) (PLA) and nano-hydroxyapatite (HA) microcomposites and used them to form 3D self-assembled hybrid cell-microparticle bone-like spheroids.

EXPERIMENTAL METHODS

A polydimethylsiloxane (PDMS) intermediate mold containing squared protrusions (100 µm*100 µm* 30 µm) was prepared via standard soft lithography and inversely replicated onto a photocurable bifunctional perfluoropolyether-urethane methacrylate, forming non-wettable micromolds. PLA-HA suspensions with PLA/HA ratios of 70/10, 50/30, 30/50 and 10/70 w/w were cast onto the micromolds and later peeled off with a sacrificial poly(vinyl alcohol) film, which was further dissolved in deionized water, releasing free-standing microcomposites. PLA microparticles were prepared as a control. The microcomposites were characterized with Fourier transform infrared spectroscopy (FTIR), Scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDS) and laser scanning confocal profilometry. Microcomposites and human mesenchymal stem cells (HMSCs) were co-seeded onto in-house-made low-adherent polycarbonate film-based thermoformed microwells. We recorded the diameter and viability of the hybrid cell-microcomposites with brightfield microscopy and PrestoBlue™ cell viability assay at varying time points. Dead cells, cell nuclei and F-actin cytoskeletal fibers in the spheroids were labelled with LIVE/DEAD™ Fixable Dead Cell Stain, DAPI and Alexa Fluor 647 (phalloidine), respectively, and visualized with a confocal fluorescence microscope (CFM).

RESULTS AND DISCUSSION

Solid microcomposites with good shape fidelity were fabricated (Figure 1A). Increased HA content generally led to microcomposites with higher surface roughness and reduced meniscus profile on the top surface, often seen after solvent evaporation[3] (Figure 1A-C). Microcomposites successfully participated in cell-guided assembly, forming hybrid spheroids, with highest cell viability detected in PLA/HA 10/70 after 5 days. Moreover, microcomposites led to the formation of larger spheroids with irregular shapes, as opposed to spherical and smaller cell-rich spheroids (Figure 2).

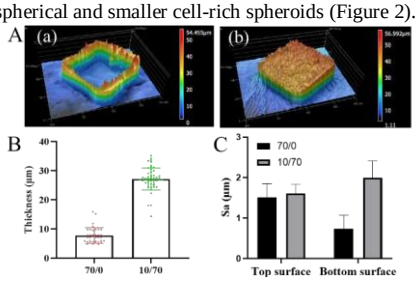

Figure 1. A) Surface profiles of (a) PLA and (b) PLA/HA 10/70 microparticles, their B) average height and C) surface roughness of the top and the bottom surfaces.

CONCLUSION

We developed a series of microcomposites with varying organic/inorganic phase ratios, pre-defined outer shapes and isotropic surface profiles and roughness, which were dependant on the PLA/HA ratio. Preliminary experiments on HMSC-microcomposite spheroid formation were successful and next, we will investigate the osteogenic properties of the hybrid spheroids.

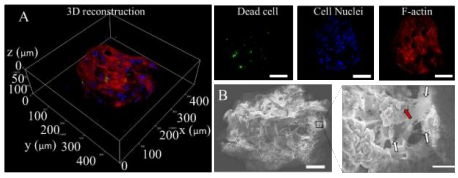

Figure 2. Morphology of Hybrid HMSCs-PLA/HA 10/70 assembly in osteogenic medium for 10 days visualized by (A) 3D CFM (scale bars: 100 µm) and B) SEM (scale bars: 50 µm (left) and 10 µm (right), red arrow: mineral deposit, white arrows: cells).

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ACKNOWLEDGMENTS

This research was supported by the China Scholarship Council (CSC) from the Ministry of Education of P.R. China, the European Union Interreg Vlaanderen-Nederland project "BIOMAT on microfluidic chip), the Dutch Province of Limburg (program "Limburg INvesteert in haar Kenniseconomie/ LINK"), the NWO Gravitation Program (project "Materials-Driven Regeneration") and the NWO Incentive Grant for Women in STEM (Project "Biotetris").

>> 10:30 - 11:30 | Tech S1 - Bioengineering immunomodulatory biomaterials

Chairpersons: Jacek Wychowaniec & David Eglin
Location: Room F

10:30 | KL Immunomodulatory - Medical device personalisation via multifunctional, immunomodulatory biomaterials; decreasing the complications and improving the healing
NIHAL ENGIN VRANA, SPARTHA MEDICAL, Strasbourg, France

10:45 | O1 Immunomodulatory - Introducing Controlled Topography onto 3D Scaffolds via Digital Light Processing to Modulate the Immune Response
Sandra CAMARERO-ESPINOSA, POLYMAT, University of the Basque Country UPV/EHU, Donostia / San Sebastián, Gipuzkoa, Spain; IKERBASQU
E, Basque Foundation for Science, Bilbao, Spain

11:00 | O2 Immunomodulatory - Nanoscale spatio-mechanical regulation of the immune signaling in cytotoxic lymphocytes
Mark SCHVARTZMAN, Ben-Gurion University of the Negev,Beer-Sheba, Israel

11:15 | O3 Immunomodulatory - Bridging the Gap Between the Immune Response and Mineralization During Fracture Healing
William Arthur LACKINGTON, Biointerfaces Lab, Empa, St. Gallen, Switzerland

MEDICAL DEVICE PERSONALISATION VIA MULTIFUNCTIONAL, IMMUNOMODULATORY BIOMATERIALS; DECREASING THE COMPLICATIONS AND IMPROVING THE HEALING

Nihal Engin Vrana[1]

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evrana@sparthamedical.eu

INTRODUCTION

Medical device related complications, mainly infections and adverse immune reactions, are a common cause of revision surgeries and medical device dysfunction. Although, the established biomaterials used in implants are biocompatible, the individual reaction to a given biomaterial can vary considerably and currently there are no methods for successfully predict such effects. In this sense, personalization of the medical devices with respect to the regenerative and immune profiles of the patients can significantly improve clinical outcomes and alleviate medical device related complications.

One way of achieving such personalization is the personalization of the tissue/ implanted medical device interface using coatings. The coating properties can be synchronized with the potential reaction of the patient to the bulk material and antimicrobial, anti-inflammatory and pro-regenerative cues can be directly incorporated into the coatings for better control over host/implant interface.

In this talk, the design criteria of such coatings and development of multifunctional coatings via biopolymer based supramolecular assemblies or thin hydrogels will be described. Examples of such coatings will be covered such as those i) which can attenuate innate immune reaction to 3D printed implants via release of a macrophage phenotype fixing cytokine cocktails in a controlled manner *in vivo*[1], ii) supramolecular assemblies of polycationic polypeptides and polyanionic polysaccharides which can be applied on complex 3D medical devices and can prevent up to 8 consecutive bacterial infections[2], iii) coatings that can delivery anti-inflammatory miRNAs and induce accelerated wound healing by first polarizing macrophages to more M2-like phenotype and subsequently inducing migration by fibroblasts while keeping antimicrobial capacities[3].

One of the roadblocks in the development of multifunctional coatings, particularly with those with multiple components, is the time-consuming and costly empirical nature of the development process. Recently, we have started to harness machine learning methodologies for predictive models of the coating component/functionality using historical literature data with complementary generation of new data to ensure high fidelity models. Such models can be based on commonly used parameters (in the first iteration we have used 23 common parameters) or can use inherent properties of the coating components using molecular descriptors. In our first attempt, a model that can predict coating thickness with respect to components has been developed, together with insights on the relative weight of different parameters on the coating properties[4].

CONCLUSION

The next generation of medical devices should take into consideration the immune profile and regenerative capacity of the patients to tailor personalized implants. Nano-/microscale coatings are versatile tools to achieve this personalization without changing the bulk properties of the medical devices. Such personalization can be achieved by advanced immune profiling tools such as antigenic epitope repertoires as demonstrated recently in the case of oral biofilms[5].

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ACKNOWLEDGMENTS

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Introducing Controlled Topography onto 3D Scaffolds via Digital Light Processing to Modulate the Immune Response

Leire Iturriaga Oñarte-Echeverria[1], Garazi Larrañaga-Jaurrieta[1,2], Sandra Ramos[1], Sandra Camarero-Espinosa[1,3*]

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INTRODUCTION

Introduction of topographical features onto 2D substrates has proven an ideal approach to control different cell processes such as proliferation, differentiation and polarization, appearing as a promising tool to modulate the innate host immune response. Despite the great potential shown on 2D substrates, extrapolation to 3D scaffolds remains a challenge and the differential results expected on curved 3D structures are unknown.[1] We have recently shown that introduction of topography or roughness to 3D additive manufactured scaffolds allows for the modulation of the immune response in vitro and in vivo.[2] However, the resulting topographical features lack structural and spatial control.

Digital light processing (DLP) allows for the fabrication of scaffolds with high structural complexity. However, its exploitation has been limited by the need of photo-crosslinkable and biocompatible liquid inks.

Here, we explored a library of low viscosity photo-crosslinkable materials. Control over the physico-chemical characteristics of the pre-polymers, allowed us to create scaffolds with topographical features below the so far reported 150 µm.[3] Further, we showed that the introduction of topographies onto 3D curved scaffolds allows to control the innate immune response in vitro.

EXPERIMENTAL METHODS

Ink synthesis: 2- and 3-arm photo-crosslinkable homopolymers and copolymers were prepared from D,L-lactide (PDLA), Ɛ-caprolactone (PCL) and a 50:50 mixture thereof (PCL-co-PDLA) by ring opening polymerization with tri- or di-glycols as co-initators. Methacrylation was performed by functionalization with methacrylic anhydride. **DLP printing:** 2D substrates and 3D tubular scaffolds were fabricated using a Titan 2 HR (Kudo 3D) with and without reactive and non-reactive diluents. **Cell culture:** RAW 247.6 mouse macrophages were expanded in DMEM and 10% heat inactivated FBS until p3. Cells were seeded at 30.000 cell.cm^{-2}. After 24h LDH release and live/dead assays were used to test biocompatibility and DNA quantification and immunofluorescence (IF) to determine cell adhesion. For polarization experiments, cells were left adhere for 24h, polarization towards M1 (LPS, IFN-Ɣ) and M2 (IL-13, IL-4) phenotypes were used as controls and stimulated for 24h. 48h after seeding, media was collected for ELISA measurements of TGF-β, TNF-α and IL-10 and cells were stained for iNOS, CD206, DAPI and F-Actin.

RESULTS AND DISCUSSION

Synthesis of 2- and 3-arm pre-polymers of Mw below 2000 kg.mol^{-1} resulted in low viscosity printable inks of PDLA, PCL-co-PDLA and PCL. 3-arm pre-polymers cross-linked faster and resulted in materials with overall higher storage moduli as shown via photorheological measurements. All materials proved biocompatible with cell viabilities >95% and LDH releases below 5%. Cell adhesion was also higher in 3-arm based substrates. Analysis of macrophage polarization in 2D via IF showed that M0 cells were predominantely on the M2 state, with few to no cells being positive for iNOS.

We further exploited 2- and 3-arm PCL-co-PDLA copolymers to create 3D curved structures with conical topographies resulting on cones with base and tip diameters and heights as small as 270 x 45 x 43 µm, the lowest that has been reported so far for our knowledge (Figure 1). Evaluation of 2D substrates and 3D tubular structures presenting these topographies demonstrated the capability of controlling macrophage polarization and revealed the importance of cell projected surface area and formation of strong focal adhesions on cell fate.

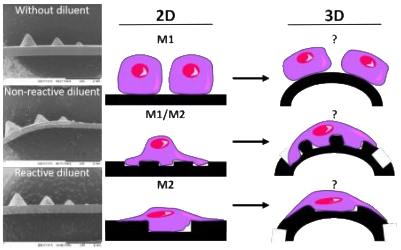

Figure 1. SEM images of conical topographies (left) and scheme depicting macrophage polarization in 2D vs 3D.

CONCLUSION

Altogether, we have developed a new biocompatible ink library that allows printing of high resolution topographical features. Introduction of controlled topographies in 3D tubular structures allowed us to study the differential effect of these in curved environments, revealing the importance of parameters such as cell surface area and formation of focal adhesions.

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ACKNOWLEDGMENTS

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Nanoscale spatio-mechanical regulation of the immune signaling in cytotoxic lymphocytes

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* marksc@bgu.ac.il

INTRODUCTION

It has been long known that cytotoxic lymphocytes – NK cells and T cells – differentiate between pathogens and healthy cells by sensing environmental chemical cues, which are delivered by the ligands expressed on the surface target cells. Yet, it is becoming progressively clear that lymphocytes also sense physical environmental cues, such as ligand arrangement, mechanical stiffness, and topography. However, the mechanism of this biophysical sensing is still mostly unexplored.

EXPERIMENTAL METHODS

Recently, we explored the role of the ligand arrangement in the immune function of NK cells using nanoengineered stimulating platforms based on patterned arrays of ligands. The first generation of such platforms was based on arrays of nanoimprinted metallic nanodots functionalized with activating ligands[1]. The next, more advanced generation of arrays came to examine how the segregation between activating and inhibitory ligands affects the inhibition of activating signaling in NK cells. The platform was based on ordered arrays of nanodots of two metals selectively functionalized with activating and inhibitory ligands, whose segregation was systematically tuned between 0 nm to 40 nm[2].

We also studied the role of environmental elasticity and topography in the function of cytotoxic lymphocytes. Here, we engineered a stimulating platform based on ligand functionalized nanowires, which were fabricated from the bottom using chemical vapor deposition, and whose surface was biochemically functionalized with activating ligands or antibodies for NK cells and T cells. In all the cases, the immune response of NK/ T cells to the ligand arrangement or surface nanotopography was assessed though nuclear translocation of Nf-kB (late signaling), degranulation marker CD107, and release of interferon gamma.

RESULTS AND DISCUSSION

The first, simplified types of ligand arras that can control only activating receptors in NK cells allowed us to discover the minimal spatial requirement of ~ 1 ligand per sq. micron needed for the activation of NK cells[1]. The arrays with controlled segregation of activating and inhibitory ligands were used to elucidate its effect of NK cell activation. Surprisingly, we found that inhibition efficiency increased with the spacing between the ligands within the probed range and rationalized this finding by physical modeling of the ligand-receptor binding kinetics[2].

In the studies of the mechano-stimulation of NK cells using nanowires, we first found that the nanowires deliver chemical, nano-topographical, and mechanical cues, whose combination produced an enhanced immune response of NK cells [3]. While patterned in microdomains, these nanowires spatially guide the cytotoxic activity of NK cells [4]. To separately reveal the effect of each cue, we recently stimulated NK cells and CD8+ T cells on nanowires with varied length and bending moduli and found that these physical parameters of nanowires greatly affect the signaling and the immune function of the lymphocytes (Fig. 1) [5],[6].

CONCLUSION

We found that the spatial distribution of activation ligands highly regulates the immune response of NK cells. Furthermore, the spatial distribution of activating and inhibitory ligands regulates the activating-inhibitory balance in these cells. Another important regulator of the activation of T cells and NK cells is their mechanical environment that comprises of the combined effect of the elasticity and topography, both span over the nanometric scale. Overall, our work provides an important insight into the way the physical cues regulate the function of NK cells and T cells

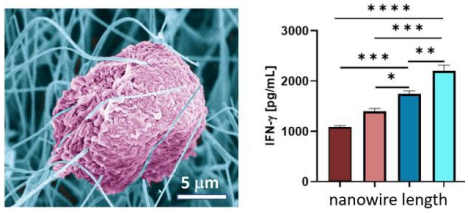

Fig. 1. *left*: false colored SEM of T cells on stimulated by nanowires, *right:* dependence of T cell activation on the nanowire length

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ACKNOWLEDGMENTS

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Bridging the Gap Between the Immune Response and Mineralization During Fracture Healing

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INTRODUCTION

Our broader understanding of the immune system's role in determining the success of intrinsic repair mechanisms has led to an increased research focus on immunomodulatory therapies[1]. These therapies could be particularly effective in abnormal fracture healing, where bone tissue engineering has thus far failed to provide safe and reliable clinical therapeutics. While current developments rely heavily on endpoint assessment of bone formation in pre-clinical studies, the effect on preceding key processes, including hematoma formation, and the immune response to fracture, are seldomly taken into account[2]. In effect, there currently exists a gap in knowledge between the immune response and mineralization during fracture healing.

The aim of this study was to engineer an *in vitro* model that facilitates studying the link between the immune response and mineralization.

EXPERIMENTAL METHODS

Collagen-hydroxyapatite (CHA) scaffolds were incubated with human whole blood, mimicking the fracture hematoma formed when the scaffold is implanted *in vivo*. The effect of blood-biomaterial interactions on the microarchitecture of the scaffold, as well as the production of key mediators (IL-1β, IL-4, IL-6, IL-8, IL-10, MCP1 and VEGF), was assessed. The response of human bone progenitor cells (HBCs) to the blood-biomaterial interactions was evaluated in terms of cell infiltration, proliferation, and mineralization in the scaffold.

RESULTS AND DISCUSSION

The interaction between blood and CHA scaffolds led to the infiltration of erythrocytes, monocytes, and platelets, and the formation of a fibrin network (*Fig. 1A*). The porous microarchitecture of the scaffold was maintained in the presence of blood, while its support for HBC infiltration was limited by the presence of blood. The scaffold stimulated a limited production of pro-inflammatory factors (IL-6 and IL-8) by blood cells (*Fig. 1B*). However, the pre-incubation of the scaffolds with blood significantly upregulated the production of IL-6 and IL-8 by HBCs by a factor of 10. The production of pro-inflammatory factors was temporally regulated, peaking between day 1 and 5, and tapered off by day 28. While blood-biomaterial interactions had no impact on the proliferation of HBCs over 28 days, blood impacted their capacity to mineralize, with a 28% reduction in calcium quantification, and a 50% reduction in intracellular alkaline phosphatase activity (*Fig. 1C*). Taken together, these data indicate that a transient hematoma-like pro-inflammatory matrix can be recapitulated *in vitro*, which can then be used to assess the effect of blood-biomaterial interactions on downstream processes of fracture healing, including mineralization. On-going experiments are assessing the capacity of blood-biomaterial interactions to alter the drug release kinetics of commonly used inducer of bone formation, while the effect of these factors on fibrin network formation and their ability to steer the immune response will be evaluated. Ultimately, the capacity of blood-biomaterial interactions to modulate the osteoinductive effects of such factaors will be determined.

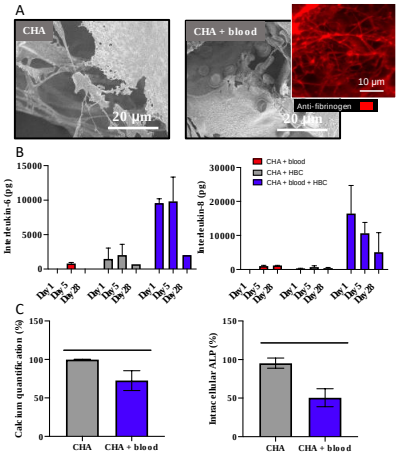

Fig. 1 The effect of blood-biomaterial interactions (A) on the microarchitecture of CHA scaffolds, (B) on the production of pro-inflammatory factors IL-6 and IL-8, and (C) on HBC mineralization.

CONCLUSION

A model mimicking the fracture haematoma could be used to delay mineralization *in vitro*. The challenge currently is to better understand the link between the immune response and mineralization, and how this response can be modulated.

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ACKNOWLEDGMENTS

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>> 11:30 - 12:30 | Tech S2 - New advances in biomaterials characterization for tissue engineering and regenerative medicine

Chairpersons: Valeria Chiono & Jochen Salber
Location: Room F

11:30 | KL1 Advances in characterization - New advances in preclinical validation tools through biofabricated human 3d tissue equivalents-on chips integrated with innovative nmr spectroscopy

Jochen SALBER, Department of Experimental Surgery/Centre for Clinical Research, Ruhr University, Bochum, Germany

11:45 | O1 Advances in characterization - Real time imaging of local O2 for quantitative analysis of O2 distribution and consumption rates during early degradation of Magnesium alloys

Ashwini Rahul AKKINENI, Centre for Translational Bone, Joint and Soft Tissue Research, University Hospital Carl Gustav Carus and Faculty of Medicine of Technische Universität Dresden, Germany

12:00 | O2 Advances in characterization - CHROMATIN COMPACTION DECREASES CELL ADHESION STRENGTH: A QUANTITATIVE APPROACH BY FLUIDIC FORCE MICROSCOPY

Julie BUISSON, Inserm UMR-1121, Laboratory of Biomaterials and Bioengineering, Centre de Recherche en Biomédecine de Strasbourg (CRBS), Strasbourg, France

12:15 | O3 Advances in characterization - How the physicochemical characteristics and protein corona modulate the activity of nanostructured lipid carriers (NLC) against Helicobacter pylori

Rute CHITAS, i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal; INEB – Instituto de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; ICBAS – Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal

New Advances in Preclinical Validation Tools through Biofabricated Human 3D Tissue Equivalents-on Chips Integrated with Innovative NMR Spectroscopy

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INTRODUCTION

Considering the EU's 3Rs initiative and the apparent growing awareness of the transferability problem of in vivo animal test results, alternative preclinical testing platforms for drugs and consumer chemicals based on human cells, spheroids and 3D tissue equivalents in combination with microfluidic systems are understandably coming increasingly into the focus of scientists, entrepreneurs, clinicians and regulators [1]. These systems, usually generalised as on chip devices, for in vitro recreation of complex human physiology could also help in the faster discovery and development of novel antimicrobial agents in the fight against the antimicrobial resistance crisis. From high-throughput analysis of compound libraries to dedicated single-candidate testing to determine toxicity, therapeutic window, pharmacokinetics and dynamics, new, especially non-destructive methods such as NMR spectroscopy with spatio-temporal analyte tracking are promising [2]. In this context, first classical investigation results on an established xenogene-free 3D human skin equivalent (HSE) and a bacterial wound infection model derived from it are compared with results of the novel NMR spectroscopy approach, which has so far only been applied on single spheroids [3,4].

EXPERIMENTAL METHODS

Preparation of HSEs: HSEs were generated stepwise from dermal and epidermal layers as described in "Fundamental in vitro 3D human skin equivalent tool development for assessing biological safety and biocompatibility – towards alternative for animal experiments" [3,4]. All NMR experiments were carried out on a Bruker Avance Neo 600 spectrometer operating at 600 MHz (14.1 T) using a cryogenic NMR probe that allows applying a maximum z-gradient of 0.5 T/m. Rectangular pulses were performed at a frequency power of 32 kHz, while power levels of selective pulses have been carefully optimized. For spatial selection, a z-gradient of 0.28 T/m was used. The dermal and bilayered HS constructs were perfused with the same medium described for 3D-CC-III [4]. Interpulse delays of echoes and Carr-Purcell-Melboom-Gill (CPMG) experiments were typically set to 1 ms [2].

RESULTS AND DISCUSSION

Replacement of collagen-I from rat tail tendon with collagen-I from human tissue donors was performed using the 3D-CC-III conditions. This was followed by a complete structural morphological and biochemical characterisation of the novel HSEs. Results from static ALI culture conditions are compared with those from a new microfluidic cultivation process. Currently, the first immunohistological CLSM, TEM and biomechanical results show differences in maturation, remodelling and stability of the dermal-epidermal constructs. Measurements of the new NMR approach for real-time characterisation of cell viability, metabolism etc. of NHDFs and NHEKs under 3D CC-III conditions are currently underway.

CONCLUSION

In this study, we successfully replaced rat tail collagen I with human collagen I. The use of human collagen already seems to have some influence on construct maturation. We currently hope that, similar to what has already been shown for spheroids, the multidisciplinary approach presented here will also allow real-time monitoring of 3D tissue equivalents on-chip. Thus, the presented method might become a valuable tool to study transport processes and metabolic adaptations to external stimuli.

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ACKNOWLEDGMENTS

HyMedPoly has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowoska-Curie grant agreement number 643050. Financial support from the Ministerium für Kultur und Wissenschaft des Landes Nordrhein-Westfalen, dem Regierenden Bürgermeister von Berlin, Senatskanzlei Wissenschaft und Forschung and the Bundesministerium für Bildung und Forschung (01KU1216I) is gratefully acknowledged.

Real time imaging of local O_2 for quantitative analysis of O_2 distribution and consumption rates during early degradation of Magnesium alloys

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INTRODUCTION

While the degradation process of magnesium (Mg) in aqueous media is well characterized, many sub processes are not fully understood. During the degradation of Mg implants, the oxygen reduction reaction (ORR) has recently been shown to play an important role in the early stages[1]. Utilizing microsensors, it was shown that O_2 consumption and degradation rate appear to be inversely related[2]. Understanding the importance of the ORR and its kinetics during Mg alloy degradation will be pivotal in predictive assessment of implant degradation behavior. A simple and modular oxygen (O_2) imaging system that employs O_2 sensor nanoparticles[3] containing O_2 sensitive platinum(II) phenyl porphyrin and an O_2 insensitive coumarin dye (both excitable at a spectral range of 400-475 nm) to image and measure O_2 consumption and distribution on the surfaces of Mg alloys when in contact with aqueous solutions.

EXPERIMENTAL METHODS

Three types of Mg alloys namely pure Mg, Mg-gadolinium (Mg-5Gd; with 5 wt.% Gd) and Mg-silver (Mg-6Ag; with 6 wt.% Ag) were used in the study. Pure Mg and the alloys were extruded and cut into discs (Ø 9 mm, height 1.5 mm). The discs were cleaned with organic solvents n-hexane, acetone and ethanol for 20 min each in ultrasound. 20 µL solution containing 5 mg/mL O_2 sensor nanoparticles in either Dulbecco's modified Eagle's medium (DMEM) or Hanks' buffered solution (HBSS) were placed on the disc surfaces and a glass coverslip (Ø 10 mm) was placed on it ensuring uniform distribution of the solution over the disc surface. This was followed by immediate commencement O_2 imaging inside a cell culture incubator (at 37 °C). Spatio-temporal changes in O_2 concentration were analyzed on images acquired every 30 s interval for 120 min. Mean O_2 consumption as a function of the distance from the disc centre, concentric rings of ten-pixel width were applied to the image stacks and the O_2 concentration within was quantified using Matlab 2020a.

RESULTS AND DISCUSSION

A very low O_2 concentration was observed immediately after adding the DMEM or HBSS solution containing O_2 sensor particles on to the disc surfaces – indicating the aggressive consumption of O_2 for ORR reaction (Fig. 1).

An overall increase in the O_2 concentration for all the samples was observed until 120 min. The rate of increase in O_2 concentration was similar for pure Mg samples in both DMEM and HBSS. In contrast, clear differences were observed for Mg alloys in DMEM and HBSS. Evolution of H_2 during the ORR had resulted in progressive development of bubbles on the surface of the discs. These H_2 bubbles apparently slowed down the ORR, as evident from the measured O_2 concentration over 120 min. The oxygen consumption in the disc centre was generally higher than towards the edges for all the samples, indicating the dependence of ORR on O_2 diffusion from the edges.

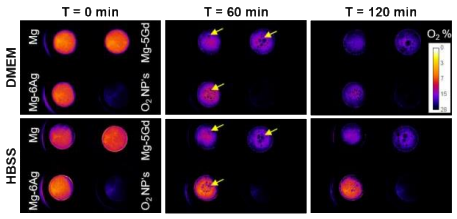

Figure 1: Images showing the progression of O_2 concentration on the disc surfaces after adding the respective solution containing O_2 sensor nanoparticles (arrows show development of bubbles).

CONCLUSION

Our work demonstrates the suitability of a modular real time imaging method using O_2 sensor nanoparticles for studying early degradation processes of Mg and its alloy. The spatio-temporal quantification of O_2 concentration shows that the degradation process was dependent on both the composition of the alloy and degradation media.

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ACKNOWLEDGMENTS

BZP acknowledges funding by the Priority Research Area KiNSIS (Kiel Nano, Surface and Interface Science).

CHROMATIN COMPACTION DECREASES CELL ADHESION STRENGTH: A QUANTITATIVE APPROACH BY FLUIDIC FORCE MICROSCOPY

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INTRODUCTION

The nucleus senses forces generated by the cell in response to the mechanical properties of biomaterials (e.g. rigidity). These external forces are transmitted from the extracellular matrix to the cytoskeleton, to the nucleus, and to the chromatin[1]. Finally, the chromatin architecture is remodeled[2].

Chromatin is the physiological carrier of genetic and epigenetic information in eukaryotes. Chromatin is classified in two architectures: 1) compacted heterochromatin genetically silent and 2) decompacted euchromatin actively transcribed. Chromatin is highly dynamic and switches between these two architectures. In the process of chromatin decompaction (found in transcription, replication, DNA damage) internal forces are generated pushing nuclear envelope outwards[3].

However, apart from the nuclear envelope, does the chromatin architecture transmit the internal forces to focal contacts regulating the cell adhesion strength? To answer this question, the advanced technique of FluidFM (fluidic force microscope), an atomic force microscope-driven micropipette[4], allows us to evaluate the adhesion strength of cells whose chromatin was altered with drugs.

EXPERIMENTAL METHODS

Cell model and drug treatments: Marsupial kidney epithelial (PtK2) cells were chosen as standard model for chromosome investigations. They exhibit shorter chromosomes compare to other mammalian species. To induce chromatin compaction, we used SA2D (sodium azide and 2-deoxyglucose: ATP depletion) or ANA (anacardic acid: inhibition of histone acetyltransferases).

Adhesion force measurement: Adhesion strength of PtK2 cells was quantified by FluidFM-based single-cell force spectroscopy[4].

Immunofluorescence microscopy: Chromatin compaction was followed by immunofluorescence experiments using antibodies specific for histone acetylation (anti-AcK) or histone methylation (anti-H3K27). To determine whether chromatin compaction regulates organization of focal adhesion contact, experiments using antibodies for vinculin and phalloidin staining for F-actin were performed.

ATP levels reversibility: We tested by videomicroscopy whether SA2D removing allowed a reversible increase in chromatin architecture. We then evaluated by FluidFM whether reversibility of chromatin compaction allows reversibility of cell adhesion strength.

Statistical analysis: Each experiment was performed at least in triplicate. Each quantification for immunofluorescence and FluidFM assay was performed on 100 and 20 cells, respectively.

RESULTS AND DISCUSSION

We show that SA2D or ANA compact chromatin, thus inhibiting chromatin acetylation and activating H3K27 histone methylation. The nuclear area decreases after adding these drugs. The chromatin compaction significantly decreases the cell adhesion strength, disrupting the actin cytoskeleton and the focal adhesion contacts, compared to untreated cells.

We show that drug removal allows the decompaction of the chromatin and thus restoration of both cell morphology and cell adhesion strength as they were before inhibition. We demonstrated that reversibility of chromatin compaction regulates the reversibility of cell adhesion strength.

We should determine how chromatin architecture regulates the cell adhesion strength. We consider two hypotheses: 1) internal forces generated by the chromatin decompaction propagate through mechanical relays (from the nuclear envelope to the cytoskeleton) activating focal adhesion contacts, 2) decompacted chromatin actively transcribed enables neo-synthesis of focal adhesion proteins.

CONCLUSION

Our results show that chromatin architecture regulates cell adhesion strength. These findings open the question of how cell rapidly coordinates the external forces sensed from mechanical properties of biomaterials and their internal genomic forces to control its adhesion. How stem cells orchestrate these external and internal forces to adjust their homeostasis is also a relevant question.

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How the physicochemical characteristics and protein corona modulate the activity of nanostructured lipid carriers (NLC) against *Helicobacter pylori*

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INTRODUCTION

The gastric pathogen *Helicobacter pylori* (Hp) infects half of the world's population[1]. Hp is associated to the development of various gastric disorders, being accountable for 90% of gastric cancers[2]. Therapeutic regimens against this bacterium rely on the use of antibiotics. However, these treatments are failing in up to 40% of the patients, mainly due to the increase of antibiotic resistance[2]. Drug-free nanostructured lipid carriers (NLC) were developed for Hp eradication, showing a bactericidal effect against Hp[3,4]. The main goal of this work is to fine-tune NLC efficiency by changing its physicochemical characteristics, namely surfactant composition, size and charge, aiming complete Hp eradication. Furthermore, the effect of NLC protein corona in its activity against Hp was evaluated.

EXPERIMENTAL METHODS

NLC were produced by hot homogenization and ultrasonication using the lipids (Precirol ATO5® and Miglyol-812®) and different surfactants: the non-ionic Tween®60 (NLC60) and Tween®80 (NLC80) to access the effect of a different surfactant and the cationic Cetyltrimethylammonium bromide-CTAB (NLC-CTAB) to access the effect of charge. NLC characterization was done by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), to determine size and charge, respectively. Concentration was measured by nanoparticle tracking analysis (NTA). In this work the effect of size, surfactant, and surface charge in NLC bactericidal activity was assessed. The effect of size was studied in NLC60 by altering the sonication parameters and three different sizes were prepared: $NLC60_{Small}$<200nm, $NLC60_{Medium}$≈200-300nm and $NLC60_{Large}$>400nm. The effect of the surfactant and charge on NLC activity was assessed using NLC60 and NLC80 (anionic) and NLC-CTAB (cationic) of approximately the same size. All NLC were tested *in vitro* against the human pathogenic Hp J99 strain. Additionally, to test NLC safety towards the gut microbiota, they were tested against other gut bacteria, namely *Escherichia coli* ATCC®25922™ and *Lactobacillus acidophilus*-01. To assess the presence of an NLC protein corona, $NLC60_{Medium}$ were incubated in different media and analyzed by liquid chromatography-mass spectrometry (MS). Moreover, to see the effect of the protein corona on NLC activity, the NLC were tested against Hp after exposure to the different media.

RESULTS AND DISCUSSION

All NLC formulations achieved complete Hp eradication. Regarding the effect of size, $NLC60_{Large}$ had a better performance achieving bactericidal activity at a lower concentration than the other sizes. In terms of effect concerning surfactant composition and charge, NLC60 and NLC80 had similar activity against Hp, achieving complete eradication at 10^{12} particles/mL. Moreover, they didn't affect the other tested bacteria, being safe to the gut microbiota. The cationic NLC-CTAB had higher bactericidal effect against Hp, achieving complete eradication at 10^{11} particles/mL. However, they also had bactericidal effect against *L. acidophilus*-01 at the same NLC concentration. The MS analysis showed protein adsorption to NLC surface. Moreover, it was observed that the protein corona delays NLC activity against Hp.

CONCLUSION

Overall, all NLC formulations were effective against Hp. NLC60 and NLC80 were selective to Hp. However, NLC-CTAB was not, affecting *L. acidophilus*-01, and thus, this formulation was not further explored. The presence of a protein corona was confirmed, and it was observed that it delays NLC bactericidal effect. These results support the NLC potential as a non-antibiotic alternative treatment to control Hp gastric infection.

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>> 15:30 - 16:30 | WS - Wiley

Chairpersons: Irem Bayindir-Buchhalter & Nicola Contessi Negrini
Location: Room E

15:30 | KL Wiley - Scientific Publishing with Impact
Irem BAYINDIR-BUCHHALTER, Wiley-VCH, Weinheim, Germany

16:00 | O1 Wiley - Mechanotransduction in High Aspect Ratio Nanostructured Meta-biomaterials
Khashayar MODARESIFAR, Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, Delft, The Netherlands

16:15 | O2 Wiley - Development of a Vascularized Tissue Model: Focus on the Stromal/Vascular Interaction and on 3D Imaging
Alessandra DELLAQUILA, Université Paris Cité, INSERM U1148, Paris, France

Scientific Publishing with Impact

Irem Bayindir-Buchhalter[1*]

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Growing research output is a must to advance a scientific career. It is therefore important that editors, reviewers and readers understand the merits of your work once your manuscript is ready for submission.

In her talk, Dr. Irem Bayindir-Buchhalter, Editor-in-Chief in Wiley's Materials Science and Physics team editing the *Advanced* family of journals, will explain the peer review process.

She will focus on how editors evaluate a manuscript and what their decisions are based on.

There will be several tips on structuring a manuscript, selecting the right journal, and transferring your manuscript if it does not make it in the journal you choose.

The talk will also address publishing integrity concerns, from plagiarism to authorship rights and conflicts of interest.

Come by for an informative discussion about how to write articles that not only successfully navigate the peer-review process, but are also discovered, read, cited, and make an impact in the research community.

Mechanotransduction in High Aspect Ratio Nanostructured Meta-biomaterials

Khashayar Modaresifar*, Mahya Ganjian, Pedro J. Díaz-Payno, Maria Klimopoulou, Lidy E. Fratila-Apachitei, Amir A. Zadpoor

Department of Biomechanical Engineering, Faculty of Mechanical, Maritime, and Materials Engineering, Delft University of Technology, Delft, The Netherlands

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INTRODUCTION

High aspect ratio nanostructured surfaces offer a combination of rare properties and functionalities which frame them as meta-biomaterials [1]. High aspect ratio nanopillars of black Ti, fabricated through dry etching of the Ti surface, have been shown to simultaneously kill the pathogenic bacteria (*e.g.*, *S. aureus*) and enhance matrix mineralization in preosteoblast cells [2]. Such functionalities make bTi a proper candidate for the fabrication of orthopedic implants where both prevention of infections and promoting osseointegration are of high interest. Yet the exact mechanisms by which these nanostructures direct the cell fate is not completely known. Here, we hypothesized that the nanopillars affect the adhesion and contractility of the cells as well as certain transcriptional factors in cells, resulting in differential levels of osteogenic markers expression.

EXPERIMENTAL METHODS

A previously described ICP RIE protocol [2] was used to create high aspect ratio nanopillars on titanium surfaces. Ti specimens were etched for 10 min with Cl_2 and Ar gases (flow rates of 30 and 2.5 sccm, respectively) under the chamber pressure of 2 Pa and at 40 °C. The etched specimens were then characterized by SEM and EDS.

Human mesenchymal stem cells (hMSCs) were expanded and seeded on sterilized flat Ti and bTi samples using α-MEM culture medium. After 1 day of culture, cells were stained for vinculin, actin, nucleus, and Yes-associated protein (YAP). Runx2 staining was also performed after 9 days of culture. ImageJ software was used to quantify cell morphological characteristics and the signal intensity of the expressed proteins to reveal the effects of different surface topographies.

To study the role of focal adhesion kinase (FAK), Rho-associated protein kinase (ROCK), and YAP in the regulation of Runx2 as an osteogenic marker in hMSCs when exposed to high aspect ratio nanopillars, each of those factors was inhibited in separate dedicated experiments. 10 µM PF-573228, 10 µM Y-27632, and 10 µM Verteporfin were added to the culture medium upon cell seeding and medium refreshing to inhibit FAK, ROCK, and YAP, respectively. In each condition, Runx2 was stained after 9 days of culture, and quantifications were done similar to the previous part. The statistical analysis was performed using GraphPad Prism v.9.2.0 and a *p*-value below 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

bTi nanopillars had a height between 700 nm and 1 µm with an average aspect ratio of 12.1. Ti and O were found to be the main elements composing the surface.

While the high aspect ratio nanopillars did not impair the attachment, survival, and metabolic activity of the cells, they gave rise to a cell morphology that was different from the one observed on the polished flat surfaces. The altered cell morphology was also associated with differences in the organization of the cytoskeleton and the formation of FAs. Our findings showed that the cell nucleus is more elongated and less rounded on the bTi surfaces as compared to the flat Ti which might be linked to the different distribution of FAs. Moreover, the intensities of nuclear YAP and Runx2 were significantly higher in cells residing on bTi surfaces (Figure 1).

The inhibition of both FAK and ROCK significantly altered the morphological characteristics of hMSCs residing on bTi surfaces. These inhibitions were also correlated with a significantly decreased Runx2 signal intensity. These results indicate that the regulation of adhesion and contractility of hMSCs on nanopillars and their subsequent osteogenic marker expression are highly dependent on the activity of FAK and ROCK. The effect of YAP inhibition on cell morphology was not as evident as that of FAK and ROCK but it also resulted in a decrease in Runx2 intensity on bTi surfaces.

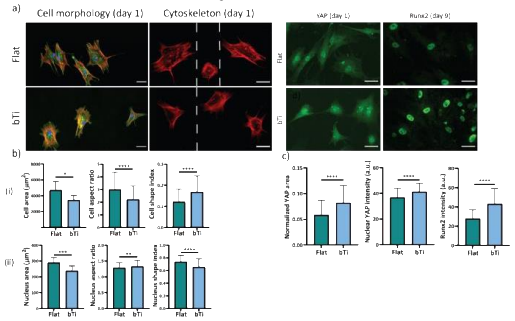

Figure 1

CONCLUSION

High aspect ratio bTi nanopillars enhance Runx2 expression in hMSCs by manipulating cell adhesion as regulated by FAK, cell contractility as regulated by ROCK, and the nuclear translocation of transcriptional factors such as YAP.

ACKNOWLEDGMENTS

This research has received funding from European Research Council (Grant no: 677575).

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Development of a Vascularized Tissue Model: Focus on the Stromal/Vascular Interaction and on 3D Imaging

Alessandra Dellaquila[1*], France Lam[2], Susanne Bolte[2], Didier Letourneur[1], Teresa Simon-Yarza[1*]

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INTRODUCTION

The development *in vitro* of pre-vascularized models is a key point for obtaining functional tissue engineered models. Notably, the use of constructs containing an artificial vascular network would allow researchers to carry on *in vitro* studies for drug and disease modeling and ensure a better integration of the constructs within the host tissue, thus enhancing its regeneration[1,2].

In this study, we propose a vascularized functional scaffold, consisting of a vascular network and a stromal component, with specific focus on: (i) the influence of the stromal component on the vasculature and its angiogenesis within the matrix and (ii) the vasculature response to external stimuli, such as hypoxia and drugs administration. Moreover, to overcome the current limitations of 2D histological procedures, often used for biomaterials, the imaging studies are conducted mainly by light sheet microcopy (LSM), a 3D high resolution imaging technique suitable for thick and complex tissue constructs[3].

EXPERIMENTAL METHODS

Polysaccharide-based scaffolds containing tubular microchannels (50-100 micrometers in diameter) are engineered by a combination of subtractive templating and freeze-drying techniques, as previously described[4].

The vascular network is obtained by culturing endothelial cells from umbilical vein (HUVECs) within the channels. Human fibroblasts from dermis (NHDFs) are added to the model to replicate the stromal component. The vasculature formation is analyzed over time by studying the metabolic activity (Resazurin-based assay), the expression of angiogenetic factors (multiplex protein assay) and the production of oxidative species (nitric oxide studies) and the monoculture model (HUVECs only) compared to the coculture model. Furthermore, the effect of the extracellular matrix (ECM) components produced by the stroma on the construct is investigated. In parallel, the scaffold formulation and processing are adapted for optimal imaging by LSM and confocal microscopy. Particularly, to preserve the scaffold's features, we are developing clearing procedures by adaptation of aqueous solution-based protocols[5].

RESULTS AND DISCUSSION

Our results confirm the increased expression of pro-angiogenic factors, such as VEGF, bFGF and PDGF, in the co-cultured matrices compared to the HUVECs-only constructs and the increased production of ECM proteins in presence of fibroblasts, that could enhance the sprouting process within the matrix pores (**Figure 1A-B**). The imaging by LSM can be performed without the need for clearing in the first days of culture, a result that will permit us to run real-time imaging of the vascularization process within the constructs. For longer culturing times, the matrices become opaquer due to an important secretion of ECM components, an issue that we have addressed successfully by developing a clearing protocol that re-establishes the matrix transparency without damaging the channel geometry nor morphology (**Figure 1C**).

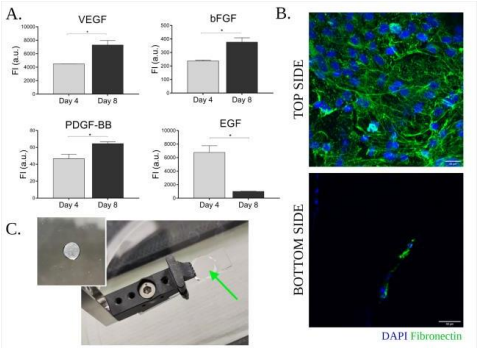

Figure 1. A. Results indicate the increased expression of VEGF, bFGF and PDGF in a coculture model (day 8 vs d4 of culture, fluorescence intensity-FI values). EGF expression decreases after the proliferating phase. **B.** Deposition of fibronectin (green) by NHDFs is observed on the top of the scaffold, where they are cultured, while no production is observed on the bottom side, unseeded. **C.** Scaffold photograph before (inset) and after (green arrow) the clearing process, optimized for our tissue constructs. Created with BioRender.com.

CONCLUSION

To summarize, this work aims to develop a complex 3D model including a stromal component and a functional vascular network. The interactions between the cell types and the influence of the stroma on the angiogenetic process *in vitro* are investigated. Particular attention is paid to evaluate these tissue models by 3D high-resolution imaging techniques, methods that will open up new ways of studying large living tissue constructs.

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ACKNOWLEDGMENTS

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>> 15:30 - 16:30 | PSOP-03 - Osteoarticular tissue engineering

Chairpersons: Jérôme Guicheux & Cristina Martins
Location: Room A

15:30 | O1 Osteoarticular - Comparison of the degradation behavior of WE43-based magnesium screws with and without PEO-surface modification in a miniature pig model

Heilwig FISCHER, 1 Center for Musculoskeletal Surgery, Charité – Universitätsklinikum Berlin, Berlin, Germany 2 Department of Oral and Maxillofacial Surgery, Charité – Universitätsklinikum Berlin, Berlin, Germany 3 Berlin Institute of Health at Charité – Universitätsmedizin Berlin, Berlin, Germany

15:45 | O2 Osteoarticular - Effects of Nanostructured and Microstructured Calcium Phosphates on In Vitro Osteoblast Response

Edgar B. MONTUFAR, Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

16:00 | O3 Osteoarticular - Multiscale Characterization and Biological Assessment of Pyrophosphate-Stabilized Amorphous Calcium Carbonate Doped with Bioactive Ions

Christèle COMBES, CIRIMAT, Université de Toulouse, CNRS, TOULOUSE INP - ENSIACET, Toulouse, France

16:15 | O4 Osteoarticular - Targeting HIF Pathway for Personalised Bone Regeneration in Diabetic Patients

Adriana-Monica RADU, Division of Surgery and Interventional Sciences, University College London, London, United Kingdom

Comparison of the degradation behavior of WE43-based magnesium screws with and without PEO-surface modification in a miniature pig model

Heilwig Fischer[1,2,3*], Katharina Schmidt-Bleek[4], Ole Jung[5], Ralf Smeets[6], Georg N. Duda[4], Alexander Kopp[7], Max Heiland[2], Carsten Rendenbach[2]

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INTRODUCTION

Magnesium is a promising candidate for use as a resorbable osteosynthesis material due to its mechanical properties and biocompatibility[1]. During magnesium degradation, hydrogen gas becomes vacant. Once implanted, if the amount of hydrogen gas exceeds the resorption capacity of the local tissue, hydrogen is accumulated and impairs osseointegration, which further leads to osteosynthesis failure[2]. In vitro, a reduction in degradation rate and by that a reduction of the initial gas formation could be shown when a plasma electrolytic oxidation (PEO) surface modification was used[3]. Thus, this study aims to compare the degradation rate, and bone quantity formed surrounding an Mg alloy (WE43MEO) with or without PEO in a minipig model for a long-time period (6 and 12 months).

EXPERIMENTAL METHODS

WE43-based magnesium screws and -plates (4-hole plates, outer dimensions: 32.5 x 5.5 x 2.0 mm, screws: 11 mm length, 2.3 mm in diameter) were implanted in the humerus and femur of Göttinger Minipigs. Implants and adjacent bone were quantified after 6 and 12 months with both micro-CT and histomorphometric evaluation after the preparation of non-decalcified sections and Giemsa staining.

RESULTS AND DISCUSSION

After 6 months, the screws showed a difference in the residual mean volume of 28.89 % for WE43 versus 62.95 % for WE43-PEO (Mann-Whitney Test, p=0.0273). An increase in bone formation appeared in the bone marrow of the non-surface modified implants (33.28 %) when compared to the PEO samples (18.48 %) (Mann-Whitney Test, p=0.0477). After 12 months, no significant difference in the implant volumes could be shown (p>0.05). Histological evaluation revealed a higher amount of bone formation above the surface-modified implants of 6.95 mm^2 (WE43-PEO) when compared to 2.61 mm^2 of the WE43 samples after 6 months (Mann-Whitney Test, p=0.0498) and then again after 12 months 4.25 mm^2 (WE43-PEO) versus 1.30 mm^2 (WE43) (Mann-Whitney Test, p=0,1397). Bone Implant Contact (BIC) was also assessed, and after 6 months 18.18 % of BIC was found in WE43 samples while for WE43-PEO samples the BIC was 51,65 % (Mann-Whitney Test, p=0,0156). The relation of bone volume per total volume of the adjacent bone was reduced in all implants in comparison to normal bone.

CONCLUSION

PEO surface modification significantly reduces the degradation rate of the fixation devices and improved osseointegration of the surface-modified screws. After 6 months, a significantly higher amount of bone could be found above the surface-modified implants as well as a higher BIC, when compared to non-surface modified devices.

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ACKNOWLEDGMENTS

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Effects of Nanostructured and Microstructured Calcium Phosphates on *In Vitro* Osteoblast Response

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INTRODUCTION

Several works studied the roles of nanostructured calcium deficient hydroxyapatite (CDHA, Ca/P = 1.50) on cell responses. These pioneer works mainly compare plate- and needle-like CDHA nano size crystals, but has not been possible to study the role stoichiometry and crystalline composition as until now only CDHA can be biomimetically processed resulting in nanostructured surfaces. In this work a novel method was developed to obtain beta tricalcium phosphate (β-TCP) and hydroxyapatite (HA, Ca/P > 1.50) with equivalent nanostructure than CDHA, enabling the study of the osteoblast *in vitro* responses to nanostructured surfaces of different composition. It was also possible to compare for the first time nano- and micro-structured calcium phosphates (CaPs) having the same chemical composition.

EXPERIMENTAL METHODS

Nanostructured CDHA disks were obtained by hydrolysis of α-TCP powder at 37 °C. CDHA disks were subsequently carbonated. CDHA and carbonated-CDHA disks were either conventionally sintered at 1100 °C to obtain microstructured β-TCP and HA disks, respectively, or heat treated at significantly lower temperature for 1 min (heating and cooling rates > 100 °C/min) to obtain nanostructured β-TCP (N-β-TCP) and nanostructured HA (N-HA) disks. The microstructure and crystalline composition were thoroughly characterized. Saos2 cells were cultured in McCoy's proliferation medium up to 28 days. The metabolic activity and dsDNA amount were determined during the culture as well as the focal adhesions and cell morphology. The osteogenic differentiation was accessed by immunostaining.

RESULTS AND DISCUSSION

Fig. 1 shows that N-β-TCP and N-HA had the same plate-like crystalline nanostructure than CDHA (SSA above 10 m^2/g), whereas β-TCP and HA had polyhedral grains (SSA below 1 m^2/g). XRD and FTIR confirmed the crystalline composition of the materials (data not shown). CDHA, N-β-TCP and β-TCP had a CaP ratio of 1.50, whereas N-HA and HA of 1.62 and 1.57, respectively. Thus, the increment of the Ca/P ratio prevented the transformation of CDHA into β-TCP, resulting in a more stoichiometric N-HA than CDHA. All the nanostructured CaPs produced equivalent Ca and P depletion of the cell culture medium, significantly below than the microstructured ones, suggesting that microstructural features control the local ionic reactivity rather than composition in a process driven by kinetics.

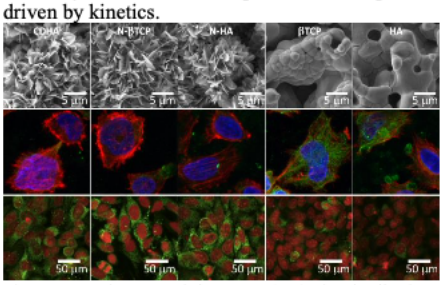

Fig. 1. SEM images of the CaPs (top), focal adhesions (green) and actin cytoskeleton (red) at day 1 (centre), and nuclei (red) and collagen type I (green) staining at day 14 (bottom). All are direct contact cell cultures.

The cells after 24 h of culture were round in the nanostructured CaPs with few focal adhesions, while in the microstructured materials presented significantly more focal adhesions and were extended (Fig. 1). In agreement, slightly faster cell growth was observed in the CaPs with polyhedral grains. The effects of the surface topography were also observed in the expression of collagen I, an early marker of osteoblast maturation. In general, the osteoblasts on the nanostructured CaPs expressed the largest amount of collagen without evident differences between them (Fig. 1).

CONCLUSION

CaP with different crystalline composition and stoichiometry than CDHA were obtained for the first time retaining the plate-like nano-crystalline morphology. The biological interactions were minimally affected by the composition, showing all the materials with the same surface structure similar ionic reactivity and cell responses, but more mature osteoblasts were found on the nanostructured CaPs.

ACKNOWLEDGMENTS

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Multiscale Characterization and Biological Assessment of Pyrophosphate-Stabilized Amorphous Calcium Carbonate Doped with Bioactive Ions

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INTRODUCTION

Calcium carbonate (CC) is often used for biomedical applications due to its excellent biocompatibility[1]. Among all the crystalline polymorphs, amorphous calcium carbonate (ACC) is the less stable and consequently the most reactive in solution. In addition, it had demonstrated an *in vitro* bioactivity[2], and *in vivo* abilities for drug release and bone remodeling[3]. Various syntheses are detailed in the literature, aiming to stabilize ACC against crystallization into a more thermodynamically stable CC phase, mostly by the addition of organic or inorganic materials. For the latter, orthophosphate ions as well as polyphosphates with high chain length had been successfully introduced as ACC stabilization additives[4], but the use of the pyrophosphate dimer is poorly documented. Yet, besides its calcite crystallization inhibition properties, it can be hydrolyzed *in vivo* by enzymes, leading to orthophosphate ions necessary for bone rebuilding. Moreover, it can modulate the ACC dissolution rate and consequently the calcium, carbonate and phosphate release. The purposes of the study are to understand the influence of the presence of pyrophosphate (PyACC), doping ions (Sr^{2+}, Zn^{2+} or Cu^{2+}, SrPyACC, ZnPyACC or CuPyACC, respectively) and/or a biocompatible polysaccharide, alginate (AlgPyACC), on the evolution in aqueous media (water and simulated body fluid (SBF)) of these different ACC powders of biological interest and their in vitro biomineralization properties through the quantification of alkaline phosphatase (ALP) activity.

EXPERIMENTAL METHODS

PyACC powders were synthetized by a co-precipitation method from a calcium solution and a pyrophosphate-containing carbonate one, the alginate or doping ions being introduced in the latter when required. They were thoroughly characterized (XRD, FTIR spectroscopy, SEM and TEM, chemical titrations, TGA-DTA, SAXS and WAXS, solid-state NMR). The powders were then placed into either water or SBF at a known solid over liquid ratio, and let for evolution at 37°C for 6 hours to 28 days. After filtration, washing and drying, both the powder and the filtrate were kept for new characterizations (XRD, FTIR, SEM for the powder and chemical titrations for the liquid). In parallel, cytocompatibility of these powders was assessed *in vitro* by MTT assays on 3T3 cells as well as alkaline phosphatase (ALP) activity in order to quantify the biomineralization capacity. For the latter, powders suspensions were added to SaOS-2 cells containing well-plate and incubated for 5 days with addition of a mineralization activation cocktail after 2 days.

RESULTS AND DISCUSSION

When added in small proportions during the synthesis of PyACC, pyrophosphate seemed to orient the CC precipitation toward a mix of calcite, vaterite and ACC (whose respective proportions depend on the Py amount) rather than calcite only. In a sufficient amount, the crystallization was completely inhibited and a fully amorphous CC obtained. Moreover, a fractal nanoscale organization of the PyACC powders was evidenced with the existence of 2-3 nm clusters, composed by a calcite-like core as described into the literature[5]. We also demonstrated that PyACC could integrate high amounts of doping ions (Sr^{2+}, Zn^{2+} or Cu^{2+}) that could give additional bioactive properties (angiogenesis, osteoblastic activity and/or anti-inflammatory effect). The cation-doped PyACC powders presented an extended stability in aqueous media, remaining amorphous for longer times than the undoped one and slowly releasing active ions without any burst effect. Alginate-PyACC remained amorphous for almost the whole evolution time. Furthermore, the association of PyACC with zinc or strontium ions or alginate polymer conferred an enhanced cytocompatibility of the materials towards fibroblast cells. In addition, zinc doping also seemed to increase ALP activity and hence the biomineralization capability.

CONCLUSION

All together, these results could allow us to understand the correlation between the nanoscale organization, the release kinetics of doped pyrophosphate-stabilized ACC materials and finally their *in vitro* bioactive properties, that is a key point for biomaterials in view of bone substitute applications.

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Targeting HIF Pathway for Personalised Bone Regeneration in Diabetic Patients

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INTRODUCTION

Bone remodeling in healthy individuals is characterised by a balance in osteoblast and osteoclast functions. Bone fragility[1] is common in diabetic patients, and is partly attributed to impaired osteoclast function[2]. Diabetic patients also present a high risk of fractures (up to 6.75 fold increase in hip fractures) and have increased healing time[1]. Low oxygen concentration (hypoxia) is recognised as a key regulator of osteoclasts formation and function via the Hypoxia Inducible Factor-1α (HIF-1α) pathway[3]. In diabetes, cells ability to sense changes in oxygen tension is diminished, and HIF pathway is impaired, leading to the suggestion for the use of artificial HIF-1 stabilization[4]. Here, we investigate the role of known HIF mimetics, Cobalt Chloride ($CoCl_2$), dimethyloxalylglycine (DMOG), Desferrioxamine (DFO) and Deferasirox (DFX) in restoring osteoclasts function. In normoxia, these HIF mimetics prevent HIF-1α destruction via different mechanisms and regulate the differentiation of osteoclasts. Thus, the release of HIF mimetics from biomaterials could be used to restore bone regeneration in diabetic patients.

EXPERIMENTAL METHODS

A selected subclone of the heterogeneous RAW 264.7 cells with maximised TRAP-5b activity was used in this study. The cells were cultured in DMEM, supplemented with 10% (v/v) foetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin (P/S). Before seeding, cells were preconditioned for at least two weeks in 4.94 mM and 22.28 mM glucose levels. Low glucose (LG) and hyperglycemia (H) media were used as simplified *in vitro* models for healthy and diabetic patients[4]. Cells were seeded at $3x10^3$ cells/cm^2, passage number 15 and differentiated into osteoclasts by adding 3 ng/mL RANKL. Co^{2+} (12.5-200 µM), DMOG (100-400 µM), DFO (2-20 µM) and DFX (1-20 µM) treatments were applied twice. Osteoclasts formation was investigated on day 5 using Tartrate-Resistant Acid Phosphatase (TRAP) staining, and the specific enzyme involved in the bone resorption process activity (TRAP-5b) was determined. Each control and treatment condition was repeated at least four times. Statistical analysis was performed using GraphPad Prism v.9. The means were compared using one-way ANOVA with Holm-Sidak's post-hoc test for multiple comparison.

RESULTS AND DISCUSSION

The HIF mimetics Co^{2+} (25-50 µM), DMOG (100-200 µM), DFO (2, 20 µM) and DFX (1-2 µM) restore osteoclasts function otherwise hindered by hyperglycemia to the levels present in low glucose as determined by TRAP-5b activity (Fig.1d). Additionally, osteoclasts number and the total surface area they cover are increased by HIF mimetics in hyperglycemia (Fig.1c). These results will further be validated by resorption on dentine discs.

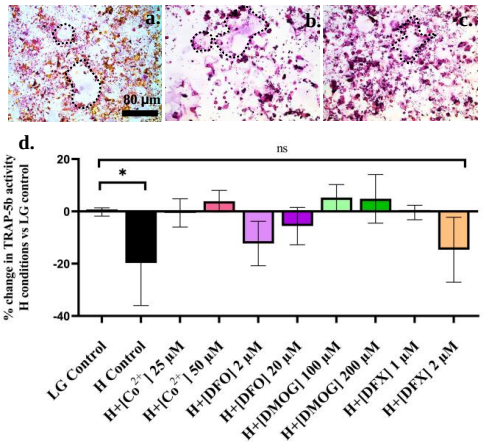

Figure 1. Osteoclasts formation and their specific enzyme activity. (a) TRAP stained osteoclasts in the LG control, (b) H control and (c) H + 25 µM Co^{2+}. Osteoclasts are stained in purple. Images were taken at 4x magnification. (d) TRAP-5b activity in LG control, H controls and H + HIF mimetic conditions. Values represent mean ± SD, *$p<0.05$.

CONCLUSION

HIF mimetics restored hyperglycemia impaired osteoclastogenesis to levels present in normal glucose conditions. These results support the suitability of HIF mimetics for the next generation of bone tissue engineering materials for diabetic patients, such as the controlled release of Co^{2+} from bioactive glasses.

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>> 15:30 - 16:30 | PSOP-04 - Bioderived materials

Chairpersons: Emmanuel Pauthe & Karine Anselme
Location: Room H

15:30 | O1 Bioderived - Bioinks derived from human amniotic membrane and proof-of-principle application to bioprinting

Léo COMPERAT, Inserm U1026, Tissue Bioengineering, ART BioPrint, F-33076, Bordeaux, France

15:45 | O2 Bioderived - Engineering a Tendon-Bone Enthesis-Mimicking Hybrid Matrix for Repair of Irreparable Rotator Cuff Tears

Chaozong LIU, Department of Orthopaedics & Musculoskeletal Science, Division of Surgery & Interventional Science, University College London (UCL), Royal National Orthopaedic Hospital, London, United Kingdom

16:00 | O3 Bioderived - Production of mineralised collagen films using electrophoretic deposition

Katrina STAUNTON, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

16:15 | O4 Bioderived - Tuning the bioactivity of MSC-secreted ECM through control of substrate stiffness

Daniel HEATH, Department of Biomedical Engineering, University of Melbourne, Parkville, Australia

Bioinks derived from human amniotic membrane and proof-of-principle application to bioprinting

Léo Comperat[1]*, Lise Chagot[1], Sarah Massot[1], Chantal Médina[1], Nathalie Dusserre[1], Marie-Laure Stachowicz[1], Jean-Christophe Fricain[1,2], Hugo de Oliveira[1]

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INTRODUCTION

Placental tissues are considered as surgical waste and can be obtained after selective caesarean surgery. It can then be considered as an abundant, available, and cost effective human tissue with little ethical issues. Thanks to its biological properties it has been used in the clinics for more than 100 years, particularly in ophthalmology and dermatology (1, 2). Its biological properties are relevant in a regenerative approach, namely: able to promote wound healing, low immune response, antifibrotic, antimicrobial, rich source of growth factors, cytokines, interesting mechanical properties and naturally composed by ECM components (hyaluronic acid, collagens, laminin, fibronectin, and proteoglycans) (3). As such, it represents an attractive source of human ECM that can find use of tissue engineering applications.

3D bioprinting has emerged as a technological approach with the potential to address unsolved question in tissue engineering and regenerative medicine. One of the main limitations associated with bioprinting remains the lack of suitable natural biomaterial inks that can better support cell maturation, following bioprinting.

As such, we developed a bioink derived from two human amniotic membrane layers (*i.e.* amnion and chorion), aiming for human physiology representability in regeneration approaches and *in vitro* models.

EXPERIMENTAL METHODS

After decellularization, digestion and methacrylation of amnion and chorion, we could establish two different biomaterial inks with different properties. The objective was to attain a more in depth characterization of both sources and to establish their inherent capacity to be processed using microextrusion bioprinting.

Different methodologies of matrix digestion were tested and compared in relation to the capacity to retain relevant growth factors. As to validate its capacity to be used in bioprinting, we combined the obtained bioinks with methacrylated hyaluronic acid, and optimized its rheological and biological characteristics.

Cell integration was assessed by live dead assay of human umbilical vein cord endothelial cells (HUVECs) bioprinted with the developed hydrogels. Angiogenesis was evaluated by confocal observation and cellular staining (PECAM, CD-31 and αSMA) on HUVECs and human skin fibroblasts (HSF) cocultured within the bioinks.

RESULTS AND DISCUSSION

We could show that the concentration of human growth factor was higher in chorion than in amnion, particularly concerning angiogenesis (see *e.g.* in **Fig. 1**)
The composite hydrogels were shown to sustain the bioprinting of HUVECs, with high viability (see **Fig. 2**) and to support the maturation of cocultures between HUVECs and HSF.

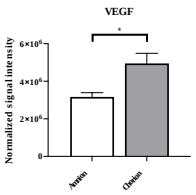

Fig. 1: Human growth factor assay on amnion and chorion samples, for VEGF (n=6).

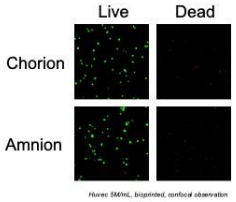

Fig. 2: Live dead assay of human umbilical vein cord endothelial cells (HUVECs) bioprinted with the developed hydrogels, resulted in viable cells after 24 hours (1/10000).

CONCLUSION

We could demonstrate that amniotic membrane derived ECM can be successfully processed into highly printable, stable, and human relevant bioinks. They contain a significant amount of growth factors, and cell-binding sequences and we could show that they support cellular integration and response within the 3D constructs. Therefore, we believe they are a strong alternative to animal ECM derived biomaterials such as bovine or rat type I collagen, while at the same time being more relevant for human studies in regenerative medicine, drug discovery and tissue engineering.

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Engineering a Tendon-Bone Enthesis-Mimicking Hybrid Matrix for Repair of Irreparable Rotator Cuff Tears

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INTRODUCTION

Rotator cuff tears in the shoulder are common, with over 275,000 repairs performed annually in the United States[1], aiming to reattach the musculotendinous unit back to bone. However, progressive fatty infiltration and tendon retraction leads to difficulty in returning the tendon to its anatomic attachment[2]. Furthermore, tendon-bone repair does not recreate the enthesis, the native microanatomical structure of graded mineralisation at the tendon insertion, forming poorly organised scar tissue, and leading to post-operative failure rates of up to 94% for large and massive tears[3]. Since demineralised animal bone matrix has shown promise as a natural scaffold to enhance enthesis healing[4], we are aiming to create a novel soft-hard tissue connector device, specific to repairing/bridging the tendon-bone injury in significant rotator cuff tears, by employing decellularised animal bone partially demineralised at one end for soft tissue continuation.

EXPERIMENTAL METHODS

Optimisation samples of 15x10x5mm^3, trialled as separate cancellous and cortical bone samples, were cut from porcine femoral condyles and shafts, respectively. Samples underwent 1-week progressive stepwise decellularisation and a partial demineralisation process of half wax embedding and acid bathing to obtain the soft-hard enthesis-mimicking hybrid matrix. Samples were characterised histologically for the presence or absence of cellular staining in both peripheral and central tissue areas (n=3 for each cortical/cancellous, test/PBS control and peripheral/central group), and mechanical properties were measured before and after processing using a BioDent reference point indentation (RPI) tester.

RESULTS AND DISCUSSION

X-ray examinations confirmed that a tendon-bone enthesis-mimicking hybrid matrix was successfully obtained, as shown in **Fig. 1**. The matrix featured a hard bone section and soft tendon section, with a gradient in structure and mechanical properties. *In vitro* evaluation revealed that cellular staining was absent in peripheral and central cancellous samples and reduced in cortical samples compared to controls (**Fig. 2**), whilst cancellous samples decreased in wet mass after decellularisation by 45.3% ($p<0.001$). Decellularisation did not appreciably affect mechanical properties, as RPI measurements associated with toughness (total indentation depth, indentation depth increase) and elasticity (1st cycle unloading slope) showed no consistent changes after decellularisation. Half wax embedding provided predictable and defined control of the mineralised-demineralised interface position during acid bathing.

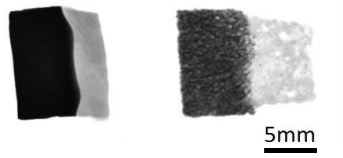

Fig. 1: Inverted X-ray image of bone(dark)-tendon(light) enthesis-mimicking hybrid matrix; cortical (left) and cancellous (right) samples.

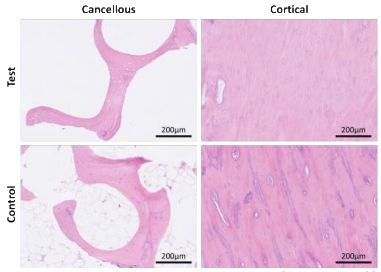

Fig. 2: Histology examinations confirming effective cell removal by the decellularisation process. H&E.

CONCLUSION

Initial optimisation trials show promise in the design of a soft-hard hybrid tissue scaffold as an immune compatible xenograft to provide new therapeutic options for irreparable rotator cuff tears. Decellularisation through sonication and vacuum negative pressure are essential for timely decellularisation whilst maintaining mechanical properties. Further studies of biological, structural and chemical characterisation are underway to assess device validity, before *in vivo* animal model trials and potential clinical translation.

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Production of mineralised collagen films using electrophoretic deposition

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INTRODUCTION

Bone is an inorganic-organic composite material with a hierarchical structure built from mineralised collagen fibrils. A recent bone mineral model proposes citrate molecules bridge between the apatitic core of bone mineral platelets, residing in the disordered surface region best described by an octacalcium phosphate (OCP) phase[1]. Novel collagen film production using electrophoretic deposition (EPD)[2], has inspired research into the technique for the applicability of the production of bone implants. To achieve successful EPD a stable suspension is required with low conductivity and particles with a non-zero zeta potential. These characteristics of mineralised collagen need to be determined and altered to produce EPD mineralised collagen films.

EXPERIMENTAL METHODS

Collagen slurry: 1 wt% insoluble dermal collagen was rehydrated in 0.05 M acetic acid for 36 hrs, homogenised and dialysed against deionised (DI) water.

Synthesis of OCP-CIT: Citric acid solution (1.8969 g, 60 ml) was maintained at 37 ˚C and pH adjusted to 5.52 using 50% NaOH solution. α-tricalcium phosphate (TCP, 0.6 g) was added and pH adjusted to 6.50. The suspension was stirred at 200 rpm for 4 days and air-dried.

OCP-CIT/collagen suspension: 1.8969 g of citric acid and 50% NaOH were added simultaneously to 60 ml collagen slurry stirred at 200 rpm at 37 ˚C to reach a final pH of 5.52. 0.6 g α-TCP was added to the collagen slurry and pH adjusted to 6.50 before stirring for 4 days. The mineralised suspension was homogenised, hyaluronic acid (HyAc) sodium salt added (0.25 - 1 mg/ml) and then dialysed against DI water.

Zeta potential: Measurements were taken using Zetasizer Nano-ZS (Malvern instruments) at 25 ˚C.

EPD film production: 1.2 cm EPD cell consisting of a 316L steel electrodes separated by a 0.075 cm thick dialysed gelatine sheet. 10 V was applied across the cathode (suspension) and anode (DI water) for 10 mins. The film was air dried before dissolving gelatine in DI water at 37 ˚C. The isolated film was rinsed and dried.

X-ray diffraction (XRD): XRD diffraction were taken using Bruker D8 DAVINCI with a step size of 0.01˚ from 2θ = 3 – 60˚, 30 rotations/min.

RESULTS AND DISCUSSION

The OCP-CIT/collagen suspension after dialysis has a pH between 7-9, which has a low zeta potential (Fig. 1). The zeta potential can be changed by adjusting beyond this pH range, however this results in a large increase in conductivity and instability to the OCP-CIT phase. HyAc is a component of the extra cellular matrix and has been widely used in bone regeneration due to its potential for improving osteogenesis and mineralisation[3]. In the field of EPD, the negatively charged hyaluronate chains are also beneficial due to their ability to change the zeta potential of particles in suspension. Here, HyAc decreases the zeta potential of the mineralised collagen, enabling successful EPD of the suspension. By adding the HyAc sodium salt before the dialysis procedure, the increase in conductivity is reduced. The XRD pattern of mineralised collagen film confirms the presence of OCP-CIT mineral phase and the broad peaks correspond to collagen (Fig. 2).

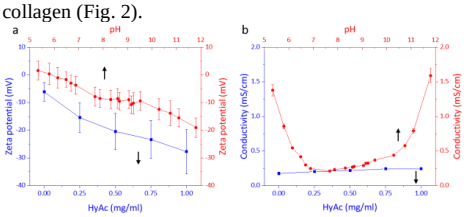

Fig. 1: The zeta potential (a) and conductivity (b) of OCP-CIT/collagen suspensions as a function of the pH (red circle) and HyAc concentration (blue square).

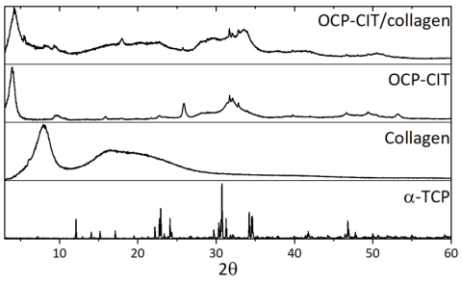

Fig. 2: XRD patterns of OCP-CIT/collagen 1 mg/ml HyAc and collagen films, OCP-CIT and α-TCP powders.

CONCLUSION

Inspired by a recent bone mineral model and a novel EPD collagen film technique, this work determined relevant characteristics of mineralised collagen showing an additive was necessary for EPD. HyAc significantly changes the zeta potential of mineralised collagen, enabling EPD film production with XRD characterisation confirming the presence of an OCP-CIT phase in the film.

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ACKNOWLEDGMENTS

Funding by EPSRC Doctoral Training Programme (KJS).

Tuning the bioactivity of MSC-secreted ECM through control of substrate stiffness

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INTRODUCTION

Mesenchymal stromal cells (MSCs) are used in hundreds of clinical trials for cellular and regenerative therapies. The low prevalence of MSCs in the human body and the high number of MSCs required for clinical applications (~10^6 MSCs/kg body weight) demand that tissue-isolated MSCs undergo substantial expansion *ex vivo*. However, the cells senesce and lose their desired "stemness" properties during expansion. We previously showed that MSCs retain more of their stemness properties when cultured on MSC-derived decellularized extracellular matrix (dECM), compared with conventional substrates.[1-3] However, previous work has produced dECM on rigid glass and tissue culture plastic surfaces, and differentiation of primary cells on these surfaces skews towards osteogenesis. Substrate stiffness and viscoelasticity are known regulators of stem cell behavior. As such, in this work we assess the impact of substrate stiffness on dECM microarchitecture and composition. We then assess how dECM on surfaces with varying stiffnesses influenced the proliferation and differentiation of primary MSCs.

EXPERIMENTAL METHODS

Polyacrylamide hydrogels were produced through the copolymerization of acrylamide and bisacrylamide monomers. As previously reported, monolithic hydrogels with a single stiffness and linear gradient stiffness hydrogels were produced that spanned a range of physiologically relevant stiffnesses (4-40 kPa). The hydrogels were covalently modified with collagen to facilitate cell adhesion, as previously reported.[1-2]

MSCs were allowed to deposit ECM on the surfaces, and the cultures were decellularized according to previously reported procedures.[3] The microarchitecture and composition of the ECM was assessed through confocal microscopy.

Primary MSCs were seeded onto the dECM substrates, and their proliferation and differentiation were assessed according to our previously published protocols.

RESULTS AND DISCUSSION

dECM on substrates with a stiffness $\geq$20kPa exhibited a more fibrous and aligned microarchitecture, while dECMs on softer substrates exhibited no noticeable alignment within the structure.

MSC proliferation and differentiation were found to vary with both substrate stiffness and with the presence or absence of dECM. Specifically, MSCs on the 4kPa substrates with dECM exhibited the most robust proliferation. In contrast, MSCs on the stiffest substrates with dECM exhibited the most robust osteogenic differentiation.

CONCLUSION

These results illustrate that substate stiffness can be used in cooperation with MSC-derived dECM to better control the behavior of MSCs cultured on the surfaces. Specifically, the judicious choice of substrate stiffness with dECM can lead to a more proliferative MSC phenotype or improved osteogenic differentiation. These results will inform the design of future tissue engineering scaffolds for regenerative medicine applications.

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ACKNOWLEDGMENTS

This work was supported by the Victorian Medical Research Acceleration Fund (2019-Round 3) and an ARC Future Fellowship (FT190100280). We gratefully acknowledge the support of the University of Melbourne and an Australian Government Research Training Program Scholarship.

We would like to thank patients who generously consented to use of their samples in this research, clinical research midwives Ms Moira Stewart and Ms Sue Duggan, lab technician Ms Janet Stevenson for technical advice, Dr Tian Zheng for assistance with atomic force microscopy measurements, and Dr Hemayet Uddin for assistance with zeta potential characterization. We would also like to thank the Melbourne Centre for Nanofabrication and the Materials Characterisation and Fabrication Platform for allowing the use of their instruments.

>> 15:30 - 16:30 | PSOP-05 - Surface functionalization

Chairpersons: Marie-Christine Durrieu & Aldo Boccacini
Location: Room B

15:30 | O1 Surface - Bone Adhesive with High Mechanical Strength and Natural Bone Replacing Capabilities Shows Clinical Potential in Implant Fixation to Osteoporotic Bone.
Gerard INSLEY, PBC Biomed, Shannon, Ireland

15:45 | O2 Surface - Development of tough 3D-printed biomimetic calcium phosphate scaffolds for bone regeneration
Maria-Pau GINEBRA, Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering, Universitat Politècnica de Catalunya; Barcelona Research Centre for Multiscale Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain

16:00 | O3 Surface - Bone sialoprotein immobilized in collagen type I induces bone regeneration in vitro and in vivo
Ulrike RITZ, Department of Orthopedics and Traumatology, University Medical Center of the Johannes Gutenberg-University, Mainz, Germany

16:15 | O4 Surface functionnalization - Surface Modifications on Titanium Alloy : A Promising Way to Understand and Fight Implant- Related Infection
Frédéric VELARD, University of Reims Champagne-Ardenne, Reims, France

Bone Adhesive with High Mechanical Strength and Natural Bone Replacing Capabilities Shows Clinical Potential in Implant Fixation to Osteoporotic Bone.

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INTRODUCTION

Osteoporosis is a silent epidemic, where 1 in 3 women and 1 in 5 men are predicted to experience osteoporotic fractures and a likely repeat fracture [1]. For example, rotator cuff repairs have a retear rate of 35-90% [2] due to bone anchor pullout and suture tear. Bone cements increase augmentation between bone and implant, however current clinically used cements such as PMMA and calcium phosphate cements (CPCs) either remain as a foreign substitute in the body or lack adequate mechanical properties.

A novel adhesive cement was developed from observations of a Molluscs ability to adhere to wet rocks – a key feature for adhesion to biological tissues, such as bone. The combination of the building-block-like structure of CPCs and introduction of the adhesive molecule, phosphoserine; forms OsStic, a bone cement that balances biodegradability with good adhesive mechanical properties (Figure 1 [3]). Preclinical studies have shown its high mechanical adhesive strength along with capabilities in adhering bone together and its replacement with natural bone during the healing period.

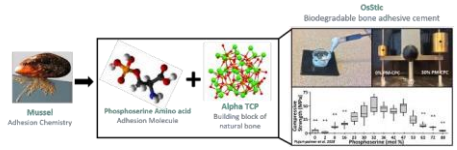

Figure 1

In this study, three bone cements were analysed: PMMA (Simplex), CPCs (Hydroset) and the novel bone adhesive (OsStic), to assess the performance of bone cements in improving functionality of bone anchors in rotator cuff repairs for osteoporotic patients.

EXPERIMENTAL METHODS

A custom-designed experimental setup was built to test pullout strengths of two commercial bone anchors (Smith & Nephew, Arthrex) with the three bone cements in osteoporotic saw-bone models (n=12). 8mm wide and 17.5mm deep cavities were predrilled into the sawbones and filled with bone cement, mimicking operating conditions. Insertion of anchors followed specific set times relevant to the cement used. Once cured, the sawbone-anchor-cement models were fixed in the mechanical testing machine and sutures gripped for tensile loading at 5mm/min displacement using ASTM standards. Pullout strengths were compared across the three bone cements for each bone anchor.

RESULTS AND DISCUSSION

Highest pullout strengths (>700N) were measured for both OsStic (n=2) and PMMA (n=3) in Smith & Nephew and Arthrex anchors, respectively. Hydroset, a calcium-phosphate cement had lowest pullout strengths (<700N, n=4) in both anchor designs (Figure 2). Overall, OsStic and PMMA showed highest pullout strength across all three cements. The specific design of the anchors showed to have significant effects on the pullout performance in the cements, especially notable in OsStic and PMMA, where OsStic preformed best in Smith & Nephew and PMMA preformed best in Arthrex. This inidicates the importance of the interaction between anchor geometry/bone cement and resulting combined funciton.

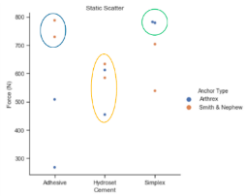

Figure 2

CONCLUSION

The comparable pullout strengths between OsStic and PMMA, a synthetic bone cement, highlights the high mechanical strength of OsStic, which eventually biodegrades and is replaced by natural bone, resulting in highly desirable clinical properties for a bone cement. Difference in cement performance was also observed across different anchor designs, highlighting the importance and need for carefully designed anchors to enhance augmentation between implant-cement-bone. Overall, this pilot study presents the first bone adhesive cement used for implant augmentation, and shows the potential of OsStic in future clinical applications.

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Development of tough 3D-printed biomimetic calcium phosphate scaffolds for bone regeneration

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INTRODUCTION

Direct ink Writing enables the fabrication of bone scaffolds with a precise control of both external shape and internal porosity. The development of reactive calcium phosphate (CaP) inks that are able to harden at body temperature through a setting reaction has brought numerous advantages. Besides resulting in biomimetic compositions, closer to the mineral phase of bone and avoiding shrinkage problems[1], it opens the door to the combination with organic second phases and the design of composite scaffolds. Polycaprolactone (PCL) has drawn much attention for biomedical applications due to its high toughness and slow degradation compared to other polyesters[2]. This study aims at overcoming the inherent brittleness of 3D-printed CaP ceramic scaffolds by using PCL organogels as binders in alpha-tricalcium phosphate (α-TCP) based inks. Moreover, the addition of polyethylene glycol (PEG) is explored as a plasticizer[3]. Our goal is to analyze the effects of the PCL and PEG contents as well as of the ceramic loading on the rheological properties of the ink and the mechanical, physico-chemical and biological properties of the 3D printed scaffolds.

EXPERIMENTAL METHODS

The inks were prepared by loading PCL organogels (15-20 wt.%), containing different amounts of PEG (0-10 wt.%), with increasing proportions of α-TCP powder. An α-TCP ink prepared with Pluronic F127 hydrogel as a binder was used as a control (C ink). Rheological studies were carried out in a rotational rheometer. 3D printed bar-shaped scaffolds with a nozzle diameter of 410 µm and a distance between filaments of 250 µm, were used to assess the mechanical properties by 3-point bending tests. Printability and shape fidelity, adhesion between printed filaments and microstructure of the scaffolds were assessed by optical and scanning electron microscopy (SEM). Porosity was quantified by mercury intrusion porosimetry (MIP), specific surface area (SSA) by nitrogen adsorption and the skeletal density by helium pycnometry. The in-vitro cell behavior of the PCL containing scaffolds and the C ink was evaluated using human mesenchymal stem cells. Cell adhesion, viability and proliferation were assessed at 24 h and 7 days.

RESULTS AND DISCUSSION

The rheological behavior of the PCL ink showed an elastic to plastic transition, in contrast to the typical viscoelastic behavior presented by the C ink. A minimum ceramic loading was required for optimal printability. The addition of PEG enhanced the adhesion between printed layers (Fig. 1A and B). However, a too high concentration of PEG (10 wt.%) resulted in a plastic behavior of the as-printed samples. The addition of PEG did not modify the SSA (15.2-16.5 m^2/g), the skeletal density (2.54-2.59 g/cm^3) and the microporosity. The presence of both polymers did not disturb the setting reaction and an entangled network of needle-like CDHA nanocrystals was obtained in all cases, with PCL fibers homogenously distributed (Fig. 1C and D). Regarding the mechanical properties, the use of the PCL organogels resulted in a 2-fold increase of the bending strength, and a 5-fold increase of the work of fracture compared to the C ink, therefore reducing significantly the brittleness of the ceramic scaffolds. Excellent cell adhesion and proliferation was found on the PCL containing scaffolds.

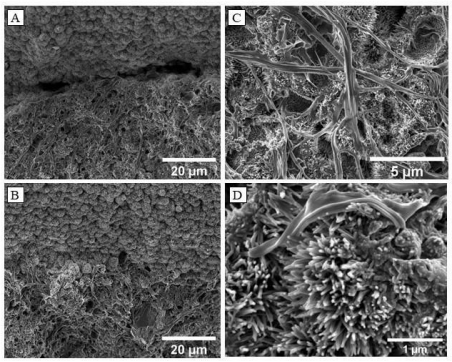

Fig. 1. Scanning electron microscopy images. A) Layer adhesion of PEG0. B) Layer adhesion of PEG5. C and D) Microstructure of the PCL containing scaffolds.

CONCLUSION

The incorporation of biocompatible and biodegradable polymers in the binder of self-setting inks is a promising method for enhancing the mechanical properties and reducing the brittleness of calcium phosphate bone scaffolds, preserving the excellent biological properties.

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ACKNOWLEDGMENTS

Spanish Ministry of Science and Innovation through PID2019-103892RB-I00/AEI/10.13039/501100011033 and FPU scholarship of LdM

Bone sialoprotein immobilized in collagen type I induces bone regeneration in vitro and in vivo

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INTRODUCTION

Bone fractures of critical size still present a major problem for orthopedists and trauma surgeons today. Frequently, this results in impaired fracture healing, in which the bone itself does not regenerate completely - pseudarthrosis develops in 5 - 10 % of all fractures (1). The gold standard for treating pseudarthrosis is autologous bone grafting, however, this therapy is associated with many disadvantages, e.g. second surgery, limited available material, etc. New biomaterials combined with bioactive molecules that promote bone regeneration could provide new therapeutic options. Bone sialoprotein (BSP) as a component of the extracellular matrix represents a promising bioactive molecule to induce osteogenic properties (2). However, published studies show that the carrier material to or into which BSP is coupled seems to play a crucial role in the effectiveness of BSP (3). Therefore, the aim of this study was to characterize the role of BSP immobilized in collagen I both in vitro and in vivo and to define an optimal setting for the effectiveness of BSP in order to develop a new bioactive osteogenic biomaterial.

EXPERIMENTAL METHODS

Primary human osteoblasts were cultured with and without BSP in a three-dimensional collagen gel and the influence of BSP on both proliferation and viability (AlamarBlue assay) as well as on gene expression of specific markers was analyzed (real-time PCR). Subsequently, the influence of BSP was investigated in vivo in two different rat models with critical size bone defects. The first model was a calvaria defect model in which collagen gels with immobilized BSP were placed; the second was a femur defect model in which a polylactide cylinder was printed with a 3D-filament printer (Ultimaker2+) and manually filled with collagen + BSP using different concentrations. In both models, bone regeneration was analyzed by X-ray during the standing period and by µCt and histology after standing periods of 4 and 8 weeks, respectively.

RESULTS AND DISCUSSION

The in vitro studies showed a slightly positive effect of BSP on the proliferation of osteoblasts. Regarding the effect of BSP on gene expression in both an osteoblast monoculture as well as in a coculture model with endothelial cells, we observed a significant increase in gene expression of the transcription factor Runx2 and the osteogenic marker osteopontin. The first in vivo model showed that BSP bound in collagen type I induced a slightly positive effect in the Calvaria defect with respect to bone regeneration compared to a collagen gel without BSP. In contrast in the second in vivo model employed, BSP bound in collagen type I and applied in a PLA cylinder showed a significant increase in bone regeneration compared to the group without BSP in a critical size femoral defect in the rat. Figure 1 summarizes the effects of BSP on bone regeneration in vitro and in vivo.

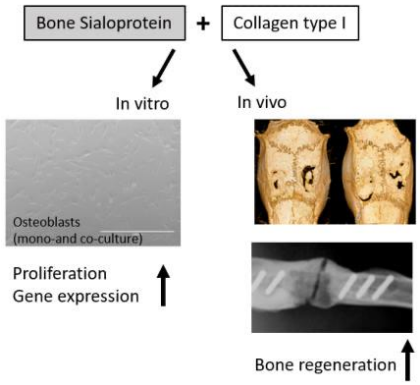

Figure 1: Effects of BSP immobilized in collagen type I on bone regeneration in vitro and in vivo.

CONCLUSION

Our study shows a significant positive effect of BSP on bone regeneration in combination with type I collagen. This fact offers many possibilities to use BSP modified collagens as bone graft substitutes; since collagen type I is already used in many medical applications (4), modification with BSP offers a simple modification with enormous potential for the therapy of poorly healing bone fractures.

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Surface Modifications on Titanium Alloy : A Promising Way to Understand and Fight Implant-Related Infection

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INTRODUCTION

The need for joint prostheses is continuously increasing due to the aging of the population. Unfortunately, even taking into account prophylaxis and aseptic surgical techniques, the risk of bone and joint infections does not decrease (approximately 1-2% of procedures). Prosthetic or peri-prosthetic osteoarticular infections are defined as postoperative osteomyelitis (bone infection), characterized by bacterial colonization leading to an inflammatory reaction and bone resorption[1]. In such a clinical context, it becomes urgent to consider new strategies and develop new molecules to treat acute and chronic infections due to bacteria, especially when they are forming biofilm. Indeed, several publications have shown the significant influence of culture conditions on the structure and formation of biofilm[2], but also the influence of the surface on which the biofilm develops[3]. It is also possible that these surfaces play an antibiofilm role actively (functionalization of an antimicrobial molecule) or passively through mechanical or physical properties. Here we investigate different surface topography for their ability to influence bacterial adhesion and biofilm formation.

EXPERIMENTAL METHODS

Materials: Titanium disks (Ti6Al4V, 12 mm diameter) were textured using a 1030nm (infrared) femtosecond laser (20W). We prepared 11 different LIPSS (Laser-induced periodic surface structures) made of ripples, cones, pillars or lamellae of various depth and periods.
Bacteria: SH1000, methicillin-sensitive *S. aureus* strain was grown in minimal medium under hypoxic conditions using the GenBag system. Overnight cultures were diluted to a final absorbance of 0.01. Bacteria were then seeded on titanium disks and incubated at 37°C for 24 h.
Biofilm evaluation by scanning electron microscopy: Disks were washed twice in PBS, then fixed in 2.5% glutaraldehyde at room temperature for 1 h. After two distilled water rinses, biofilms were dehydrated in graded ethanol solutions (50, 70, 90, and 100% twice) for 10 min. Biofilms were finally desiccated in a drop of hexamethyldisilazane, air dryed, then sputtered with a thin gold–palladium film using a JEOL ion sputter JFC 1100 instrument. Biofilms were observed using a JEOL JSM-7900F microscope.
Biofilm evaluation by confocal laser scanning microscopy: Disks were washed twice in PBS and stained with SYTO™ 9 at 1 µM and propidium iodide at 20 µM to label live and damaged or "dead" bacteria. After 30 min of incubation, each disk was washed two times with PBS and covered with glass coverslip before observation with confocal laser scanning microscopy (LSM 710 NLO, Zeiss). Fluorochromes-labeled bactera were imaged and their volume quantified using IMARIS software (Imaris, RRID:SCR_007370).
Statistics: Non parametric Wilcoxon-Mann-Whitney test was performed to assess significance of our results (n=6 data per condition, $p<0.05$) (StatXact7,Cytel Studio).

RESULTS AND DISCUSSION

On differently textured titanium, we observed an organization of bacterial community and a quantity of adhered bacteria that varied according to the types of motifs. Live/Dead staining evidence that some textures on titanium showed a reduced number of adhered bacteria compared to control surface, but also an increase of dead bacteria proportion (almost 50 % of dead bacteria). Of particular interest, SEM analyses did not reveal any morphological alterations of bacteria wall, suggesting that surface may impact the metabolism and biofilm onset program. Of interest, we tested albumin or fibronectin coating of titanium prior to bacteria seeding to mimic first seconds of implant setting inside patients. We evidenced that proteins fewly modified SH1000 behaviour, even if a slight trend to the increase of bacteria number may be appreciated.

CONCLUSION

We evidenced at least two textures of interest on titanium to develop antibacterial surface intended to be used on bone implant.

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ACKNOWLEDGMENTS

The authors would like to thank Institut Carnot MICA (InvitrOS exploratory program) and ESCMID (InvitrOS Research Grant program) for providing financial support to this project, and PICT imagery facility for scanning electron microscopy and confocal laser scanning microscopy.

>> 15:30 - 16:30 | PSOP-06 - Biomaterials processing

Chairpersons: Michael Gelinski & Riccardo Levato
Location: Room C

15:30 | O1 Processing - Fetuin A functionalization of biodegradable nonwovens towards biomimetic guided bone regeneration

Stefan OSCHATZ, Institute for Biomedical Engineering, Rostock University Medical Center, Rostock, Germany

15:45 | O2 Processing - The freeze-casting process: a promising tool for the fabrication of macroporous composite scaffolds for bone substitution

Jérémy SOULIE, CIRIMAT, Université de Toulouse, CNRS, Toulouse INP - ENSIACET, Toulouse, France

16:00 | O3 Processing - Three-dimensional Architectural Design and Assessment of Ice-templated Collagen Scaffolds for Tissue Engineering Applications

Huijie Lillian ZHANG, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

16:15 | O4 Processing - Versatile method for high-throughput fabrication of microparticles with defined geometries and size for biomedical applications

Marta MACIEL, CEB -Center of Biological Engineering, University of Minho, Braga, Portugal; CICECO – Institute of Materials of Aveiro, University of Aveiro, Aveiro, Portugal

Fetuin A functionalization of biodegradable nonwovens towards biomimetic guided bone regeneration

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INTRODUCTION

Hard tissue defects remain a challenge in regenerative medicine, particularly in dental, maxillofacial and tumor-related surgery. Autologous replacement is the material of choice, but availability is limited and collection is often accompanied by harvesting-associated co-morbidities at the donor site. Synthetic materials, most frequently calcium phosphate (CaP) cements and ceramics, represent an attractive option, but are often insufficient in terms of osteoconduction, osteoinduction or osteogenesis.[1]

EXPERIMENTAL METHODS

In this work, biodegradable PLLA-co-PEG nonwovens were manufactured by electrospinning from polymer solution using a mixture of $CHCl_3$/trifluoroethanol as solvent. Fetuin A was covalently linked to the surface of the fibers using common EDC/NHS coupling.[2] To generate superficial functional groups, the nonwoven material has been preliminary treated with O_2-plasma (Fig. 1 A). Protein loading after functionalization has been determined using a Fetuin A specific enzyme-linked immunosorbent assay on the remaining supernatant after protein coupling. CaP affinity of the functionalized nonwovens in comparison to the unmodified nonwoven has been investigated with a tailored *in vitro*-calcification protocol.[3,4] For this, a metastable solution of calcium phosphate in HEPES buffer was used, adjusted for a CaP ion product of 9 mM^2, resulting in a calcium/phosphate ratio as in hydroxyapatite. Samples were incubated for 7 days in the calcification medium, subsequently rinsed with distilled water and dried under reduced pressure. CaP load was quantified using a photochemical Arsenazo III assay and morphological characterization of the mineralization was performed using SEM imaging.

RESULTS AND DISCUSSION

Fetuin A exhibits a distinct calcium affinity.[5] Superficially immobilized Fetuin A complexes calcium ions from the surrounding medium, which in turn act as crystallization nuclei, initializing biomimetic hydroxyapatite crystallite mineralization (Fig. 1 C). CaP formation takes place homogeneously distributed over the entire surface of the functionalized nonwoven. In contrast to the use of seed crystals, the entire nonwoven fibers are thus surrounded by a CaP layer, maintaining the fibrous morphology of the nonwoven.

In terms of the base material, nonwovens exhibit unique properties, as for instance an exceptionally high surface-to-volume ratio. Moreover, nonwovens possess a porous fiber interstice, mimicking biological extracellular matrix architecture. The porous morphology supports cell infiltration and could enhance new bone formation. The structural properties of the nonwovens allow for facile implantation and versatile application, as these materials are highly flexible and can easily adapt to the shape of complex hard tissue defects.

CONCLUSION

Fetuin A modification of biodegradable nonwovens appears to be a novel and feasible approach towards enhanced biomineralization of rather unpolar polymer-based biomaterials. Our initial functionalization results indicate a distinct enabling of CaP crystal growth on the material surface, as well as in the material bulk under *in vitro* conditions, compared to the untreated nonwoven. Fetuin A functionalized nonwovens show promising properties and potential for application in oral and maxillofacial bone regeneration, or for improved bone fracture healing with osteosynthesis implants.

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The support of Rostock University Medical Center within the FORUN program is gratefully acknowledged.

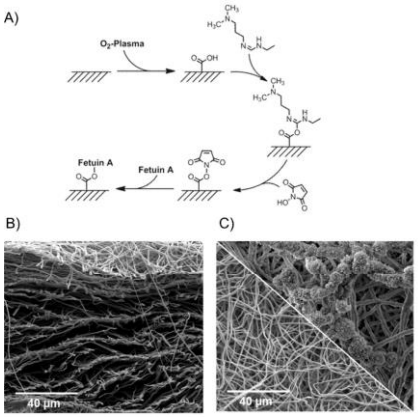

Fig.1: A) Schematic of Fetuin A functionalization by EDC/NHS coupling. B) Cross sectional SEM image of PLLA-co-PEG nonwoven. C) SEM images of pure PLLA-co-PEG nonwoven compared to Fetuin A functionalized PLLA-co-PEG nonwoven after accelerated *in vitro* calcification.

The freeze-casting process: a promising tool for the fabrication of macroporous composite scaffolds for bone substitution

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INTRODUCTION

Numerous scaffolds have been developed in the field of bone substitutes to fill defects larger than 1 cm3 in non-bearing sites. Ideally, such scaffolds should: i) stimulate the new bone formation by enhancing cell differentiation, proliferation and adhesion) iii) exhibit adequate macropore size and volume in order to allow cell colonization and angiogenesis), iv) maintain mechanical properties during handling by surgeons. Some inorganic materials, such as calcium phosphate or bioactive glasses, partially meet some of these criteria. Nevertheless, the mechanical cohesion of such materials is often obtained after high temperature treatment that prevents the shaping of metastable materials or their association with active (bio)molecules. Moreover, they have high brittleness and low elasticity, which is prohibitive for their cut by the surgeon before the medical device implantation. These drawbacks can be avoided using polymeric-based composites[1]. Inorganic fillers have been associated either to natural or synthetic polymer matrices. Dense composites subjected to high mechanical stress in bearing sites (like interference screw) can be molded by injection but the treatment of a non-bearing site such as mandibular bone requires lower mechanical properties but higher macroporosity to enhance new bone formation (colonization, angiogenesis) into the defect and mimick mandibular trabecular bone: 70-80% of porosity and pore size of 300-400 µm with partial anisotropic orientation of porosity/trabecules[2]. Porous composites could be elaborated by various processes: electrospinning, hard template methods, 3D-printing, supercritical CO_2, each having their own advantages.

The present work is focused on the freeze-casting[3] technique that is based on the control of solvent crystal nucleation/growth and its subsequent sublimation without any issue related to template removal. Through several examples, the aim of this contribution is to highlight correlations between formulation parameters (nature/size of inorganic fillers, nature/functionalization/length of polymers, solvent nature/amount), the process parameters (freezing temperature) and the final properties of scaffolds.

EXPERIMENTAL METHODS

Several (nano)fillers were synthesized by co-precipitation or sol-gel routes and fully characterized (XRD, NMR, DLS, granulometry, TEM). The latter were associated with synthetic or biosourced polymers via functionalization, in situ synthesis or simple mixing and the corresponding suspensions were freeze-cast. Resulting scaffolds were studied via SEM, porometry, X-Ray microtomography, SAXS. Compression and degradation tests were also carried out.

RESULTS AND DISCUSSION

It was demonstrated, for instance, that the PDLLA-grafting[4] onto the surface of bioactive glass nanoparticles improves their dispersion in the pore walls and their spatial distribution within the whole scaffold and finally their recovery rate[5]. We also showed that the apatite/PLGA ratio has a strong influence on the Young's modulus (decreasing the polymer segment mobility) and in vitro degradation properties (buffering effect)[6]. Porosity orientation and size have also been proven to be process-driven, in particular via the freezing temperature and direction. Finally, appropriate processes allowed to integrate ionic/ molecular active agents in fillers (exfoliating Layered Double Hydroxide or doping bioactive glasses or apatite).

CONCLUSION

All these results demonstrated that well-chosen smart (nano)filler/polymers associated to the freeze-casting technique lead to well-controlled scaffolds with tunable properties allowing them to adapt to different bone pathologies.

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Three-dimensional Architectural Design and Assessment of Ice-templated Collagen Scaffolds for Tissue Engineering Applications

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INTRODUCTION

Ice-templating is a versatile technique to produce porous scaffolds, and architectures can be controlled by carefully designing the temperature profiles during the freezing process[1]. However, the precise control of structures in three-dimensional (3D) space remains a problem. In this work, we describe a method to produce collagen scaffolds with controlled pore alignment and orientation, and we propose a modelling toolkit for the rapid design and prediction of the final 3D network. The scaffold structures were characterised using X-ray microtomography (μCT). Mechanical testing was performed under compression and tension and the thermal stability of collagen was also assessed.

EXPERIMENTAL METHODS

1 wt% collagen in 0.05 M acetic acid was directionally frozen in a custom-made device, and the structures were imaged using μCT. The scaffolds were then chemically crosslinked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and N-hydroxysuccinimide (EDC/NHS) at 10% concentration of that reported previously in the literature[2]. The pore orientation was controlled via heat patch configuration and base design. The development of different designs was aided by finite element modelling in COMSOL (Fig. 1a-c) to achieve specific orientations in 3D. Fourier transform infrared spectroscopy (FTIR) studies from room temperature to 80 ℃ were used to investigate the denaturation of wet collagen near heat patches. The mechanical tests were undertaken by immersing scaffolds in water, using a 25 N load (Fig. 2a). Testing direction and loading cycles were studied.

RESULTS AND DISCUSSION

The directional solidification technique successfully produced scaffolds with highly aligned porosity, and the simulation results matched with the μCT images well. A single heat patch was found to divert the pore orientation towards the heat input while maintaining macroscopic alignment (Fig. 1a). Five heat patches arranged around a cylindrical design with sequential heating configurations led to a circumferential shift of pore direction in 3D (Fig. 1b). FTIR studies suggested that wet collagen maintained triple-helix structures when the temperature and exposure time increased up to 80 ℃ and 2 hours, providing guidance for heat patch designs. Additionally, the base design was found to influence the pore orientation without any heat patch inclusion (Fig. 1c-d). Under tension, the average failure strain was measured as 56±7 %, and the failure stress was 75±17 kPa. The anisotropy in compressive mechanical properties is shown in Fig. 2b. Three distinct regions were identified on the stress-strain curve when tested in direction 1, and a higher elastic modulus 6.2±1.5 kPa was noted in contrast to 1.0±0.2 kPa in direction 2. Furthermore, the position-controlled cyclic loading indicated a phase lag between strain and force. Permanent initial stretch was found in all samples.

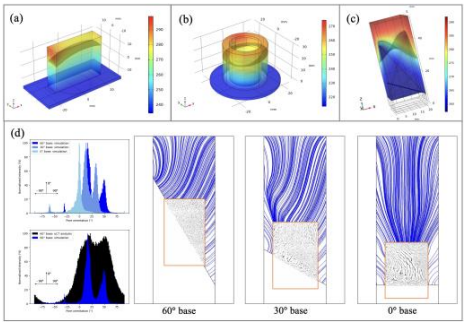

Fig. 1 Simulation of the temperature profile (unit: K) during the solidification process and comparison with μCT images. The black surfaces in simulations represent the ice freezing front (IFF) location. (a) Simulation of cuboid design with one heat patch. (b) Simulation of hollow cylindrical design with five heat patches distributed on the inner circumferential plane. (c) Simulation of curve shift design, where its base has a total angle shift of 120°, from -60° to 60°. (d) The qualitative and quantitative comparison between temp filed simulation (blue) and μCT images (black).

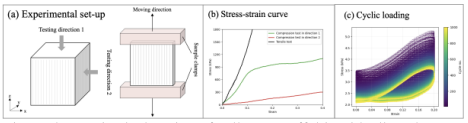

Fig. 2 The mechanical testing of collagen scaffolds with aligned porosity. (a) Schematic diagram of experimental set-up (left: compression; right: tension). (b-c) The typical stress-strain curves for (b) compression and tensile tests, and (c) cyclic tensile loading.

CONCLUSION

Simulations provided an accurate model of the structures produced experimentally. FTIR revealed that the use of the heat patches to control architecture did not lead to collagen degradation. The elastic modulus along the pore alignment was measured as 6.2±1.5 kPa hence suitable for applications in neural, cardiac and skeletal context. This work offers new insights into recapitulating complex structural features of native tissues.

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Versatile method for high-throughput fabrication of microparticles with defined geometries and size for biomedical applications

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INTRODUCTION

Recent trends in tissue engineering towards a minimalist approach of materials' use are focusing in microparticles to act as both cell carriers and modulators of cell fate.[1] The design of these particles is a key factor in cell modulation as biophysical properties such as topography, material, stiffness, size and shape affect cell response and outcomes.[2] They can also be easily manipulated to release biochemical cues such as drugs, molecules or growth factors in a controlled manner during applications like drug delivery or tissue regeneration.[3] However, simple protocols aiming for the production of a high number of particles with different characteristics such as shape, remain scarce.[4] Our goal is to develop a method that could be able to produce defined shaped microparticles in an easy way and broadly applicable to design microparticles made of different materials.

EXPERIMENTAL METHODS

3D printed templates with defined geometry (circles and squares) were prepared by a cast molding technique using polydimethylsiloxane (PDMS). Briefly, a PLA mold was designed in SolidWorks and produced in a 3D printer with the defined shapes and size, allowing the fabrication of a PDMS counter mold with several pillars (Figure 1A-C). The general method can then be employed consisting of: (i) the deposition of a sacrificial material layer at the top of the pillars and (ii) followed, by the application of the pretended material solution used for microparticles' fabrication. The release of the microparticles is then simply achieved by sacrifice of the first layer used to coat the PDMS pillars. In this context, a 10 wt% gelatin was used as the sacrificial layer and the silica source solution (aqueous 100 mM TMOS) was applied. After drying, the templates were put in 60ºC water bath and the microparticles released (Figure 1D). Human adipose mesenchymal stem cells were stained with cell Vybrant dye (DIO) and seeded in these microparticles (Figure 1E). To proof the method versatility the same type of template was used to produce chitosan microsheets. The sacrificial layer was achieved with 2% alginate solution further cross-linked with calcium solution. A EDTA bath was used to allow the release of these microsheets (data not shown).

RESULTS AND DISCUSSION

Thin microparticles can be produced with different shapes, sizes and materials using a 2-step protocol (Step 1: a sacrificial layer that shouldn't chemically interact with the microsheet material and 2: the microsheet precursor solution should be able to form a film). Just by selecting different sacrificial layers, the microparticles can be formed setting simple details and released from the template. Although these results only presented the materials used in both steps as solution systems, future experiments could explore other materials in different phases.

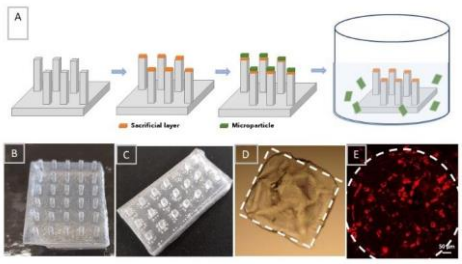

Fig. 1 – A. Schematic illustration of the method. B and C) PDMS templates (circles and squares, respectively). D) Square silica microparticle. E) Circle microparticle with cells (red).

CONCLUSION

Microparticles' design is becoming an important feature for tissue engineering. Their importance in the field of tissue engineering, specially, in bottom-up strategies is increasing. In the future, microparticles production scale-up will be a critical point for clinical translation or others fields in bioengineering, as the techniques available so far are still slowly developing. There is a clear need for faster, inexpensive, and simpler approaches that can produce in large amounts such kind of materials. A simple template and straightforward method were developed and easily adapted to the different type of materials from inorganic to natural polymers.

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ACKNOWLEDGMENTS

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>> 15:30 - 16:30 | PSOP-07 - Soft tissue engineering

Chairpersons: Alexandra P. Marques & Micheal Doser
Location: Room F

15:30 | O1 Soft tissue - Pathogen-Resistant Dermal Clay Dressings for Healing Burns, Wounds, and Scars

Kausik MUKHOPADHYAY, Department of Materials Science and Engineering, University of Central Florida. Orlando. USA

15:45 | O2 Soft Tissue - Porous Poly(glycerol sebacate)-based Scaffolds for Adipose Tissue Engineering Applications

Rachel FURMIDGE, Materials Science and Engineering, Kroto Research Institute, The University of Sheffield, Sheffield, UK

16:00 | O3 Soft Tissue - Advanced Synthetic Materials for 3D Printing Applications and its Use as Scaffolds for Airway Regeneration.

Luis SORIANO, School of Pharmacy and Biomolecular Sciences, RCSI, Dublin, Ireland; Tissue Engineering Research Group, RCSI, Dublin, Ireland; SFI Centre for Research in Medical Devices (CÚRAM), NUI, Galway, Ireland

16:15 | O4 Soft Tissue - A PGSAA-based adhesive filler for perianal fistulas

Elodie LLUSAR, TISSIUM, Paris, France

Pathogen-Resistant Dermal Clay Dressings for Healing Burns, Wounds, and Scars

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Introduction: Globally, more than 11 million people are victims of severe burn injuries each year[1]. A large number of those patients undergo hemorrhagic shock due to severe burns and pain inflicted from burn wounds and often develop multiple organ failure and infection[2]. As such, nearly 4 million victims require further hospitalization and advanced treatments. Treatments for burns include topical agents that have several disadvantages, including contact dermatitis, increased antibiotic resistance, toxicity through absorption, and disruption of microbiome on the skin[3]. The purpose of our study is to engineer a unique bandage system using a sustainable approach and evaluate its capacity to reduce the proliferation of the most common skin wound pathogens and heal scars. Herein, we present a cost-effective, microbe-resistant material that can be used for burn wounds. Our goal is to understand and apply the fundamentals of materials and chemical interactions and develop a product that can be contoured as a bandage and easily used in casualties.

Experimental Methods: This novel microbe-resistant material has been made of organo-modified, metal-exchanged clay scaffolds. Synthesis of hybrid clay films were carried out using optimization of metal and trimethyl glycine (TMG) precursors with Bentonite clay. TMG variants were synthesized based on reported procedures for developing free-standing clay films. Typical synthetic steps to make the hybrid clay films are: (i) mechanical mixing of clay, TMG precursor and deionized water in different ratios, (ii) to this viscous solution, calculated amount of metal salt or organic precursors are added and the clay is exfoliated, (iii) the solution poured and made to films using heat-mediated slurry coating applicator. Once dry, the hybrid clay films are cut in sizes and stored for testing and characterization. The films were tested against gram-negative and gram-positive bacteria. Single colonies of the bacterial strains were individually inoculated in LB broth at 120 rpm at 37 °C until the 0.5 optical density (O.D.) was achieved. The final O.D. of the bacterial solution was measured by UV-Vis and adjusted to 0.1 for studying the antibacterial activity[4].

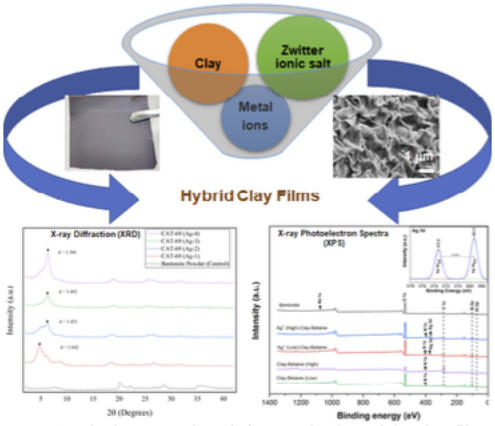

Fig 1. Synthetic approach and characterization of the clay films using powder XRD, XPS and SEM imaging.

Results and Discussion: Several modifications of the clay-based hybrid films were carried out with and without metal precursors, e.g. silver, copper, bismuth and sulfanilamide. Powder-XRD, ATR-FTIR, XPS, ICP-OES, DMA, SEM, analyses were carried out to ascertain the integrity of the clay aluminosilicate structure, strength, elemental compositions and hydrophilic properties of the films that are essential healing cascade. DMA tests have shown mechanical durability through increasing basal spacing from TMG incorporation in the clay layers through exfoliation. The substantial shift of the $d_{(100)}$ spacing (Fig 1, XRD) from 1.14 nm in pristine Bentonite clay to extremely ordered 1.81 nm for TMG-Ag functionalized clay films indicate a pseudo tri-layer NH_4^+-alkyl arrangement in the clay. The increase in basal spacings also helps incorporate the metal ions or organic molecules that impart the antimicrobial or antifungal properties in the films. XPS analyses (Fig 1) show presence of Ag $3d_{3/2}$ and Ag $3d_{5/2}$ with binding energy values at 372.2 eV, 367.2 eV, and 400.1 eV for N 1s from the TMG present in clay, further validated by the –C≡N frequency at 1394 cm^{-1} from ATR-FTIR analyses. Pathogen resistance of the clay samples was studied using *E. coli* and *S. aureus* using zones of inhibition for 18 hours with the Kirby Bauer disk susceptibility test that showed 100% efficacy. A 2nd degree burn protocol using control, Silvadene cream and our clay dressing for porcine trial exhibited a superior performance for the clay system upon comparison with burn wound healing features (Fig 2).

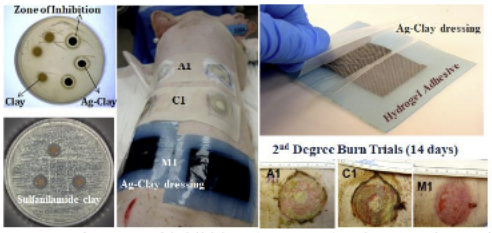

Fig 2. *Left*: Zone of inhibition (*S. aureus*) using Ag-clay and Sulfanilamide-clay; *Middle & Right*: 2nd degree burn trial for porcine model to test wound healing: Silvadene (A1), Control (C1), Ag-Clay (M1).

Conclusion: The hybrid clay films are a cost-effective solution to deter infections. It has been possible to develop cost effective approach to engineer durable clay films with advantages over the current topical systems for burns, antibacterial and antifungal efficacy. We are currently engaged in anti-fungal efficacy and 3rd degree burn model studies.

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Porous Poly(glycerol sebacate)-based Scaffolds for Adipose Tissue Engineering Applications

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INTRODUCTION

Autologous fat grafting is one of the most commonly performed procedures by plastic surgeons, used for reconstructive surgeries to repair soft tissues. However, the technique is not without limitations. Primarily, there is a reported 40-60% reduction in graft volume following transplantation, thought to be caused by poor survival of mature adipocytes once implanted following a lack of revascularization at the donor site, leading to tissue necrosis and its subsequent resorption[1]. Therefore, there is a clinical need for an improved fat graft that can maintain volume following implantation.

Adipose tissue engineering strategies aim to develop appropriate biomaterials that can facilitate the survival and revascularization of transplanted adipose tissue. Poly(glycerol sebacate) (PGS) is a synthetic biomaterial with a similar structure to triglycerides, and which is highly suited to adipose tissue engineering, as it is much softer than commonly used biomaterials and is also highly elastomeric, mimicking the mechanical properties of adipose tissue[2]. Following the methacrylation of PGS (PGS-M)[3], it can be photocrosslinked, and, by altering the degree of methacrylation, its mechanical and biodegradation properties can be tuned to meet the requirements of the target tissue. By designing and optimizing PGS-M scaffolds, we aim to create porous scaffolds that can be combined with adipose tissue to improve the volume retention of autologous fat grafts.

EXPERIMENTAL METHODS

PGS was synthesised and methacrylated to different degrees (40%, 60% and 80%). High internal phase emulsion templating of PGS-M pre-polymer was used to create a 'water-in-oil' emulsion, which was cured into porous three-dimensional scaffolds within cylindrical moulds, then sectioned into ~1 mm thick ~0.6 cm diameter discs. The parameters of scaffold fabrication were altered to create scaffolds with varying mechanical properties and porous structures. The mechanical properties of PGS-M scaffolds were measured through compressive testing and cyclic loading. Scaffolds were seeded with adipose-derived stromal cells (ADSCs), which are MSC-like adipocyte precursor stem cells. Resazurin reduction assay was used to assess cell metabolic activity of ADSCs cultured on PGS-M scaffolds, as an indication of cell viability. Histological analysis was performed to assess the migration of ADSCs into PGS-M scaffolds at different time points throughout culture (14 days).

RESULTS AND DISCUSSION

PGS-M was synthesised and found to have a degree of methacrylation (DM) within an acceptable range of the theoretical DM (40%, 60% and 80%). Scaffolds were fabricated with a range of pore sizes and degrees of methacrylation. PGS-M scaffolds were fabricated into porous polyHIPE structures with over 74% porosity and crosslinked to form polymerised HIPEs (polyHIPEs). PGS-M scaffolds were highly flexible and showed elastic recovery. The mechanical properties of PGS-M scaffolds were found to be within the range of adipose tissue. The metabolic activity of ADSCs cultured on PGS-M scaffolds increased over the time course of the experiment (14 days), indicating that the cells were proliferating on the scaffolds. The migration of ADSCs into the scaffold could be observed following 14 days of *in vitro* culture.

CONCLUSION

When seeded onto PGS-M scaffolds, ADSCs proliferate and infiltrate into the scaffold, suggesting that PGS-M scaffolds provide a suitable environment for cell survival and growth. PGS-M scaffolds have mechanical properties similar to those of adipose tissue and appear to mimic the nature of adipose tissue to dampen compressive forces. We envisage that, when implanted into a patient, these mechanical properties will aid the scaffolds in feeling natural to the patient. Future work will involve optimising the scaffolds to promote the differentiation of ADSCs into mature adipocytes, perhaps via the incorporation of factors that stimulate adipogenesis such as dexamethasone. Further, the fabrication of PGS-M scaffolds into a smaller geometry, such as porous microspheres, will be investigated, so that they can be injected into patients via a hypodermic needle, rather than surgically implanting the scaffolds.

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Advanced Synthetic Materials for 3D Printing Applications and its Use as Scaffolds for Airway Regeneration.

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INTRODUCTION

Three dimensional (3D) printing is a popular and versatile tool used in tissue-engineered applications allowing the development of 3D printed scaffolds using a wide range of biocompatible materials[1]. However, there is a need for the development of advanced printable materials to overcome the limitations of current synthetic polymers that can suffer from reduced cell attachment and growth when used as standalone materials[2]. Herein, we investigated the use of novel polyesters (P1 and P2) that allow for post-polymerization modifications in comparison to conventional 3D printed polymers (PCL) for tissue engineering applications.

EXPERIMENTAL METHODS

P1 and P2 were synthesized and 3D printed (3DP) into a square lattice-like structure while PCL 37 kDa and PCL 50 kDa were employed as controls. Gel permeation chromatography (GPC) and differential scanning calorimetry (DSC) were used to characterise the 3DP polymers and its printability was evaluated measuring the mean fiber diameter and the weight of the lattices. Mechanical properties of the lattices were evaluated using uniaxial compression.

3DP lattices were combined with collagen-based scaffolds using custom freeze drying processes for the formation of a porous network to support the growth of Calu-3 bronchial epithelial cells used in airway tissue engineering applications. Scaffolds were seeded with $3x10^5$ cells/scaffold and their biocompatibility was evaluated by metabolic activity assays and DNA quantification over 10 days in culture.

RESULTS AND DISCUSSION

P1 and P2 were successfully synthesised with a molecular weight (M_w) of 66.70 kDa and 110.2 kDa respectively. Thermal analysis using DSC revealed a narrow melting temperature (T_m) range between 45-47°C and a crystallization temperature (T_c) between 29-31°C for P1 and P2, considerably lower than that of PCL (57°C). Both polymers were 3DP for the first time showing suitable printability with a narrow weight distribution range (Fig.1A) and fiber mean dimeter of 365.3±4.2µm and 336.9±16.2 µm, similar to PCL 50kDa and 37kDa respectively (Fig1.B). The longitudinal compressive modulus of P1 and P2 was 1.67±0.38 MPa and 5.09±0.8 MPa, similar to PCL 50kDa and 37kDa respectively.

After seeding the 3DP lattices combined with collagen using Calu-3 cells, exponential growth was seen in PCL 37kDa and P2 scaffolds as well as in collagen-only control scaffolds, with a significant increase at day 10 in comparison to day 1 and day 3. While no exponential growth was seen for PCL 50kDa scaffolds, a decreased metabolic activity over time was observed for P1 scaffolds (Fig2A). These results were further confirmed by DNA quantification on the scaffolds with similar DNA content for P2 and collagen-only scaffolds and a significant decrease observed for P1 scaffolds (Fig. 2B).

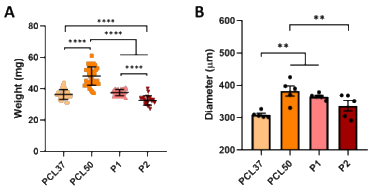

Figure 1. (A) Weight distribution and (B) fiber mean diameter of the 3DP lattices.

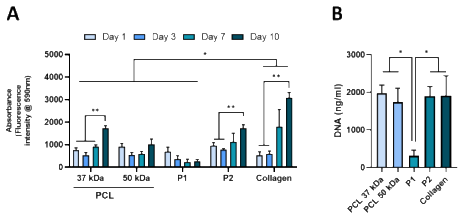

Figure 2. (A) Metabolic activity of Calu-3 cells seeded on 3DP scaffolds at day 1, 3, 7 and 10 of culture. (B) DNA quantification at day 10 in culture (*p<0.05, **p<0.01).

CONCLUSION

Novel synthetic polymers (P1, P2) are 3D printable materials with properties similar to commonly used printable biomaterials (PCL). Moreover, P2 represents a promising biomaterial that supports cellular attachment and growth of airway cells and could be potentially employed in airway tissue engineering applications.

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ACKNOWLEDGMENTS

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A PGSAA-based adhesive filler for perianal fistulas

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INTRODUCTION

Perianal fistula is a pathological connection between the anal canal and perianal skin, which most commonly develops from an anal abscess or in the context of inflammatory bowel diseases. Treating such fistulas while preserving patient's continence is a clinical challenge, as current sphincter-preserving approaches have low success rates[1].

To answer this clinical need, we aim to develop a filler which conforms to the fistula tract, is not extruded prior healing, by adhering to tissue, and enables fistula healing. The filler is based on an adhesive polymer, poly(glycerol-co-sebacate) acrylate-based adhesive (PGSAA). In this study, the adhesive performance of two filler formulations with different PGSAA content were evaluated both *ex vivo* and *in vivo* in a porcine model of perianal fistulas.

EXPERIMENTAL METHODS

Ex vivo adhesion of PGSAA-based fillers was benchmarked to fibrin glue (Tisseel, Baxter). Adhesion to porcine muscle tissue was measured by lapshear on a mechanical tester following ASTM standard F2255-05.

A porcine model, based on the work of Buchanan *et al.*[2], was developed for *in vivo* testing. Briefly, four-centimeter-long perianal fistulas were created in 2 pigs by insertion of catheters and dilators from the anal canal to the perianal skin (Figure 1). Three fistulas were created in the first animal, four in the second. Drains were left in the defect for 3 weeks to promote the formation of fistulas tracts lined with granulation tissue. After drains removal, the seven fistulas created were debrided using abrasive brushes, flushed with saline, and filled with two formulations of PGSAA-based fillers (n=3 for the formulation with the lowest adhesion, n=4 for the other). The pastes were injected with cannulas from the internal to the external opening, in a retrograde manner. The internal orifices on the mucosa were closed with sutures.

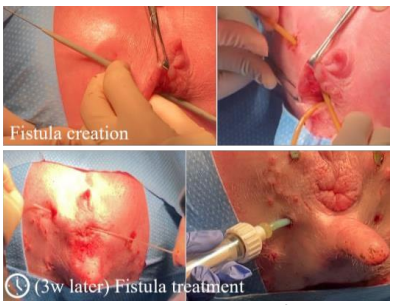

Figure 1. Pictures illustrating fistula creation, and treatment with debridement and filler injection.

The animals resumed normal diet and exercise until sacrifice one week after treatment: the fistulas were cut open to assess presence or absence of the fillers.

RESULTS AND DISCUSSION

Ex vivo adhesion to porcine rump was either similar or superior to fibrin glue for the two PGSAA-based fillers studied, respectively 1.2- or 3-folds difference. Injection of fibrin glue is a sphincter-preserving technique for perianal fistula treatment for which reported success rates are variable, and usually below 50%[3-5]. Poor outcomes have been attributed to fast resorption[5] before healing occurs, and to extrusion[6] due to the liquid consistency and limited adhesion of fibrin.

The PGSAA-based fillers were successfully injected in porcine perianal fistulas after debridement of the tracts. The filler with the highest adhesion was still in place in the fistula tracts at sacrifice one week after treatment. However, for the filler with lower adhesion (similar to fibrin glue), most of the products were dislodged a week after treatment. It is unclear whether the products were extruded from the external orifice on the skin (that was left open) or the internal orifice in the anal canal. On the anal canal side, the sutures placed during treatment did not hold and were gone after a week.

CONCLUSION

We designed adhesive fillers based on PGSAA polymer that were evaluated *ex vivo* and in a porcine model of perianal fistulas. A week after treatment, the filler with the highest adhesion was not dislodged from the tracts despite mechanical constraints and being exposed to stools on the anal canal side. These results demonstrated that PGSAA-based fillers are promising for perianal fistula repair. Further animal testing is required to demonstrate the integration of the product with the surrounding tissue and impact on fistula healing.

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ACKNOWLEDGMENTS

The authors would like to thank the Crohn's and Colitis Foundation for providing financial support to the *in vivo* testing in this project.

>> 17:15 - 18:45 | Canadian Society / ESB

Chairpersons: Maryam Tabrizian & Nicolas L'Heureux
Location: Room E

17:15 | KL1 Canadian Society & ESB - Targeted Gene Delivery of Interleukin-10 via Polymer Nanoparticles to Reduce the Inflammation in Atherosclerosis

Maryam TABRIZIAN, Department of Biomedical Engineering; Faculty of Dentistry and Oral Health Sciences, Montreal, Canada

17:30 | KL2 Canadian Society & ESB - Innovative 3D Autologous and Immunocompetent Skin Model Reconstructed by Tissue Engineering

Emilie ATTIOGBE, Centre de Recherche en Organogénèse Expérimentale de l'Université Laval (LOEX), Québec, QC, Canada / Centre de Recherche du CHU de Québec-Université Laval, Québec, QC, Canada

17:45 | O1 Canadian Society & ESB - Non-woven Textiles for Medical Implants: Mechanical Performances Improvement

Frederic HEIM, Laboratoire de Physique et Mécanique Textiles (LPMT), ENSISA, Mulhouse, France

18:00 | O2 Canadian Society & ESB - Effect of Synthetic INGAP-P on human islet insulin secretion and gene expression

JAMES PORTER, Dept. of Biological and Biomedical Engineering, McGill University, Montreal, Canada

18:15 | O3 Canadian Society & ESB - Proteomics as a tool to gain next level insights into photo-crosslinkable biopolymer modifications

Nele PIEN, Polymer Chemistry and Biomaterials Research Group, Centre of Macromolecular Chemistry (CMaC), Ghent University, Ghent, Belgium and Laboratory for Biomaterials and Bioengineering, CRC-I, Laval University, Quebec, Canada

18:30 | O4 Canadian Society & ESB - Method for Covalent Bonding of Heparin to Carbon Nanotube-Coated Metal Surfaces

Lynn HEIN, Mechanical Engineering, McGill University, Montreal, Canada; Chemical Engineering, McGill University, Montreal, Canada

Targeted Gene Delivery of Interleukin-10 via Polymer Nanoparticles Reduces the Inflammation in Atherosclerosis

Nicholas Distasio[1], Stephanie Lehoux[3], Maryam Tabrizian[1,2]

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INTRODUCTION

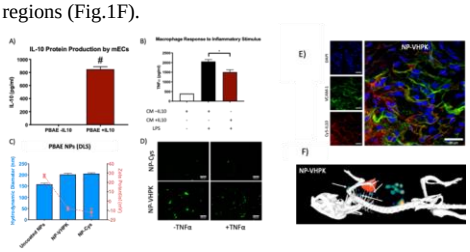
Atherosclerosis arises from lipid-laden deposits that develop over time in branched or curved segments of the blood vessels that experience abnormal shear stress[1]. Endothelial cells (ECs) in these regions overlie the growing plaques and overexpress vascular cell adhesion molecule-1 (VCAM-1). We have developed a nanoplex (NP) by self-assembly of a poly(ɓ-amino ester) (PBAE) and plasmid DNA encoding the anti-inflammatory cytokine IL-10 within the context of atherosclerosis[2]. The NPs was coated with a VCAM1-specific pegylated peptide for targeting the atherosclerotic plaque. Here in, we present the *in vitro* and *in vivo* characterization of these nanoplexes and their faith in attenuating inflammatory response in atherosclerotic plaque.

EXPERIMENTAL METHODS

For synthetizing PBAEs, acrylate monomers and amine monomers were mixed at a molar ratio of 1.2:1, dissolved in 500 mg/ml DMSO at 90ºC and stirred for 48h. Polymer synthesis and nanoparticle size, charge, and dispersity were tracked via ^{1}H-NMR and dynamic light scattering (DLS), respectively. Primary mouse endothelial cells (mECs) were extracted from the lungs of C57BL/6 mice and HUVECs were purchased from a commercial provider. Polymers were mixed with DNA at a weight ratio of 30:1 or 60:1. Transfection efficiency was measured via nanoparticles containing a GFP plasmid using flow cytometry in HUVECs and mECs. IL-10 transfection using polymer nanoparticles was quantified via qPCR and ELISA in HUVECs and mECs. Media collected from ECs 72h after transfection was given to RAW264.7 macrophages with IL-10 signaling verified by western blotting. The response of these macrophages to inflammation following stimuli (TNFa, LPS) was also characterized via western blot and ELISA. Surface plasmon resonance with imaging was used to characterize affinity-based interactions between NPs containing pIL-10 and VCAM-1 grafted on the sensor surface. NPs containing Cy3-pIL-10 were added to TNFα-activated mECs under static or dynamic flow conditions in an Ibidi µ-slide and uptake was assessed via flow cytometry and fluorescence microscopy. ApoE$^{-/-}$ and LDLR$^{-/-}$ mice were used to induce atherosclerotic plaque formation. Tissues containing plaque were harvested, cryo-sectioned and aortas were freshly prepared *en face*. Targeted and non-targeted NPs (Cy3- or Cy5-pIL-10) were co-stained with VCAM-1. For live imaging and biodistribution, NPs were intravenously injected in atherosclerotic mice and imaging was performed on live mice and on harvested organs using the IVIS Spectrum live imaging system.

RESULTS AND DISCUSSION

PBAE nanoplexes were able to transfect mECs, leading to significant production of IL-10 (Fig.1A). IL10-transfected mECs protected macrophages from inflammatory stimulus (Fig.1B). VCAM-1 targeted peptide coating (NP-VHPK) lowered the surface charge of NPs (+30 mV to -10 mV) while slightly increasing the size (180 to 200nm) (Fig.1C). mECs were activated with TNFα to overexpress VCAM-1, and then NPs were incubated with GFP plasmid DNA. Increased uptake and transfection were observed with TNFα and NP-VHPK, which targets VCAM-1 (Fig.1D). NP-VHPK was found to bind the endothelium *ex vivo* when incubated with aortic arches from an atherosclerotic mouse model (Fig.1E). 2h after injection, NP-VHPK displays a similar biodistribution as most NPs, with a majority going to the liver, spleen, kidneys, and the lung, although NP-VHPK had a stronger signal in these endothelial-rich regions than NP-Cys. 3D tomographic imaging showed NP-VHPK localized near the heart in atherosclerotic plaque regions (Fig.1F).



Conclusion: IL-10 gene therapy can attenuate inflammation in the context of atherosclerosis and this strategy can be targeted to sites of plaque formation.

Figure 1. mEC transfection with PBAE nanoplexes to IL-10 production (A) and conditioned media from these cells is anti-inflammatory in macrophages (B). VCAM-1 targeted NPs are negatively charged (C) and can transfect mECs with an increase observed after inflammatory stimulation with TNFα (D). VCAM-1 targeted NPs (NP-VHPK) bind VCAM-1 specifically: *ex vivo* tissue immunostaining (E). When injected *in vivo*, NP-VHPK is detectable near heart in possible atherosclerotic tissues (F).

CONCLUSION

IL-10 gene therapy can attenuate inflammation in the context of atherosclerosis and this strategy can be targeted to sites of plaque formation.

ACKNOWLEDGMENTS: Canadian Institutes for Health Research operating grants.

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Innovative 3D Autologous and Immunocompetent Skin Model Reconstructed by Tissue Engineering

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INTRODUCTION

The relevance of studying skin disease in animals is limited due to immunological and physiological differences. Many efforts have been made on skin tissue engineering to develop *in vitro* skin models. However, existing models fail to reproduce the immune complexity of human skin. Indeed, most of them concentrate on a few types of immune cells (one to three at most). Moreover, those cells are either derived from cell lines or differentiated from blood progenitors due to the difficulty to obtain skin resident immune cells. These cells are then included in epidermal or full skin reconstructs that often lack vasculature and come from heterologous donors, possibly interfering with the study of immune reactions.

To fill this gap, a 3D autologous skin model was developed, containing endothelial cells and cutaneous resident immune cells.

EXPERIMENTAL METHODS

A cell extraction technique was firstly developed to isolate resident skin cells from the same donor (keratinocytes, fibroblasts, immune and endothelial cells). From this isolation, an autologous *in vitro* 3D human skin model was developed, using a self-assembled method. First, autologous dermal fibroblasts were cultivated and produced a dense extracellular matrix similar to the *in vivo* one. Matrix-embedded cells formed a manipulatable sheet as previously described by our group. The model was then constructed by cell sheet superposition, by three steps: a dermal fibroblasts cell sheet, a collagen lattice containing the whole dermal cell isolate including immune and endothelial cells, finally a second dermal fibroblasts cell sheet seeded with keratinocytes.

RESULTS AND DISCUSSION

Cell viability and identification were performed through flow cytometry using a set of specific surface markers. Cells from the dermis were split between fibroblasts (70%), immune cells (20%) and endothelial cells (10%). Freshly isolated dermal immune cells were characterized as resident skin macrophages (CD45+, CD14+, CD163+); T lymphocytes (CD45+, CD3+); dendritic cells (CD45+, CD14-, CD1a+) and mast cells (CD45+, CD117+). Lymphocytes and mast cells were found in a higher extend (5-7%) than macrophages and dendritic cells (3%). Mast cells were found to be frost sensitive and did not survive freezing.

3D model was characterized by fully differentiated epidermis, capillary-like networks formed through self-assembly by endothelial cells and the presence and survival of lymphocytes, macrophages and dendritic cells identified through immunofluorescence staining. Skin substitute supplemented with resident immune cells and endothelial cells displayed a higher proliferation rate at the epidermal basal layer as revealed by Ki67 staining compared to the model containing only fibroblasts and keratinocytes.

Immune cells number remained stable throughout the culture and did not exfiltrate the 3D model after 14 days in culture. Following an immunological stimulation, skin reconstructs responded by an inflammatory response with contribution of immune cells. Further analyses are ongoing to complete the immunocompetence of this model.

CONCLUSION

This study describes an innovative autologous skin model which, to our knowledge, summarizes the resident skin immune system in a more realistic way than available skin models. It provides a relevant tool to study cellular interactions and the contribution of the immune system in skin development and inflammatory responses.

Non-woven Textiles for Medical Implants: Mechanical Performances Improvement

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INTRODUCTION

Textile materials have been largely used as medical implants over the last decades. These materials provide outstanding mechanical strength combined with unique flexibility due to the discontinuous configuration of the fibrous construction. However, the long-term success of these implants relies on their ability to interact with the surrounding biological tissues[1]. Actually, one key challenge is to control the Foreign Body Reaction (FBR). In this context, non-woven fibrous constructions made from small fibers and larger pores are expected to limit fibrotic tissue development. However, their strength is not large enough to withstand strong loading conditions and the stress applied to a vascular graft, a hernia mesh or a heart valve. The purpose of this work was to investigate if non-woven substrates can be reinforced by embroidery towards strength increase.

EXPERIMENTAL METHODS

Non-woven samples were produced from both melt-blowing and electro-spinning techniques, reinforced with a stitching yarn and tested regarding several performances : ultimate tensile strength, burst strength and strength loss after fatigue stress. Several stitching parameters were considered: distance between stitches, number of stitch lines (1, 2 or 3) and line geometry (horizontal H, vertical L, cross X) (Fig.1). The performance values obtained after reinforcement were compared with values obtained for control samples.

RESULTS AND DISCUSSION

From the obtained results several observations can be made. First it comes out that it is possible to obtain non-woven mats characterized by a fibre diameter between 3 and 6 µm with both the melt-blowing and the electro-spinning techniques. The cohesion between the fibres is however dependent on the production process. Melt-blown structures are globally stiffer. Second, it has been shown that the extension strength can be increased by up to 100 % for the ES material and 50% for the MB material by inserting a reinforcement yarn in the fibrous non-woven construction. In that approach, the X pattern appears to be more efficient that the H or L patterns. Regarding the number of reinforcement lines, the burst strength increases in correlation with the number of reinforcement lines in the MB material (Fig.2). At last, it comes out that after cyclic loading, the strength of both materials goes down due to the cycling interaction between the reinforcement yarn and the non-woven mats. This degrading effect is more pronounced for the ES mat. However, the remaining strength values after cycling tends to remain above the values obtained for non-reinforced control samples.

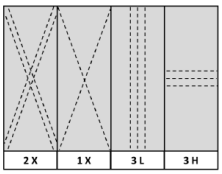

Fig.1 Stitching patterns

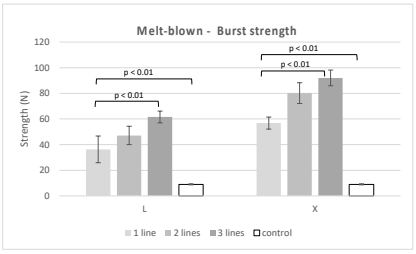

Fig. 2 burst strength vs stitching pattern

CONCLUSION

These preliminary results confirm that it is possible to enhance and control the strength of non-woven materials by adjusting the embroidery reinforcement design. However, further fatigue tests are necessary to be performed on a longer duration, to confirm the results obtained and validate the strength enhancement.

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Effect of Synthetic INGAP-P on human islet insulin secretion and gene expression

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INTRODUCTION: Autoimmune destruction of pancreatic islets leads to loss of blood homeostasis and type 1 diabetes (T1D). This study aims to characterize human islets in vitro, towards improving live cell transplantations for T1D. This includes developing a multiplex hormone [1] and gene panel of donated human cells, to help qualify alternate suitable sources for graft cells. These can include viral immortalized human cell insulinomas, beta/endothelial cocultured spheroids, and other guided differentiation of cell lineages. Novel techniques [2] are required for the direct interrogation of cell functionality and viability for transplant, to obtain comprehensive data to inform tissue culture studies and eventually improve diagnostic methods towards personalized treatment options.

EXPERIMENTAL METHODS: We employed ELISA insulin detection (Fig. 1) and DNA quantification to carryout initial islet evaluation. Cells were lysed and subjected to a gene expression assay by RT-qPCR. A panel of islet genes related to the insulin secretion pathway were measured for regulation of mRNA. Live cell confocal image fluorescent staining was used to examine islet morphology and viability (Fig. 2). Potential islet cell replacement types were tested for their insulin respond to glucose. Novel biosensing techniques are investigated to improve the workflow of in vitro islet analysis. Microfluidic chambers interfaced with sensitive electrode arrays were used to detect insulin in solution in static and continuous assays. Multiplexed hormone detection for islet characterization was investigated using SPRi to allow high throughput biomarker analysis. 3D printing and other microfabrication were used towards incorporation of testing components into point of care application.

RESULTS AND DISCUSSION: Different human donors showed changes in the insulin response to glucose. Those with highest insulin output showed distinct gene regulation patterns compared to other donors. Gene studies pointed towards the effect of age on islets, as well as an interplay of glucagon and glut2 gene regulation in most donors. Islet-replacement candidates in EndoC-BH1 and MIN6

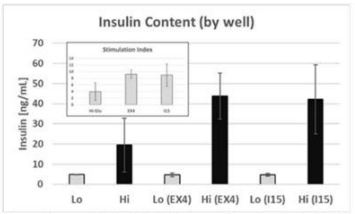

Figure 1: human islets GSIS: high glucose (negative control) with the addition of Exendin-4 (EX4, positive control) and INGAP-P (peptide sample). Inset: Stimulation index

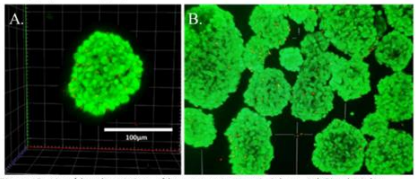

Figure 2: Live/dead staining of human pancreatic islets. A.) Single-islet imaging reveals >99% viability B.) Population imaging shows consistent viability and morphology across the batch

spheroids showed suitable response of insulin when exposed to higher glucose concentrations. Newly developed biosensing strategies successfully detected insulin and other islet secretions in miniaturized and dynamic fluid environments.

CONCLUSION: These studies help to build the base of knowledge of islet behavior and the ability of renewable cell replacements to out-perform native primary cells. Continued development of in-vitro characterization and handling will help to understand and treat autoimmune destruction of beta cells in type 1 diabetes.

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Proteomics as a tool to gain next level insights into photo-crosslinkable biopolymer modifications

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INTRODUCTION

The distribution of photo-crosslinkable moieties onto a protein backbone can affect a biomaterial's crosslinking behavior, and therefore also its mechanical and biological properties. A profound insight in this respect is essential for biomaterials exploited in tissue engineering and regenerative medicine. In the present work, photo-crosslinkable moieties have been introduced on the primary amine groups of: (i) a recombinant collagen peptide (RCPhC1) with a known amino acid (AA) sequence, and (ii) bovine skin collagen (COL BS) with an unknown AA sequence. The degree of substitution (DS) can be quantified with two conventional techniques: an ortho-phthalic dialdehyde (OPA) assay and ^{1}H-NMR spectroscopy. However, neither of both provides information on the exact type and location of the modified AAs. Therefore, proteomic analysis will be evaluated herein as a tool to identify functionalized AAs as well as the exact position of photo-crosslinkable moieties along the AA sequence, thereby enabling an in-depth, unprecedented characterization of functionalized photo-crosslinkable biopolymers. Moreover, our strategy enables the visualization of the spatial distribution of the modifications within the overall structure of the protein.

EXPERIMENTAL METHODS

The methacrylation of the biopolymers was performed as previously described.[1] Next, ^{1}H-NMR spectroscopy and OPA were used to quantify the DS. Samples for proteomics analysis were prepared using a modified enhanced filter aided sample preparation and measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) protein analyses.[2-3] For the 3D protein structure prediction of the proteins, the online I-TASSER platform was used.

RESULTS AND DISCUSSION

Neither ^{1}H-NMR spectroscopy nor OPA enabled the identification and the localization of the introduced functionalities. Conversely, proteomic analysis enabled: (i) the identification of the peptides and modified AAs, and (ii) the localization of the introduced photo-crosslinkable groups. Whereas the identification and localization of the modified peptides is important, the quantification of modifications present along the protein backbone is as essential. The DS could be determined by ^{1}H-NMR spectroscopy and OPA. However, with proteomic analysis, some challenges were encountered when quantifying the photo-crosslinkable moieties: (i) the presence of redundant peptide sequences, and (ii) a higher DS (corresponding with more photo-crosslinkable moieties) resulting in more steric hindrance. Despite these challenges, proteomic analysis was able to provide insight in the modification frequency of specific modification sites, enabling the calculation of an average DS. Based on the proteomic analysis and the obtained information on the localization of the modified groups, it was possible to 3D model the biopolymers. Moreover, it permitted the identification, localization and distribution of each unmodified and modified AA in its 3D structural conformation, providing crucial insight in the overall distribution of the modified sites along the protein backbone.

CONCLUSION

Proteomic analysis cannot (yet) be used as a stand-alone technique to fully characterize a modified (photo-crosslinkable) biopolymer because it only provides an average DS based on the modification frequencies. However, it is the only technique that enables the identification and localization of the functionalized AA along with supplying the required information for establishing the 3D model. This enabled to gain unprecedented insight in the distribution of the introduced functionalities along the protein backbone which is crucial with respect to reproducibility and regulatory aspects for its use as a biomaterial for tissue engineering and regenerative medicine applications, and further unravelling of the efficiency of the biopolymer modification process and the effect on the crosslinked network.

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Method for Covalent Bonding of Heparin to Carbon Nanotube-Coated Metal Surfaces

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INTRODUCTION

Multi-walled carbon nanotubes (CNTs) are a promising substrate for drug immobilization and release [1]. While a lot of work regarding the use of CNTs as individual drug carriers has been performed, the idea of employing them as a drug-loaded interface on metallic implant surfaces is still less popular. The unique property pool and tuneability of CNTs make them an attractive candidate for a coating medium to enhance the blood and biocompatibility of metallic surfaces. As such, a mean to covalently bind heparin, a pharmaceutical blood thinner, to CNTs on the surface of 316L stainless steel (SS), a common metal implant material, has been developed.

EXPERIMENTAL METHODS

CNTs were directly grown on 316L SS mesh substrates following a thermal chemical vapor deposition (CVD) method, which was previously reported by Jorge et al. [2]. The CNTs had to be removed from the SS substrate and deposited onto a pristine SS mesh coupon since the high-temperature process for CNT synthesis caused the corrosion resistance of the SS substrate to degrade completely. First, the CNTs underwent functionalization by argon (Ar)/oxygen (O_2)/ethane (C_2H_6) plasma at flow rates of 500/5/1 sccm, respectively, to produce negatively charged CNTs as has been described by Hordy et al. [3]. The negatively charged CNTs were then sonicated in RO water for 15 min to break them off the SS mesh. Subsequently, electrodeposition of CNTs onto a pristine 2 x 5 cm SS mesh coupon was carried out by applying a DC voltage of 20 V for 60 min to the CNT-RO water suspension. The deposited CNT forest was again functionalized by continuous, non-thermal ammonia plasma to graft amine (-NH_2) onto the surface of the CNT forest. The carboxyl groups (-COOH) of heparin were then bonded to the -NH_2 groups on the CNT surface using crosslinking agents. Characterization of the CNTs was carried out by SEM, XPS and TGA-IR. Qualitative and quantitative analysis of heparin loading was achieved by XPS and Toluidine Blue O assay [4], respectively.

RESULTS AND DISCUSSION

The CNTs synthesized by CVD were ~30–40 nm in diameter and 15-20 µm in length. After Ar/O_2/C_2H_6 plasma functionalization, the concentration of CNT surface oxygen increased from zero to 21%, mostly due to the presence of hydroxyl (-C-OH), carbonyl (-C=O), and carboxyl (-COOH) groups [3]. Electrodeposition of the negatively charged CNTs on the pristine SS mesh coupon was achieved without the use of an external surfactant. The CNT coating had a uniform, black appearance with good adhesion to the SS mesh sample. The functionalization of the deposited CNTs by ammonia plasma resulted in an increase of the surface nitrogen concertation from zero to 16%. The presence of polar groups on the CNT surface caused it to be hydrophilic which is crucial for its biocompatibility. The presence of primary and secondary amine groups was confirmed by TGA-IR. XPS spectra of the heparin-loaded CNT samples showed the appearance of a sulphur peak which is attributable to the presence of heparin (cf. *Fig. 1*). Quantification by Toluidine Blue assay proved an average heparin loading efficiency of 30-40%.

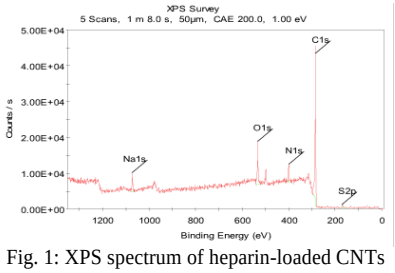

Fig. 1: XPS spectrum of heparin-loaded CNTs

CONCLUSION

The ability to covalently bind heparin on 316L SS surfaces covered with CNTs has been demonstrated. Further characterization of the electrodeposited CNT coating has yet to be performed. The performance of alternative loading mechanisms, as well as associated drug release profiles will be examined in the near future.

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ACKNOWLEDGMENTS

The authors would like to thank the National Research Council of Canada for the TGA-IR measurements and the Natural Sciences and Engineering Research Council of Canada for the funding of this project.

>> 17:15 - 18:45 | SFB / ESB

Chairpersons: Maria Grazia Raucci & Joachim Kohn
Location: Room F

17:15 | KL1 SFB ESB - Photoinduced Hydrogel Network Reorganization Modulates Intestinal Organoid Epithelial Shape to Template Crypt Formation

Max YAVITT, Chemical and Biological Engineering, University of Colorado Boulder, Boulder, Colorado, USA; BioFrontiers Institute, Boulder, Colorado, United States

17:45 | KL2 SFB ESB - Artificial Extracellular Matrix Scaffolds Enhance Maturation of Human Stem Cell-Derived Neurons

Zaida ALVAREZ PINTO, Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, USA//Institute for Bioengineering of Catalonia, IBEC, Barcelona, Spain

18:15 | O1 SFB ESB - Hydrogel Viscoelasticity Modulates Fusion of Mesenchymal Stem Cell Spheroids

Mani DIBA, Laboratory for Cell and Tissue Engineering, John A. Paulson School of Engineering and Applied Sciences, Harvard University, Cambridge, MA, USA

18:30 | O2 SFB ESB - Investigating the impact of Ti3C2TX (MXene) within an accommodative intraocular lens design on the development of posterior capsular opacification

Grace COOKSLEY, School of Pharmacy and Biomolecular Sciences, University of Brighton, Brighton, UK; Rayner Intraocular Lenses, Limited., The Ridley Innovation Centre, Worthing, UK

Photoinduced Hydrogel Network Reorganization Modulates Intestinal Organoid Epithelial Shape to Template Crypt Formation

F. Max Yavitt[1,2*], Michael Blatchley[1,2], Peter Dempsey[3], Kristi Anseth[1,2]
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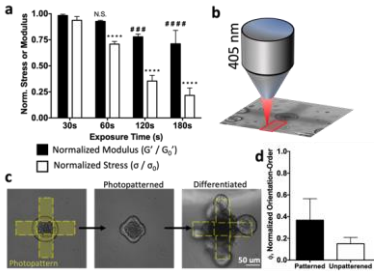
INTRODUCTION. Intestinal organoids (IOs) are 3D cellular aggregates that self-organize from stem cells to recapitulate the structure and function of the intestines. When encapsulated within a supportive matrix, crypts housing intestinal stem cells project from a central, enclosed lumen, mimicking the crypt-villus architecture that is characteristic of the intestine. However, crypt formation is a stochastic process that introduces phenotypic heterogeneity, demonstrated by the distribution in crypt sizes and spacings, and cellular populations seen *in vitro*. Such heterogeneity limits the translation of organoids towards clinical applications, including the interpretation of results from drug screens[1].

To address culture heterogeneity, we aim to influence organoid developmental programs to generate more uniform crypt structures. Cell shape and tissue morphology are known to be strong regulators of stem cell behavior[2], yet the role of dynamic changes in cell shape in directing fate specification of IOs is unknown. This question has been difficult to address due to the inability to control local epithelial shape within individual organoids, a deficiency that stems from a lack of appropriate adaptable materials. Here, we apply photopatterned light to allyl sulfide hydrogels[3], which undergo photoinduced bond reorganization to spatially relax the hydrogel matrix surrounding encapsulated organoids. Relaxation of stress causes a disruption of the mechanical equilibrium between the expanding organoid and the compressive hydrogel, which enables an epithelial bud to protrude into the photopatterned region, altering the epithelial curvature. Control over pattern dimensions allows for control over local epithelial curvature, enabling investigations into the effect of changes in epithelial curvature on crypt development. This work highlights user-directed control over tissue morphology as a tool to influence crypt formation, generating more uniform morphologies and reducing culture heterogeneity

EXPERIMENTAL METHODS. Hydrogels were formed by the reaction of DBCO functionalized PEG macromers with allyl sulfide containing, diazide functionalized crosslinkers. Hydrogels were equilibrated with a solution containing the photoinitiator, lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) and a rheometer (TA Instruments DHR-3) with a parallel plate and light curing attachment was used to measure stress relaxation and storage modulus as a function of irradiation time. IOs were dissociated into single cells, encapsulated into the allyl sulfide crosslinked hydrogels, and cultured in growth media. Cell laden hydrogels were equilibrated with LAP, and a 405 nm light from a confocal microscope (Zeiss LSM 710) was used to create shape-specific patterns proximal to the growing organoids. Photopatterned organoids were then photodegraded[3] and exposed to differentiation media conditions to initiate crypt formation. After two days, organoids were fixed and the angle of crypt formation was measured using ImageJ and used to calculate the spatial orientation order parameter[4], which describes crypt alignment. For rheology and cell culture, N=3 hydrogels were used per condition and compared using one-way ANOVA with Tukey's Test.

RESULTS AND DISCUSSION. Exposure of cell-laden hydrogels to light initiated bond rearrangement in the allyl sulfide crosslinks, enables stress relaxation (white) with minimal degradation (black, Figure 1a). A 405 nm laser was used to locally modulate hydrogel viscoelastic properties, changing the epithelial shape within the patterned regions (Figure 1b). This technique was used to pattern 4 distinct buds within organoids, which were then exposed to differentiation conditions to initiate crypt formation (Figure 1c). Crypts were more likely to form from these regions of photopatterned shape change, as seen by an increase in the orientation order parameter (black) compared to unpatterned organoids (white), indicating that crypts are more aligned (Figure 1d).



CONCLUSION. Differences in local epithelial shape are thought to direct the morphology of developing IO crypts. However, hydrogels typically used for organoid culture are incapable of dynamically modulating organoid shape *in situ*. We report the development of a photoadaptable hydrogel platform that enables spatial and temporal control over organoid epithelial shape. Intestinal crypts are more likely to form from these regions of photopatterned shape change, indicating that the initial cell shape can be used to template crypt formation, thus generating more homogenous crypt structures. Current experiments are focused on investigating the biomechanical pathways that relate changes in tissue morphology to stem cell fate specification events.

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ACKNOWLEDGMENTS. The authors would like to thank the National Institutes of Health (R01 DK120921, F31 DK126427) for financial support.

Artificial Extracellular Matrix Scaffolds Enhance Maturation of Human Stem Cell-Derived Neurons

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INTRODUCTION

Human induced pluripotent stem cell (iPSC) technologies offer a unique resource for modeling neurological diseases. However, iPSC models are fraught with technical limitations including abnormal aggregation and inefficient maturation of differentiated neurons. Current ECM approaches for culturing iPSC-derived neurons in vitro are inconsistent, cost-inefficient, and ultimately fall short in their ability to promote neuronal maturation in part due to the absence of synergistic cues derived from the architecture, chemical composition and molecular dynamics of the native extracellular matrix (ECM). Supramolecular polymers, have emerged as new materials that can have ordered structures and tunable dynamics, in contrast to ordinary hydrogels that are typically based on crosslinked covalent polymers. [(1)] Here, we investigated three peptide amphiphiles (PAs) supramolecular matrices displaying on their surfaces a laminin derived sequence (IKVAV), which differ in the intensity of molecular motion within the fibers. We used these matrices to probe how they impact on the maturation of human iPSC-derived neurons.

EXPERIMENTAL METHODS

The three PAs were first analyzed using the coarse-grained MARTINI force field. Experimentally, we used Cryo-TEM and SEM that confirmed that the three IKVAV-PAs self-assemble into nanofibers. The secondary structure of each IKVAV-PA was analyzed by WAXS, CD analysis and FTIR spectroscopy. The intramolecular dynamic was verified using measurements of fluorescence depolarization (FD) of the PA nanofibers and nuclear magnetic resonance (NMR). [(2)] Human motor neurons (MNs) were generated from hiPSCs by utilizing a sequential cocktail of small molecules that first promote the formation of ventral-caudal neural progenitors, which are differentiated into postmitotic neurons. [(3)] High resolution microscopy, western blot, proteomic and electrophysiology analysis were used to analyze MNs maturation.

RESULTS AND DISCUSSION

The newly designed IKVAV-PAs have an almost identical chemical composition, except for 4 amino acids in the non-bioactive domain that makes the IKVAV epitope be displayed in a more or less mobile fashion within the nanofiber. We find that nanofibers with greater intensity of internal supramolecular motion have enhanced bioactivity toward iPSC-derived motor and cortical neurons. Proteomic, biochemical and functional assays reveal that scaffolds with highly mobile molecules triggered the ITGB1 pathway, exhibited increased survival, reduced aggregation and became morphologically and electrophysiological mature, relative to neurons grown on IKVAV PAs with less motion or commercial laminin coatings. Our work demonstrates the importance of incorporating dynamically controllable features into synthetic ECM scaffolds that can provide significant improvements to stem cell-based neuronal models.

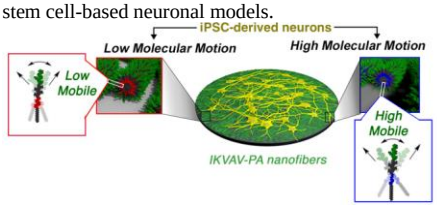

Figure 1. Schematic of the IKVAV-PAs' rationale.

CONCLUSION

The PA-based ECM mimetic technology offers a number of biological and technical advantages relative to current approaches for culturing stem cell derived neurons in vitro. Firstly, the continuous engagement of the ITGB1 receptor mediates the inherently-driven, increased level of functional maturation. Secondly, neuronal aggregation is drastically inhibited, essentially recapitulating the plating conditions that were previously attainable only by co-culturing with a primary glial feeder layer. Thirdly, the ease and complete control over their fabrication, ensure a consistent and economical product that can be widely used across labs.

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ACKNOWLEDGMENTS

"The authors would like to thank the US National Institutes of Health (NIH), National Institute on Neurological Disorders and Stroke (NINDS) R21NS107761 and the Center for Regenerative Nanomedicine (CRN) at the Simpson Querrey Institute for providing financial support to this project".

Hydrogel Viscoelasticity Modulates Fusion of Mesenchymal Stem Cell Spheroids

Mani Diba[1,2,§,¤], David T. Wu[1,2,3,§], Stephanie Yang[1,3], Benjamin R. Freedman[1,2]
Alberto Elosegui-Artola[1,2], David J. Mooney[1,2,*]

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INTRODUCTION

Multicellular spheroids and organoids are promising tools for *in vitro* modelling and *in vivo* regeneration of various tissues and organs.[1] Several biofabrication strategies have demonstrated that the fusion of these cellular building blocks can enable the fabrication of tissue-like constructs, capturing complex aspects of native *in vivo* environments.[2-4] Nevertheless, despite increasing evidence that matrix viscoelasticity directs cellular behavior,[5] its effect on spheroid fusion has not been studied. Therefore, this study aims to investigate the effect of hydrogel viscoelasticity on cell migration from mesenchymal stem cell (MSC) spheroids and the consequent fusion of these multicellular clusters. We hypothesized that hydrogel matrices with a fast stress relaxation behavior would facilitate the fusion of MSC spheroids.

EXPERIMENTAL METHODS

Hydrogels were prepared through calcium cross-linking of sodium alginate with various molecular weights and characterized using oscillatory rheology. Spheroids were formed from mouse- or human-derived MSCs using a forced aggregation technique. To enable the quantification of inter-spheroid distances in 2D images, spheroid encapsulation was carried out within a single geometric plane by a two-step casting of alginate disks with uniform mechanical properties. After each time point of culture in media supplemented with or without platelet-derived growth factor (PDGF; 10 ng/mL), the samples were fixated using formaldehyde, and stained using Phalloidin (cytoskeleton) and Hoechst (nucleus). Imaging was carried out using confocal microscopy and image analyses were performed using the CellProfiler™. Statistical analyses were performed using one-way analysis of variance followed by Tukey's multiple comparisons test.

RESULTS AND DISCUSSION

Two hydrogel compositions with similar elasticity (storage moduli ~4±1 kPa) were engineered for spheroid encapsulation, which exhibited fast (stress half-life = 89±59 s) or slow (stress half-life = 467±142 s) stress relaxation behavior. Fast-relaxing (FR) hydrogels facilitated cell migration from the spheroids, as the area per spheroid increased significantly during the culture period (Fig. 1). In contrast, spheroids encapsulated in slow-relaxing (SR) hydrogels shrank (Fig. 1A) or did not change (Fig. 1B) for up to 5 days of culture, indicating the critical role of matrix viscoelasticity in spheroid behavior. The supplementation of the culture media with PDGF, as a potent activator of cellular migration, significantly enhanced cell migration from spheroids in FR hydrogels (Fig 2B vs. 2A). However, PDGF was not effective in SR hydrogels, indicating that this biochemical signal does not override the effect of viscoelasticity on the spheroids. When spheroids were positioned within a ~150 µm distance (surface-to-surface), significant inter-spheroid fusion was observed as a result of the enhanced cell migration in these hydrogels (Fig. 2C, D).

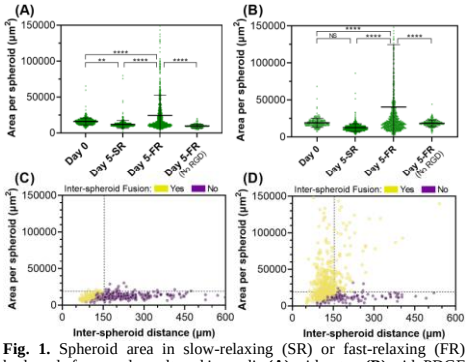

Fig. 1. Spheroid area in slow-relaxing (SR) or fast-relaxing (FR) hydrogels for samples cultured in media **(A)** without or **(B)** with PDGF supplementation. Scatter plots of spheroid area as a function of inter-spheroid distance (center-to-center) for **(C)** SR or **(D)** FR hydrogels cultured for 5 days in PDGF-supplemented media. (**C,D**) Horizontal and vertical dashed lines indicate the average spheroid area and the inter-spheroid distance of touching spheroids at Day 0, respectively. All spheroids were made of mouse bone marrow MSCs. NS, ** and **** indicate P<0.005, P<0.0001, and Not Significant, respectively.

CONCLUSION

FR hydrogels facilitate cell migration from MSC spheroids, which can be further enhanced by employing PDGF. This enhanced migration enables the fusion of neighboring spheroids, while this phenomenon is not observed in SR hydrogels. Ongoing experiments focus on unravelling the molecular mechanism of migration and fusion in this system. Our results emphasize the critical role of hydrogel viscoelasticity and have wide implications for the use MSC spheroids in regenerative therapies or the fabrication of *in vitro* models.

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ACKNOWLEDGMENTS

The authors would like to acknowledge the National Institutes of Health Grants (R01-CA223255-04, R01-DE013349-19, K99-AG-065495) and the Osteology Foundation Young Researcher Grant (21-032) for providing financial support to this project.

Investigating the impact of Ti_3C_2Tx (MXene) within an accommodative intraocular lens design on the development of posterior capsular opacification

Grace Cooksley [1][2] Joseph Lacey [2] Marcus Dymond [1] Yury Gogotsi [3] Susan Sandeman [1]

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Introduction

Cataract surgery restores visual acuity for patients however, the natural ability of the lens to focus for near and distance vision, known as accommodation, is lost. Moreover, patients have a 20-50% risk of developing posterior capsule opacification (PCO) [1]. PCO is treated by Nd:YAG laser ablation although this procedure has many complications [1]. Biomaterial developments in accommodative IOL design should therefore consider optimisation strategies which make a return of accommodative focus feasible whilst not exacerbating propensity to activate pathways leading to PCO formation. MXenes are a family of two-dimensional transition metal carbides and/or nitrides with a unique combination of properties. Ward et al., (2020) [2] used $Ti_3C_2T_x$ in an adjustable focus lens model to provide changes in dioptric range for an accommodative IOL design. However, MXenes have yet to be investigated regarding their impact on the developmental pathways of PCO. The aim of this study was to investigate the impact of MXene on LEC behaviour linked to inflammation and epithelial-mesenchymal transition (EMT) pathways contributing to PCO. It was hypothesised that $Ti_3C_2T_x$ may be used to repress pathways leading to hyper-inflammation and EMT.

Methods

Delaminated $Ti_3C_2T_x$ MXene was synthesised using the MILD method and characterised using dynamic light scattering and UV-spectroscopy. Expression of IL-1β and -6 by human lens epithelial cells treated with $Ti_3C_2T_x$ coatings were quantified using enzyme immunoassay and real-time polymerase chain reaction. Detection of mesenchymal markers was conducted using immunocytochemistry and western blots analysis. *In vitro* migration assays were completed using the scratch assay technique.

Results and Discussion

On direct contact with $Ti_3C_2T_x$ colloidal solution and coatings, HLE-B3 and FHL124 lens cells did not express IL-6 and IL-8. After priming of HLE-B3 with IL-1β, post-treatment of $Ti_3C_2T_x$ colloidal solution suppressed all expression of IL-6 and IL-8 after only 1-hour exposure. Moreover, $Ti_3C_2T_x$ coatings suppressed IL-6 and IL-8 expression by HLE-B3 cells, confirmed by ELISA and rt-PCR, suggesting a potential cell membrane interaction by $Ti_3C_2T_x$. $Ti_3C_2T_x$ did not upregulate mesenchymal markers and significantly reduced expression of vimentin in cells treated with $Ti_3C_2T_x$ and TGF-β2. Nevertheless, wound closure rate was significantly increased in cells incubated with $Ti_3C_2T_x$ coatings.

Conclusion

$Ti_3C_2T_x$ MXene may suppress pro-inflammatory pathways on interaction with the cell membrane. The presence of $Ti_3C_2T_x$ coatings did not upregulate mesenchymal markers despite increasing the wound closure rate. The study will continue by developing an *in vitro* transwell insert model of inflammation using a co-culture of FHL124 cells and THP-1 monocytes and silicone-based capsular bag model.

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Acknowledgements

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>> 17:15 - 18:45 | BIOMATERIALS AWARDS - ELSEVIER

Chairpersons: Abhay Pandit & Ana Pego
Location: Room B

17:15 | O1 - Design of Advanced Polymer Biomaterials for the Treatment of Autoimmune Disease

Nicholas A PEPPAS, Department of Biomedical Engineering, Department of Chemical Engineering, Department of Pediatrics, Surgery and Perioperative Care, Dell Medical School, and Division of Molecular Pharmaceutics and Drug Delivery, The University of Texas at Austin, Austin, USA

17:45 | O2 - Engineered biomaterials for lymph node drug delivery and disease modeling enable next-generation approaches in cancer immunotherapy

Susan N. THOMAS, Woodruff School of Mechanical Engineering and Parker H. Petit Institute of Bioengineering and Bioscience, Georgia Institute of Technology, Atlanta, GA, USA

18:15 | O3 - "Smart" Biodegradable Materials with Desired Shapes, Structures and Electroactive Functions at Nano/Micro-Scales For Medical Applications

Thanh DUC NGUYEN, Department of Mechanical Engineering, Department of Biomedical Engineering, Institute of Materials Science, University of Connecticut, USA

Design of Advanced Polymer Biomaterials for the Treatment of Autoimmune Disease.

Nicholas A Peppas
[1]Department of Biomedical Engineering, [2]Department of Chemical Engineering
[3]Department of Pediatrics, [4]Surgery and Perioperative Care, Dell Medical School, and
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INTRODUCTION

Engineering the molecular design of intelligent biomaterials by controlling structure, recognition and specificity is the first step in coordinating and duplicating complex biological and physiological processes. Recent developments in siRNA and protein delivery have been directed towards the preparation of targeted formulations for protein delivery to specific sites, use of environmentally-responsive polymers to achieve pH- or temperature-triggered delivery, usually in modulated mode, and improvement of the behavior of their mucoadhesive behavior and cell recognition. We address design and synthesis characteristics of novel crosslinked networks capable of protein release as well as artificial molecular structures capable of specific molecular recognition of biological molecules. Molecular imprinting and microimprinting techniques, which create stereo-specific three-dimensional binding cavities based on a biological compound of interest can lead to preparation of biomimetic materials for intelligent drug delivery, drug targeting, and tissue engineering. We have been successful in synthesizing novel glucose- and protein-binding molecules based on non-covalent directed interactions formed via molecular imprinting techniques within aqueous media. We have also developed structurally superior materials to serve as effective carriers for siRNA delivery to combat Crohn disease and ulcerative colitis.

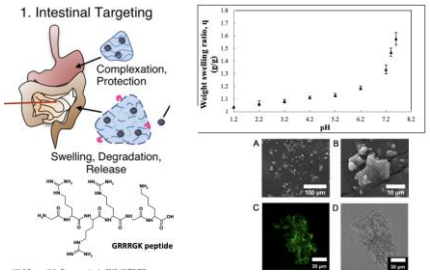
EXPERIMENTAL METHODS, RESULTS AND DISCUSSION

Biodegradable, cationic nanogels were synthesized via a controlled radical emulsion polymerization scheme using activators regenerated by electron transfer atom transfer radical polymerization (ARGET ATRP). Nanoparticles were synthesized with the tertiary amine methacrylate monomers 2-(diethylamino)ethyl methacrylate or 2-(diisopropylamino)ethyl methacrylate to impart pH responsiveness. Nanogels were copolymerized with poly(ethylene glycol) to impart colloidal stability and biocompatibility. Hydrophobic comonomers were used to modulate responsive properties, and a disulfide crosslinking agent was used to impart biodegradability.

Multiple sets of nanogels were synthesized including formulations that varied with respect to comonomer fraction (0 - 50 mol %), crosslinking density (0.31 to 5 mol %), cationic monomer (substitution of DEAEMA for DPAEMA from 0 to 100%), and choice of hydrophobic comonomer. In each case, nanogel composition was determined by nuclear magnetic resonance spectroscopy (NMR) to correlate nanogel composition with feed ratios.

The pH-responsive swelling profiles of nanogels formulations were determined by dynamic light scattering. Nanogels were approximately 180 nm in the swollen state and 110 nm in the collapsed state with critical swelling pH that ranged from pH 6.0 – 7.8 and volume swelling ratios from 2.5 – 5.0.

Nanogel mediated cytotoxicity and erythrocyte hemolysis were evaluated as a function of concentration. Nanogels pK_a was the strongest predictor of both cytocompatibility and hemolysis at pH 7.4. Formulations with pKa < 7.0 demonstrated limited toxicity in both assays. Hemolytic activity at pH 6.8 and below was a function of pKa, hydrophobicity, and volume swelling. Knockdown of eGFP was utilized to assess transfection efficiency of nanogels formulations. Nanogels electrostatically complexed siRNA with high efficiency. Knockdown of eGFP was enhanced by the incorporation of comonomers with increasing hydrophobicity. The cationic nanogels discussed in this work are excellent candidates for intracellular drug delivery. The described structure-function relationships will allow for rapid optimization of formulations, and the ability to synthesize cationic nanogels with targeted material properties.



CONCLUSIONS

Over the past 20 years we have concentrated on the design and development of advances nanomaterials based on crosslinked polymers for advanced targeting and delivery in specific cells for treatment of autoimmune diseases. The systems developed here have been tested for targeting of siRNAs and specific mRNAs

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Engineered biomaterials for lymph node drug delivery and disease modeling enable next-generation approaches in cancer immunotherapy

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INTRODUCTION

The advent of the cancer immunotherapy era has created wide-reaching new opportunities for drug delivery and biomaterial technologies to impact cancer therapy. Major barriers to the safer and more effective treatment of cancer using immunotherapy include achieving sufficient drug doses in target tissues at appropriate time scales as well as enabling drug synergies by co-delivery of combination therapies. More effective modeling of immunotherapy effects in disease-relevant preclinical models also represents a critical hurdle to the effective translation of newly developed therapies, that could likewise enable the development of personalized immunotherapy approaches to improve patient care. This talk will highlight recent advances from the Thomas lab in addressing current major challenges to achieving more effective cancer immunotherapy by leveraging engineered biomaterials. These include enabling technologies for controlled delivery of therapeutics to lymph nodes[1-4], tissues where anti-tumor immune responses are initiated and regulated, and modeling the tumor immune microenvironment for immunotherapeutic drug screening[5].

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"Smart" Biodegradable Materials with Desired Shapes, Structures and Electroactive Functions at Nano/Micro-Scales For Medical Applications

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INTRODUCTION

The ability to transform polymers, proteins and other safe medical materials into desired 3D forms/shapes/structures at nano and micro scales with "smart" electroactive functions, while sustaining the materials' excellent biocompatibility and biodegradability, provides significant applications in different biomedical fields, ranging from tissue engineering and controlled drug/vaccine-delivery to medical implanted devices.

EXPERIMENTAL METHODS

We employed a novel 3D micro-manufacturing method, so-called SEAL (StampEd Assembly of polymer Layers), to create a unique biomaterial microarray (MA) skin patch for single-administration self-boosting vaccines and long-acting antibody therapies. Besides, using physical fabrication and thermal processing methods (without changing the material chemistry), we transformed safe biodegradable polymers (e.g. the ones used for surgical sutures) into "smart" piezoelectric materials which can self-generate electricity under applied force and vice versa. The piezoelectric materials provide the cores to develop novel biodegradable force sensors, self-charged facemask membrane, electroactive tissue scaffolds, and biodegradable ultrasound transducers which provide significant and diverse medical applications in monitoring intra-organ pressures, filtering pathogens/toxic-pollutants, regenerating damaged tissues, and delivering drugs through physiological barriers such as the blood-bran junctions, respectively.

RESULTS AND DISCUSSION:

We have achieved the MA patch which only requires a single-time skin application to deliver vaccines/biologics/drugs repeatedly over a long period at pre-determined times to simulate the effect of multiple longitudinal injections[1]. These single-time MA patches can deliver pneumococcal/SARS-COVID-2 vaccines and other biologic drugs such as therapeutic antibodies to protect animals against deadly infectious pathogens.

We have also achieved biodegradable piezoelectric materials and demonstrated the use of these materials to regenerate damaged cartilages in an osteoarthritis rabbit model[2]. The piezoelectric materials were also used to create highly functional filtering nanofiber membrane for N95-like facemasks with a long-term biodegradability[3] and biodegradable force sensor[4] which can self-vanish after monitoring intra-organ pressures. Finally, we demonstrated the achievement of a novel protein-based piezoelectric nanomaterial which enables the development of a biodegradable highly functional ultrasound transducer to deliver chemo-drugs through the blood brain barrier for treating brain cancers[5].

CONCLUSION

In brief, our highly interdisciplinary research covers different areas in single-time self-boosting MA vaccine patches, biodegradable piezoelectric force sensors, electroactive facemask filters, piezoelectric tissue-scaffolds, and biodegradable ultrasound transducers. Throughout this diverse-research program, we demonstrated the impact of the science/technologies to transform safe biomaterials into desired 3D forms/shapes/structures at nano and micro scales with "smart" electroactive functions (e.g. piezoelectric effect) to enable significant medical and healthcare applications.

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>> 17:15 - 18:45 | Biomaterials Science - The Royal Society of Chemistry

Chairpersons: Cristina Barrias & Laura Ghandi
Location: Room A

17:15 | Award RSC - Lipid nanoparticles for mRNA therapeutics, genome editing, and cell therapy

Yizhou DONG, Division of Pharmaceutics and Pharmacology, College of Pharmacy, The Ohio State University, Colombus, United States

17:45 | O1 RSC - Novel mucoadhesive chitosan-methylcellulose buccal patches with broad antibacterial activity

Lorenzo BONETTI, Dept. of Chemistry, Materials, and Chemical Engineering "G. Natta", Politecnico di Milano, 20131, Milan, Italy

18:00 | O2 RSC - Functionalization of 3D printed PLLA/PLCL bioresorbable stents with endothelial cell adhesive peptides

Victor CHAUSSE, Biomaterials, Biomechanics and Tissue Engineering Group, Dept. of Materials Science and Engineering, Barcelona East School of Engineering (EEBE), Universitat Politècnica de Catalunya (UPC), Barcelona, Spain. Barcelona Research Center in Multiscale Science and Engineering, UPC, Barcelona, Spain

18:15 | O3 RSC - Injectable Biomimetic Mussel – Based Adhesive for Bone Repair and Remodeling

Antzela TZAGIOLLARI, School of Mechanical and Manufacturing Engineering, Dublin City University, Ireland

18:30 | O4 RSC - An iodine labeled injectable hyaluronic acid hydrogel for an in vivo bicolor x-ray monitoring of a biomimetic scaffold in brain regeneration cell therapy

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Lipid nanoparticles for mRNA therapeutics, genome editing, and cell therapy

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INTRODUCTION

Messenger RNA (mRNA) has shown great promise for broad therapeutic applications. However, the efficient and safe delivery of mRNA remains a key challenge for the clinical use of mRNA-based therapeutics. Lipid and lipid-derived nanoparticles possess unique properties for mRNA delivery. In this presentation, I will describe the development of lipid-derived nanomaterials for delivery of multiple types of mRNAs and their potential applications for treating genetic disorders, cancers, and infectious diseases.

RESULTS AND DISCUSSION

We have designed and developed new lipid-like nanoparticles (LLNs) and lipid-derived nanoparticles (LNPs) for therapeutic applications. For example, TT3 LLNs restore the human factor IX (hFIX) level to normal physiological values in FIX-knockout mice. Also, TT3 LLNs as multifunctional oncolytic nanoparticles carrying therapeutic self replicating RNA eliminate established tumors and prime systemic immunity. Additionally, FTT5 LLNs efficiently deliver long mRNAs, such as human factor VIII (hFVIII) mRNA for expression of hFVIII protein in hemophilia A mice, which restore hFVIII level to normal. Also, we show that adoptive transfer of macrophages containing antimicrobial peptides linked to cathepsin B in the lysosomes (MACs) can be applied for the treatment of multi-drug resistant (MDR) bacteria-induced sepsis in mice with immunosuppression. The MACs are constructed by transfection of vitamin C lipid nanoparticles (V_CLNPs) that deliver antimicrobial peptide and cathepsin B (AMP-CatB) mRNA. The V_CLNPs allow the specific accumulation of AMP-CatB in macrophage lysosomes, which is the key location for bactericidal activities. Our results demonstrate that adoptive MAC transfer leads to the elimination of MDR bacteria, including *S. aureus* and *E. coli*, and the complete recovery of immunocompromised septic mice.

CONCLUSION

The insights into the LNPs and mRNA engineering facilitate a broader range of RNA therapeutics. For example, our work provides a new strategy for overcoming MDR bacteria-induced sepsis and opens possibilities for nanoparticle-enabled cell therapy for infectious diseases.

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The authors would like to acknowledge the support of the Early Career Investigator Award from the Bayer Hemophilia Awards Program, Research Awards from the National PKU Alliance, New Investigator Grant from the AAPS Foundation, Maximizing Investigators' Research Award R35GM119679 from the National Institute of General Medical Sciences, and the R01HL136652 grant from the National Heart, Lung, and Blood Institute for providing financial support to the projects.

Novel mucoadhesive chitosan-methylcellulose buccal patches with broad antibacterial activity

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INTRODUCTION

Mucoadhesive buccal patches are promising dosage forms for successful drug delivery, offering the distinctive advantage of long residence time on the oral mucosa, thus resulting in increased treatments effect[1]. On this topic, electrophoretic deposition (EPD) of chitosan (CS) has been demonstrated as a simple and easily tunable technique to produce buccal patches[2]. However, CS-based buccal patches may suffer from weak adhesion strength, that can impair their therapeutic effect. Methylcellulose (MC), a widely investigated biopolymer in the biomedical area[3], has been reported to determine increased mucoadhesive behavior when used in a blend with other biopolymers[4]. The aim of this work was to obtain CS-MC patches in one pot process via EPD and to investigate the possibility of incorporating gentamicin (Gt) as a model of broad-spectrum antibiotic in the so-obtained patches.

EXPERIMENTAL METHODS

EPD was used to obtain buccal patches, namely CS, CS-MC, and CS-MC-Gt. To produce the samples, CS (0.5 % w/v) alone or in blend with MC (1 % w/v MC, 1 % w/v CS) was dissolved in a 1% v/v acetic acid solution in distilled water. For CS-MC-Gt samples, Gt (2 mg/mL) was added prior to cathodic EPD. The obtained patches were first freeze dried, then analyzed from a morphological (SEM), physicochemical (TGA, FT-IR, swelling/degradation), mechanical (tensile tests, tack tests), and in vitro biological point of view (indirect cytotoxicity test, indirect antibacterial test).

RESULTS AND DISCUSSION

CS and CS-MC samples (**Fig. 1**) appeared homogeneous, with random porosity created by H_2 bubble evolution[2]. Interestingly, an increase in porosity was evidenced after the addition of MC[5].

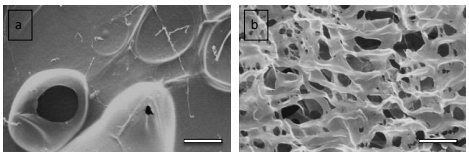

***Fig. 1** – SEM micrographs of a) CS and b) CS-MC samples. Scale bar = 20 μm.*

Both TGA and FT-IR characterization confirmed the presence of MC in the CS-MC patches. Since MC presents a neutral charge in the EPD conditions, the deposition mechanism of the blend was assumed to be caused by the establishment of weak interactions (e.g., H-bonds and hydrophobic interactions) between MC and CS[6]. Swelling and degradation tests (37 °C, 0.1 M NaCl) revealed only a slight influence of the addition of MC on the water uptake and gel fractions of the samples, suggesting a higher concentration of CS than MC in the obtained patches. Conversely, a significant difference in terms of mechanical properties was evidenced from tensile tests: E = 0.16 ± 0.05 Mpa vs. 0.04 ± 0.01 Mpa for CS and CS-MC, respectively. The reduced mechanical properties of CS-MC samples were attributed to their lower density (i.e., higher porosity). However, high mechanical performances are not required for buccal patches, conversely to an adequate mucoadhesion which is essential. In this regard, CS-MC exhibited significantly ($p < 0.05$) higher σ_{adh} (0.85 ± 0.26 kPa) and W_{adh} (1192.28 ± 602.36 Pa*mm) than CS control samples (σ_{adh} = 0.42 ± 0.22 kPa and W_{adh} = 343.13 ± 268.89 Pa*mm) (**Fig. 2**).

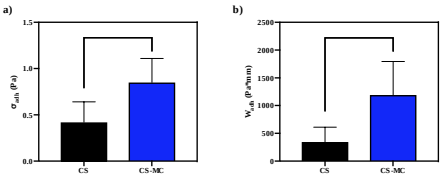

***Fig. 2** – a) Release pressure (σ_{adh}) and b) mucoadhesion work (W_{adh}) for CS and CS-MC samples.*

Lastly, the viability of cells (L929) incubated with material (CS, CS-MC, CS-MC-Gt) extracts was > 70 %, excluding possible cytotoxic effects of the materials themselves. Interestingly, CS-MC-Gt displayed remarkable antibacterial activity against both Gram positive (*S. aureus*) and Gram negative (*E. coli*) bacteria.

CONCLUSION

Novel CS-MC patches with superior (i.e., compared with CS control) mucoadhesive properties and broad antibacterial activity were obtained via a one-pot, simple, and easily tunable EPD process. The obtained patches lend themselves for the treatment of oral mucosal diseases.

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Functionalization of 3D printed PLLA/PLCL bioresorbable stents with endothelial cell adhesive peptides

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INTRODUCTION

Biomimetic surface modification with proteins or peptides that have specific cell-binding moieties is a promising approach to improve endothelialization of current bioresorbable stents (BRS)[1]. Bioactive cell-adhesive peptides such as RGDS (Arg-Gly-Asp-Ser) and YIGSR (Tyr-Ile-Gly-Ser-Arg) have been used to promote endothelial cells (ECs) adhesion and migration[2]. However, the optimal configuration and surface distribution of the peptides should be controlled to result in accelerated ECs responses.

This work presents the functionalization of novel 3D-printed polymeric BRS fabricated by solvent-cast direct-write (SC-DW) technique with a dual-peptide based platform containing RGDS and YIGSR motifs to selectively improve the adhesion and migration of ECs.

EXPERIMENTAL METHODS

Stent fabrication and characterization. Poly-L-lactic acid (PLLA) and poly(lactic-co-caprolactone) (PLCL) stents were fabricated by SC-DW cylindrical printing onto a rotating mandrel with 3 mm in diameter[3]. The ink consisted in a solution of PLLA or PLCL copolymer (95:5 lactic to caprolactone ratio) in chloroform at 10% w/v and 12.5% w/v, respectively. After fabrication, stents underwent a thermal treatment at 80ºC for 12h. Crystallinity, mechanical properties, surface topography and in vitro accelerated degradation in PBS at 50ºC over 4 months were evaluated by DSC, compression assays, SEM, GPC and ^{1}H-NMR.

Peptide synthesis and quantification. The peptidic linear sequences RGDS, YIGSR and a dual molecule containing both peptides (RGDS-YIGSR), with and without a terminal carboxyfluorescein (CF), were synthesized in solid-phase following the Fmoc/tBu strategy[4]. Then, stents were O_2-plasma activated and the biomolecules covalently attached to the surfaces using EDC/NHS. Biomolecules quantification was assessed via detachment in 1 M NaOH solution at 70°C for 12 min and subsequent fluorescent intensity analysis with a microplate reader.

Biological characterization. HUVECs adhesion and proliferation was characterized by immunofluorescence.

RESULTS AND DISCUSSION

PLLA and PLCL stents with 3 mm in diameter were successfully obtained by means of SC-DW (Figure 1a), with strut thickness of ~127 µm for PLLA and ~135 µm for PLCL series. PLLA stents presented higher crystallinity than PLCL stents (27.2% vs 21.6%). All stents underwent bulk degradation during 4 months immersion in PBS at 50ºC, with a sustained decrease in molecular weight and an increase in crystallinity as degradation proceeded. PLCL stents degraded 1.5 times faster than PLLA stents due to higher water penetration in amorphous regions.

Stent functionalization with RGDS, YIGSR and RGDS-YIGSR peptidic molecules resulted in a homogeneous coating, as shown in Figure 1b. Quantification of immobilized biomolecules revealed peptide concentrations over 700 pmol/cm^2.

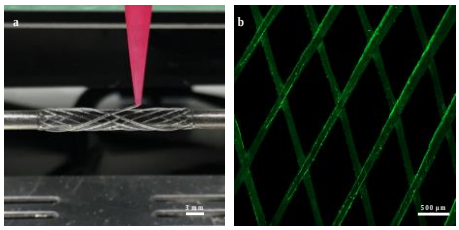

Figure 1. (a) 3D Printing of PLLA stent and (b) PLLA stent functionalized with CF-RGDS under confocal imaging.

Stents functionalized with synthesized biomolecules showed increased cell adhesion with respect to untreated PLLA and PLCL stents. Different tendencies were detected depending on the immobilized biomolecule. Similarly, proliferation of HUVECs in functionalized stents was found to be enhanced until complete colonization of the scaffold.

CONCLUSIONS

Functionalization of 3D-printed polymeric stents with bioactive molecules represents a promising strategy to enhance the biointegration of the stents for a faster endothelium recovery.

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Injectable Biomimetic Mussel – Based Adhesive for Bone Repair and Remodeling

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INTRODUCTION

Bone fractures have increased by 70% since 1990, leading to 455 million new cases in 2019, which poses significant challenges for orthopaedic surgeons [1]. Currently, the treatment of complex bone fractures involves the use of invasive techniques, and the use of calcium phosphate cements (CPC) as bone adhesives lacks clinical effectiveness. Phosphoserine, a relatively simple molecule extracted from mussels [2], combined with alpha-tricalcium phosphate (alpha-TCP) and calcium silicate (capable of inducing *in vivo* osseointegration and non-cytotoxic) can potentially achieve entirely novel properties, including better handling properties, improved bone strength under wet conditions and an accelerated capacity for osteogenesis. This study aims to design and characterise phosphoserine modified calcium phosphate cement (PM-CPC) adhesives for the bonding and healing of bone fractures and to assess their potential for minimally invasive delivery (Fig.1a).

EXPERIMENTAL METHODS

The alpha-TCP powder was synthesised using existing protocols [3] and characterised in terms of its chemical (X-ray diffraction (XRD)), and physical (particle size analysis, zeta potential, scanning electron microscopy (SEM)) properties. The PM-CPC formulation was optimised applying a Design of Experiment (DoE) approach using a Box-Behnken design (BBD) (Design-Expert V5 Software, Stat-Ease Inc., USA). The key inputs assessed were grinding cycles of alpha-TCP (2-13 cycles), liquid:powder ratio (0.2–0.5 ml/g) and the molar ratios of phosphoserine (Flamma, S.p.A. Italy) (10–50 wt.%) and calcium silicate (Sigma Aldrich, Ireland) (0–2 wt.%). The influence of these parameters on the physical, chemical, handling/injectability, setting (Gilmore needle) and static mechanical (compressive and adhesive) properties and the optimal PM-CPC composition were determined. Furthermore, the shear and tensile bonding strength of the optimal adhesive to cortical and cancellous bovine bone were determined under dry and wet conditions. Additionally, to facilitate on-demand mixing, pre-mixed formulations comprising of glycerol- and oil-based systems were developed and assessed.

RESULTS AND DISCUSSION

XRD and FTIR analysis confirmed a phase purity of 99.9% for the alpha-TCP. A negative correlation (R^2 = 0.90) was observed between particle size and number of attrition cycle (Fig.1b). Particle size was found to significantly (p-value<0.0001) influence the setting and static mechanical properties of the PM-CPC, which agrees with previous studies [2], [3]. DoE optimisation enabled identification of the optimal PM-CPC composition with a setting time of 1–2 min, compressive strength of 18 MPa and shear strength of 3.5 MPa after 72 h, following the required clinical range. A significant ($p \leq 0.001$) decrease in bone-to-bone shear adhesion and tensile strength was observed after treatment in a wet environment. However, PM-CPC provided higher adhesion compared to commercially available cements [4] (Fig.1c). Finally, both paste-based systems were found to be mixed during the delivery and injectable with suitable mechanical properties and homogeneity for on-demand mixing and delivery using the double syringe mix system (Fig.1d).

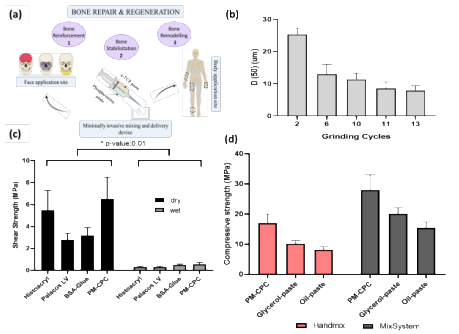

Fig. 1: (a) Overview of the PM-CPC adhesive technology, (b) effect of grinding cycles on the powder particle size, (c) comparison of bone-to-bone adhesion between different adhesives (d) mechanical properties of optimal PM-CPC cement and pastes through hand- and on-demand-mixing.

CONCLUSION

The optimal PM-CPC proposed by the DoE methodology demonstrated an injectable adhesive with optimal setting times, compressive, shear and tensile strength for the treatment of challenging bone voids and fractures. The design, characterisation and development of this novel PM-CPC adhesive paste with the associated minimally invasive delivery device represents a critical step forward in the treatment of complex bone injuries and has the potential to improve patient outcomes. The biocompatibility and ability of the PM-CPC to promote new bone formation will be assessed through *in vitro* and *in vivo* studies.

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An iodine labeled injectable hyaluronic acid hydrogel for an in *vivo* bicolor x-ray monitoring of a biomimetic scaffold in brain regeneration cell therapy

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Key words: Hydrogel scaffold, Cell therapy, bi-colour imaging

INTRODUCTION

Cell transplantation has emerged as a promising regenerative medicine therapy for ischemic stroke by replacing death cells and by stimulating endogenous self-repairing mechanisms[1]. Unfortunately, the therapeutic efficacy is compromised by a substantial loss of grafted cells[2]. Injectable hydrogels combined with stem cells have been proposed as a promising strategy to improve cell retention at the infarct site and their survival[3]. For this purpose, we proposed to use a recently developed hyaluronic acid (HA) hydrogel, that exhibits self-healing and injectability properties, as a biomimetic scaffold for delivery of transplanted cells in the brain lesion[4]. As a combined advanced therapy medicinal product candidate, an in *vivo* follow-up is required to monitor both the fate of the hydrogel scaffold and cells in a long temporal window. In this context, a radiopaque iodinated-labeled HA hydrogel (HA-I) was designed. Then, the tracking of both hydrogel scaffold and gold-labeled human adipose-derived mesenchymal stem cells (Au-hASC) in a specific and quantitative manner was assessed in *vivo* by bi-color imaging using spectral photon-counting computed tomography (SPCCT) and synchrotron K-Edge subtraction computed tomography (KES-CT).

EXPERIMENTAL METHODS

Two modified iodine-labeled hyaluronic acid (HA-I) partners were synthesized by grafting an iodine contrast agent (acetrizoic acid, TBA) on functionalized HA chains in mild conditions. The HA-I hydrogel was prepared by simply mixing in physiological conditions these two modified HA-I partners that can self-crosslink *via* boronate ester bond formation. The iodine concentration in HA-I hydrogel was quantified by ICP-MS analysis and KES-CT. Cell viability and proliferation of hASC (0.1 10^6 hASC/ 200 µL HA-I hydrogel at day 0) were evaluated in different cell culture periods. In *vivo* intracerebral injections of HA-I hydrogel as a control and Au-hASC embedded within HA-I hydrogel (0.5 10^6 Au-hASC in 10µL HA-I) in healthy rats (n=5) were monitored by magnetic resonance imaging (MRI). Then, on day 4 following the transplantation, bicolor imaging was performed using SPCCT and synchrotron KES-CT.

RESULTS AND DISCUSSION

An iodine-labeled HA hydrogel was formulated in physiological conditions with an iodine concentration [I]=2.4 mg/mL, suitable for X-Ray imaging techniques **(Figure 1A)**. In *vitro* cytotoxicity studies demonstrated a high cell viability after 7 days (> 80 % of cell viability). The dynamic rheological behaviour of the HA-I hydrogel showed that the storage moduli (G') at 25 and 37 °C displayed a plateau in the frequency range 0.01-10 Hz. The G' value (340 Pa, at 1Hz, 25°C) was suitable for a mini-invasive transplantation of Au-hASC in healthy rat brain (**Figure 1B**). Then, bicolour imaging using a KES-CT 3D reconstruction revealed cell retention of the biomimetic scaffold (Au-hASC embedded within HA-I hydrogel) (**Figure 1C**).

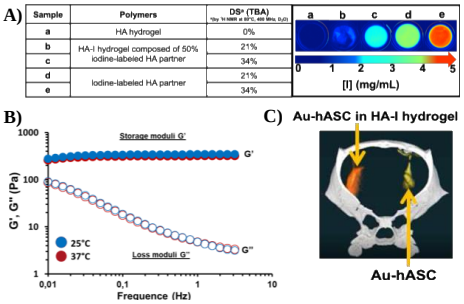

Figure 1: **A**) Iodine quantifications by KES-CT in HA hydrogels samples **B**) Dynamic rheological behavior of HA-I hydrogel. **C**) Ex *vivo* 3D image of healthy rat brain obtained with KES-CT.

CONCLUSION

A radiopaque iodine-labeled injectable HA hydrogel was successfully developed to deliver Au-hASC in healthy rat brains. Our first results demonstrate the ability to monitor the fate of stem cells on one hand and of cell-embedding hydrogel on the other hand by bicolor imaging using KES-CT. This paves the way for further investigations of this dual-labeled repair kit toward its applicability in cell therapy. One of the perspectives of this work is thus to evaluate its therapeutic effect after intracerebral administration in a rat model of ischemic stroke by coupling multimodal imaging (KES-CT) for monitoring cells and scaffold and advanced multiparametric MRI methods for evaluating tissue repair.

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ACKNOWLEDGMENTS

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>> 17:15 - 18:45 | BIOMAT & Human Repair French Network

Chairpersons: Jérôme Chevalier & Pierre Weiss
Location : Room C

17:15 | KL1 BIOMAT & Human Repair - Wireless Theranostic Smart Contact Lens for Monitoring and Control of Intraocular Pressure in Glaucoma

Hahn SEI KWANG , Department of Materials Science and Engineering, Pohang University of Science and Technology (POSTECH), Nam-gu, Pohang, Gyeongbuk, Korea

17:45 | O1 BIOMAT & Human Repair - DIAPID project : an innovative prosthesis dedicated to children

Ariane DAVID, FIMATHO, University Hospital of Lille, France, Lille

18:00 | O2 BIOMAT & Human Repair - Breast reconstruction using a bioabsorbable Tissue-Engineering Chamber for the regeneration of autologous adipose tissue: A Long Term pre-clinical review on minipigs

Julien PAYEN, Lattice Medical, Loos, France

18:15 | O3 BIOMAT & Human Repair - Fucoidan: from brown algae to a novel radiotracer in cardiovascular imaging

Cédric CHAUVIERRE, Université Paris Cité, UMRS1148, INSERM, Paris, France

18:30 | O4 BIOMAT & Human Repair - Bone Substitutes: from lab to bed

Didier MAINARD, CHRU, Nancy, France

Wireless Theranostic Smart Contact Lens for Monitoring and Control of Intraocular Pressure in Glaucoma

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INTRODUCTION

Glaucoma is a chronic eye disease requiring continuous medical care for patients' life time. Intraocular pressure (IOP) is the major risk factor that indicates the glaucoma condition and the appropriate IOP control is currently the only available medical care for the glaucoma patients. Accordingly, it is important to monitor continuous IOP changes in real time and deliver drugs in response to the IOP profile. Here, we report a new paradigm theranostic smart contact lens (CL) for both monitoring and control of IOP in glaucoma. After characterization of theranostic smart CL *in vitro*, we performed the demonstration of the theranostic smart CL for the successful monitoring and control of IOP in glaucoma animal models *in vivo*.

EXPERIMENTAL METHODS

The gold hollow nanowire (AuHNW) based IOP sensor and flexible DDS were integrated with an antenna and a ASIC chip for the wireless power transmission and communication. The theranostic smart CL was fabricated with a diameter of 7.5 mm for perfect fitting to rabbit's eyes. After the IOP was measured by CL and tonometer, each measured IOP was compared to assess the IOP monitoring of CL *in vivo*. The glaucoma was induced in both eyes of each animal by injection of methylcelluose or α-chymotrypsin. In addition, the IOP was controlled by monitoring IOP and releasing drugs every other days for 5 days with glaucoma animal models. The therapeutic effect of theranostic smart CL was assessed by retina histology and immunohistochemical analyses.

RESULTS AND DISCUSSION

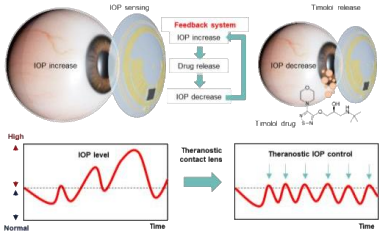

Figure 1. Schematic illustration of theranostic smart CL for both monitoring and control of IOP.

A theranostic smart CL for both monitoring and control of IOP was successfully developed with a high sensitive IOP sensor, a flexible DDS, an antenna and a chip (Fig. 1). This theranostic CL could continuously monitor IOP levels and appropriately release drugs in response to IOP levels. We designed two different types of DDS for daily and weekly uses and the sufficient drug could be loaded into each theranostic CL for IOP control (Fig. 2a). There was strong correlation between the measured IOP levels by the tonometer and the theranostic CL ($R^2 = 0.88$) (Fig. 2b). The released drugs could reduce the IOP levels of glaucoma animal models into a normal range when the IOP level was in a high range (Fig. 2c). However, drug was not released out from the theranostic CL when the IOP level was in a normal range. The retina histology revealed that the retina thickness was similar with that of normal in both treatment groups. There was a significant difference in the ganglion cell layers (GCL) and the inner nuclear layers (INL) with and without treatments (Fig. 2d). All these results confirmed the feasibility of our fully integrated theranostic smart CL for monitoring and control of the IOP with a feedback system of an IOP sensor and a DDS for futuristic glaucoma treatment.

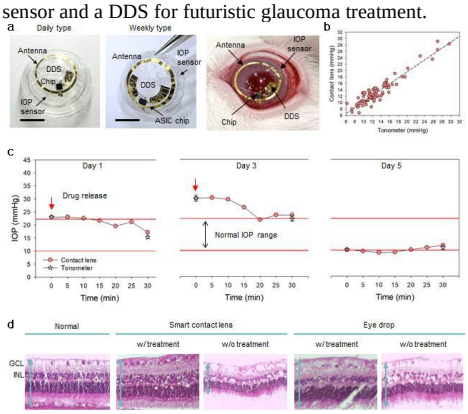

Figure 2. (a) Images of integrated theranostic smart CL. (b) The correlation of measured IOP by tonometer and CL. (c) The IOP control by theranostic smart CL. (d) The retina histology with and without treatment.

CONCLUSION

We successfully demonstrated the theranostic smart CL for both monitoring and control of IOP in glaucoma. This theranostic smart CL could maximize the therapeutic effect and minimize the side effect of drugs with a feedback system. Our theranostic smart CL would open a new avenue for the futuristic personal IOP control.

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ACKNOWLEDGMENTS

This research was supported by the Korea Medical Device Development Fund grant (2020M3E5D8105732) and Bio & Medical Technology Development Program (2021M3E5E7021473) of the National Research Foundation (NRF) funded by the Ministry of Science and ICT, Korea.

DIAPID project : an innovative prosthesis dedicated to children

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INTRODUCTION

Congenital diaphragmatic hernia (CDH) is a rare and severe disease affecting about 1 to 3500 live births. It is defined by a posterolateral defect of the diaphragmatic muscle allowing a communication between thorax and abdomen with ascension of the abdominal viscera in the thorax. Surgical closure of the defect should be performed as soon as possible to expect a spontaneous ventilation of the baby and his survival. For the largest ones, surgeons need a prosthesis to close the defect. Nowadays, there is no specific mesh and a significant incidence of recurrences, which affect the morbidity of those young patients[1].

It is therefore crucial to develop a prosthesis having adequate mechanical properties to prevent this complication and increase the survival rate.

The goal of DIAPID is to elaborate and optimize functional Polyurethan-based bi-face prosthesis made by electrospinning, withstanding an elongation upper than 300% and ultimate stress of 3-5MPa[2], having one fibrous hydrophilic side allowing enhanced cell interactions and one smooth hydrophobic side to prevent abdominal adhesion.

EXPERIMENTAL METHODS

We used medical grading thermoplastic polyurethane and several conditions of solubilisation. The bi-face prosthesis is produced in a continuous way by varying the processing parameters during electrospinning, using two types of electrospinner, a linear one (4 needles which could translate lateraly) and an annular one.

First, a Polyurethane film is produced and then, after a controlled transition stage, fibers are electrospun to make a rough fibrous and porous scaffold. We obtain different scaffolds with specific mechanical properties according to processing parameters. All scaffolds then have a biological and mechanical evaluation to assess their biocompatibility and elongation. Expanded polytetrafluoroethylene (ePTFE) is the control material for each analysis since it is the most commonly used in this indication. Briefly, for elongation we performed uniaxial stretching tests and for biocompatibility, we analyze cytotoxicity, inflammation and cell colonization. Finally, we look for chemical functionalization to optimize cell colonization.

RESULTS AND DISCUSSION

First we compared prosthesis manufactured according to the 2 modules (linear and annular) and were able to obtain membranes with a thickness clearly more homogeneous with the annular module, with 2 morphologically very distinct surfaces. The average elongation stress before failure was about 230% for all of our scaffolds against 150% for ePTFE.

From a biological point of view:

-we were able to observe the absence of contact cytotoxicity or release on murine and human macrophages at 24 and 48 hours (MTS test)

-absence of macrophage activation (murine and human) at 24 and 48 hours (no NO synthesis, or increase in the concentration of TNFalpha in the medium)

-cell colonization is present and differentiated on each of the 2 sides of the prosthesis by murin myocyts.

We then analyzed the impact of functionalization with polydopamine, which serves as an anchoring layer on the surface of the biomaterial to optimize cell attachment. We observe a good adhesion of this polydopamine on the biomaterial surface, with a marked improvement in its hydrophobicity without any modification on cytotoxicity nor macrophages activation.

CONCLUSION

This preliminary work confirms the relevance of our choice in terms of material and manufacturing technique. We must of course go further in the proof of concept by other mechanical tests (swelling, wear), in vitro biological evaluation and in vivo tests on small animals.

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ACKNOWLEDGMENTS

“The authors would like to thank the FIMATHO network and Fondation Maladies Rares for providing financial support to this project”.

Breast reconstruction using a bioabsorbable Tissue-Engineering Chamber for the regeneration of autologous adipose tissue: A Long Term pre-clinical review on minipigs

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INTRODUCTION

Breast cancer touch 1 in 8 women in the world, in 40% of cases, mastectomy is the usual treatment followed by chemotherapy or radiotherapy. Only 14% of women receive reconstructive surgery. After mastectomy, several methods are currently proposed for breast reconstruction for women: the fitting of a silicone prosthesis or the grafting of autologous adipose tissue (fat) by microsurgical technique (DIEP) or by non-vascularized transfer (lipofilling). However, these effective therapeutic possibilities have several drawbacks (cumbersome surgical procedure, multiple surgical procedures). They are also expensive for the care system.

The authors of this article have been working for 5 years on the development of a new solution: MATTISSE, an absorbable bio-prosthesis for breast reconstruction. It is an original technique making it possible to combine the advantages of current techniques to obtain an autologous reconstruction method, not providing after-effects of the donor site, making it possible to obtain a sufficient volume, in a single operating time.

With MATTISSE, the patient should recover a natural breast, without long term implant. MATTISSE is also applicable for aesthetic surgery. This technology is exploited by the company LATTICE MEDICAL

EXPERIMENTAL METHODS

The patented technology involves the use of autologous adipose tissue taken near the breast area during the surgery, and then combines with a 3D printed absorbable scaffold, help the regeneration of adipose tissue to get the desired size with just one surgery. MATTISSE is made up of a medical grade bioabsorbable polymer, turner into filament material to be 3D printed. MATTISSE is composed of two parts, a scaffold enhancing the adipose regeneration and a shell giving the volume and the shape. The use of 3D printing.. It takes from 3 to 6 months for the autologous tissue to be fully restored and 18 months for the full resorption of the polymer.

The following article shows the rationale for the use of bioabsorbable polymers in Tissue Engineering Chamber in Minipigs Models.

Vascularized adipose flaps were elevated in the inguinal region of female minipigs (n=9). Flaps were inserted into TECs that had different characteristics including porosity and the presence of a flat base. To conceive the different TECs, we used a 3D printing process meeting implantable device requirements in ISO7 clean room using medical-grade bioresorbable polymer. The 475 cc volume TECs have been sterilized using validated Ethylene Oxide process. Volumes of fat flap were assessed by MRI at various time points. At the end of the experiments, histomorphometric and immunohistological analyses were performed.

RESULTS AND DISCUSSION

We demonstrate the possibility to regenerate vascularized autologous adipose tissue inside the MATTISSE implant in a long term review (21 months). The volume
Bioresorbable TEC promoted angiogenesis and adipogenesis (up to 250 cc at day 90, corresponding to more than a 250 % increase) without predominant inflammatory response. Histologically, the expansion of adipose tissue resulted mainly from an increase in the number of adipocytes rather than cell hypertrophy.
Local and systemic effect of biopolymer resorption has been assessed regarding the ISO 10993 standards and show negligible effect with a small fibrotic tissue response <10% of the total volume.
This Minipigs study shows high performance, good reproducibility and long term volume stability after 21 months review, this viability is obtained thanks to a strong neovascularization obtained on the tissue.

CONCLUSION

This study show the requirements in terms of performance and safety to validate the pre-clinical requirements for a class III medical device under the EU MDR system. Now the company Lattice Medical has obtained a first agreement to start a pre-market clinical investigation involving 50 patients in 8 EU centers. Inclusions starts in July 2022 on Breast Reconstruction.

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Fucoidan: from brown algae to a novel radiotracer in cardiovascular imaging

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INTRODUCTION

The inflammatory pathway in atherothrombosis is characterized by complex interactions between endothelial cells, leukocytes and platelets. Among them, P-selectin is an adhesion molecule expressed at the surface of activated endothelial cells and platelets[1]. Non-invasive imaging of P-selectin has been investigated in a wide range of animal models of cardiovascular disease. In these studies, imaging agents were formulated with antibodies against P-selectin. However, these imaging methods have not been tested in patients yet.

Previously, we identified fucoidan extracted from brown algae as a polysaccharide ligand with nanomolar affinity for P-selectin[2]. Technetium-99m (^{99m}Tc)-labeled fucoidan selectively targeted P-selectin on activated human platelets *in vitro* and enabled SPECT/CT to visualize platelet-rich thrombi in animal models of endocarditis[3]. We envisioned radiolabeled fucoidan as a versatile imaging tool and recently developed ^{99m}Tc-fucoidan as a GMP-grade clinical imaging agent[4].

Here, we evaluated the safety profile and biodistribution of this new radiotracer in humans, and we provided for the first-time preliminary proof-of-principle by performing ^{99m}Tc-Fucoidan SPECT/CT in a patient with deep vein thrombosis (DVT).

EXPERIMENTAL METHODS

This report describes two imaging studies performed in a single center. The studies were conducted in accordance with the Declaration of Helsinki and in compliance with current Good Clinical Practice guidelines. The protocols were approved by the local institutional review board, and all participants provided written informed consent.

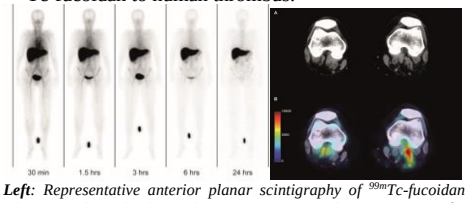
The first study (phase I) had an open-label design to assess the safety, biodistribution and dosimetry of a new diagnostic imaging agent in 10 healthy volunteers. After undergoing baseline examinations, healthy volunteers received a single injection of ^{99m}Tc-fucoidan (370 MBq), followed by whole body planar scintigraphy (t=30 min, 1.5 hrs, 3 hrs, 6 hrs and 24 hrs) and SPECT imaging (t=2 hrs). Blood decay samples and urine were collected.

The second study is an ongoing open-label diagnostic study to demonstrate proof-of-concept in patients older than 50 years with an acute DVT. Patients were recruited via the emergency room after diagnosis of DVT by compression ultrasound sonography. Within 48 hours of the initial diagnosis, patients received a single injection of ^{99m}Tc-fucoidan (370 MBq), followed by whole body planar scintigraphy and SPECT/CT imaging after 1.5 hrs.

RESULTS AND DISCUSSION

Following injection of ^{99m}Tc-fucoidan, we observed renal uptake of the radiotracer, followed by accumulation in the bladder and high activity in urine, indicating rapid renal elimination of fucoidan. Moderate physiological uptake was observed in the liver which quickly decreased over time. Radioactivity measurements were higher in plasma as compared to whole blood, indicating that a fraction of ^{99m}Tc-fucoidan bound to circulating non-activated platelets. These findings were in agreement with prior biodistribution studies of radiolabeled fucoidan in animals. Taken together, ^{99m}Tc-fucoidan had favorable imaging characteristics for visualizing P-selectin expression in a wide range of pathologies (Left).

In the second study, we reported on 1 female subject with an acute DVT of the left leg as diagnosed using ultrasound sonography. Following administration of ^{99m}Tc-fucoidan, SPECT/CT scanning of the legs revealed increased uptake of the radiotracer in the left popliteal vein as compared with the unaffected right popliteal vein in which intravenous blood pool activity can be observed (Right). This result is encouraging but requires confirmation in a larger sample size. In addition, *ex vivo* studies are needed to corroborate specific binding of ^{99m}Tc-fucoidan to human thrombus.



***Left**: Representative anterior planar scintigraphy of ^{99m}Tc-fucoidan administered to a healthy volunteer (#ID 06). **Right**: Axial views of a CT scan of a female patient presenting with an acute DVT of the left popliteal vein (**A**). ^{99m}Tc-fucoidan is visually increased in the left popliteal vein as compared with the right popliteal vein (**B**).*

CONCLUSION

In this first-in-human study, we demonstrated that ^{99m}Tc-fucoidan has a favorable safety and biodistribution profile for clinical imaging. Preliminary data suggested ^{99m}Tc-fucoidan SPECT/CT is suited for detecting enhanced P-selectin expression in patients with an acute DVT, but ongoing investigations (phase IIa) must confirm these findings.

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ACKNOWLEDGMENTS

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Bone Substitutes: from lab to bed

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INTRODUCTION

Orthopaedic surgeons are often confronted with bone defects. To fill a bone defect, they have at their disposal a large array of bone substitutes from human, animal, mineral or synthetic origin. A bone substitute is a biomaterial or a bioengineering product which can fill a bone defect in order to achieve healing and preservation of function.

BONE SUBSTITUTES FAMILY

Autograft still remains the gold standard to fill bone defects. Its supply may sometimes be insufficient and autograft harvesting causes morbidity. Deep frozen allografts may be used under various modalities ; they can be used to address virtually any clinical situation, without limitations related with the volume of the bone defect. Serological screening decreases the risk of virus transmission.

Allografts may also undergo a special processing to enhance microbiological safety, which prevents virtually all risks of viral diseases. Decalcified allografts appear to have osteoinduction properties. Synthetic bone substitutes are essentially calcium phosphates. Two of these are used in clinical application : hydroxyapatite and beta tricalcium phosphate. They are also available in a biphasic form. They are now widely used and dispense with autograft harvesting. In some indications, they may be used instead allografts. They have a macroporous structure which allows for bone ingrowth, i.e. osteoconduction . Their most important property is bioactivity, i.e the ability to establish a chemical link with bone, without interposition of fibrous tissue at the interface.

Calcium phosphate bone cements are not widely used so far. With their injectable form, they have the advantage of allowing percutaneous bone filling. They must be used following a specific handling procedure. Calcium sulphate may be associated with antibiotics and employed in septic situations.

Calcium carbonate has been very popular but it is now in competition with calcium phosphates.

The choice of a bone substitute cannot be the same whatever the clinical indication. The choice will depend upon the site and volume of the bone defect upon local and general conditions, upon the aetiology of the bone defect and upon the properties of the bone substitute.

CONCLUSION

Although, bone substitutes are now widely used in orthopaedic and trauma surgery, comparative, prospective and multicentric studies are still missing for their validation in clinical practice. Other products are available such as BMPs for bone consolidation, in association or not with bone substitutes for which clinical evaluation will also be necessary.

Research in tissue engineering is very promising for filling bone loss.

>> 10:30 - 12:30 | SYMP-04 - Use of physical forces for biomaterials design or characterization

Chairpersons: Agnès Drochon & Rui Domingues
Location: Room C

10:30 | KL Physical forces - New tools in magnetic tissue engineering
Manuela E. GOMES, University of Minho, Guimaraes, Portugal

11:00 | O1 Physical forces - Architectured mechano-hybrid-scaffold for endochondral healing of critical size bone defects
Ansgar PETERSEN, BIH Center for Regenerative Therapies at Charité – Universitätsmedizin Berlin, Berlin, Germany; Julius Wolff Institut, Charité – Universitätsmedizin Berlin, Berlin, Germany

11:15 | O2 Physical forces - High-throughput Generation of Soft Dissolvable Cell Microcarriers using In-air Microfluidics
Luanda LINS, Leijten Lab, Dept. of Developmental BioEngineering, TechMed Centre, University of Twente, Enschede, The Netherlands

11:30 | O3 Physical forces - Biological Evaluation of an In Vitro Biomimetic Platform Based on a Parallel Perfusion Bioreactor Designed to Test and Promote the Osteogenic Commitment of Cells and Biomaterials
Farah DAOU, Dept. of Health Sciences, Center for Translational Research on Autoimmune and Allergic Diseases (CAAD), Università del Piemonte Orientale (UPO), Novara, Italy

11:45 | O4 Physical forces - A novel approach for vascularization in 3D in vitro adipose tissue models
Matteo PITTON, Department of Chemistry, Materials and Chemical Engineering "G. Natta", Politecnico di Milano, Milan, Italy

12:00 | FP01 Physical forces - Perfusable Microvessel Substitutes Integrated in a Tailored Bioreactor Enable the Investigation of Chemotaxis in vitro
Mattis WACHENDÖRFER, Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany

12:05 | FP02 Physical forces - Synthesis of aligned hollow polymeric microfibers by coaxial electrospinning for the development of 3D in vitro models in perfusion bioreactors
Nazely DIBAN, Departamento de Ingenierias Química y Biomolecular, Universidad de Cantabria, Santander, Spain

12:10 | FP03 Physical forces - Photo-crosslinkable gelatin-based bio-inks as strategy towards patient-specific breast reconstruction
Lana VAN DAMME, Polymer Chemistry & Biomaterials Group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium

Architectured mechano-hybrid-scaffold for endochondral healing of critical size bone defects

Ansgar Petersen[1,2*], Martina Tortorici[1,2], Aaron Herrera[1,2], Thomas Urbaniak[2], Christoph Gayer[3], Hans Leemhuis[4]

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INTRODUCTION

Until today, no pure biomaterial strategy for bone defect regeneration does exist, even though associated low costs, off the shelf availability and a low risk of side effects are clear advantages. We have previously shown that a soft, collagen-based biomaterial with a channel-like pore architecture is able to induce endochondral ossification for the healing of critical size defects in long bones[1]. Here, we report on the incorporation of a 3D printed support structure for the mechanobiological optimization of the biomaterial at the site of implantation. We hypothesized that the resulting mechano-hybrid-scaffold (MHS) will protect the endochondral healing process against excessive stains and related structural and mechanical impairment even at long implantation duration. A successful preclinical evaluation of this material would open the route for translation towards the clinics.

EXPERIMENTAL METHODS

Different support structure architectures were designed using SolidWorks (Dassault Systems) with the goal to maximize porosity and to reduce in vivo straining below 3% at the site of implantation. For this, in vivo forces in the rat bone defect model were measured using an instrumented fixateur externe. Support structures were produced from medical grade polycaprolactone (PCL) by selective laser sintering[2]. Support structures were characterized structurally via SEM and mechanically via monoaxial compression testing. Following the design optimization, support structures were introduced into the fabrication process of the collagen-based guiding structure based on directional freezing and freeze drying. Using mechano-bioreactors[3], the resulting MHS scaffolds were characterized concerning support structure fatigue and cell recruitment into the collagenous scaffold under cyclic, in vivo-like loads ($1.2*10^6$ compression cycles). MHS were implanted into 5mm critical size defects in the rat femur stabilized by a fixateur externe without the addition of bioactive molecules or cells. Bone defect healing was studied via µ-CT and (immune)histology at week 3, 6 and 9 after implantation.

RESULTS AND DISCUSSION

In vivo forces on the femur were measured to be 1.5x body weight. To achieve a straining of ≈ 2.5%, the support structure design was iteratively optimized until a suitable stiffness of >10MPa was achieved. Optimized sintering parameters for the support structure resulted in MHS that did not show fatigue during in vitro evaluation in the bioreactor. Cell migration into the material was not impaired by the introduction of the support structure, preserving the required strong cell recruitment potential of the collagen-based guiding structure. Subcutaneous implantation of the support structure verified the biocompatibility of the laser sintered structure. Implantation of MHS into the critical-size femoral bone defect revealed a robust induction of endochondral ossification and progressive mineralization along the pre-aligned extracellular matrix fibers rich in collagen-I and fibronectin. Compared to pure collagen scaffolds used in earlier studies[1], the introduction of the support structure clearly improved the consistency of the linear extracellular matrix patterning six weeks post surgery and stabilized the endochondral healing process. This is regarded to be a consequence of a reduced scaffold straining and the additional spatial guiding function of the support structure design. Nine weeks after implantation, three out of six animals were in the process of bony bridging as verified by µ-CT and histological analysis. Currently, large animal experiments in sheep are performed to verify the MHS concept under geometrical and mechanical conditions that mimic the situation in humans.

CONCLUSION

The results of this study verify the potential of the architecturally and mechanobiologically optimized MHS to induce the regeneration of critical size bone defects. The use of clinically approved and fully degradable materials (collagen and PCL) are regarded beneficial for a future translational to the clinics.

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New tools in magnetic tissue engineering

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The poor healing ability of tendons as well as the limitations of currently used therapies have motivated tissue engineering (TE) strategies to develop living tendon substitutes. morphogenesis.

Our lab has been exploring the development of unique cell-laden 3D magnetically responsive systems that recapitulate key features of the native tissue and that can be further remotely modulated both in vitro and in vivo by the application of external magnetic stimuli.

We are exploring conventional and innovative tools such as multimaterial 3D bioprinting to design magnetic responsive systems mimicking specific aspects of tendon tissue architecture, composition and biomechanical properties, which, combined with adequate stem cells, shall render appropriate behavioural instructions to stimulate the regeneration of tendon tissue. Simultaneously, the 3D cell-laden magnetic system shall enable sophisticated 3D tissue models to unravel mechanisms behind tendon homeostasis and repair that will support the base knowledge to establish rational design criteria for the biofabrication of living tendon substitutes offering the prospect of tendon regeneration as opposed to simple tissue repair.

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High-throughput Generation of Soft Dissolvable Cell Microcarriers using In-air Microfluidics

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INTRODUCTION

3D cell culture microcarriers feature a high surface area to volume ratio, which allows more cells to connect per culture volume. Conventional microcarriers are designed to promote effective cell attachment and a high rate of cell proliferation. However, the cell harvest step is still challenging for many microcarriers as it can associate with a complex procedure, which hinders scaling-up of production, causes significant cell loss, reduces viability of harvested cells, and causes cell-product contamination [1]. In order to overcome these problems, we designed an innovative dissolvable microcarrier for bioreactor cell culture with an efficient harvest. The use of dissolvable microcarriers at the end of the cell culture is a strategy that allows to eliminate the respective separation step with benefits in terms of cell yield and cell-product purity [2]. The here presented soft, on-demand dissolvable, cell microcarriers do not require enzymatic degradation or chemical destabilization of the polymeric substrate, but rather depend only on the material used to manufacture the microcarrier. These next-gen, soft, dissolvable, scalable microcarriers were produced in monodisperse and ultra-high throughput manner by combining soft biomaterials with In-air microfluidics (IAMF), which is a scalable microtechnology that was recently invented in our lab [3].

EXPERIMENTAL METHODS

To generate microgels, a piezo-actuated microjet composed of 1% (w/v) alginate (80-120 cP) was collided with a continuous microjet of 0.01 M CaCl2 and 10% (v/v) ethanol (EtOH) allowing for Marangoni flow-driven engulfment of alginate microdroplets. Physical cross-linking occurred immediately after impacting the calcium chloride solution due to outside-in diffusion of calcium ions (Figure 1-a). Microgel size, sphericity, and monodispersity of microgels were characterized using phase contrast microscopy. Surface biofunctionalization was performed using electrostatic interaction between microgels and polyelectrolytes functionalized with RGD-peptide moieties. C2C12 myoblast were used as a model in order to analyze cell attach and proliferation on soft microcarriers in spinner flasks (shear stress: 70 rpm). Viability was assessed using live/dead staining and metabolic activity was assessed using Presto Blue.

RESULTS AND DISCUSSION

IAMF droplet generation proved to be a viable technology for the reliable ultra-high throughout generation of monodisperse soft microcarriers (220 µm, figure 1-a and -b) with excellent surface biomodification capacity. As demonstrated in Figure 1-c, after three days of culture, numerous cells had adhered to the surface of IAMFs microcarriers. Nine days post-seeding, many of the IAMFs microcarriers were linked together by cells, and numerous tridimensional structures were created by interaction between adherent cells. Moreover, a continuous increase in total viable cell number was demonstrated for at least nine days (Figure 1-c, Figure 1-d), with a daily proliferation yield >20%. Microcarriers could be cytocompatibly and rapidly be dissolved by scavenging alginate's calcium ions via exposure to phosphate buffered saline enabling effective harvest of expanded cells.

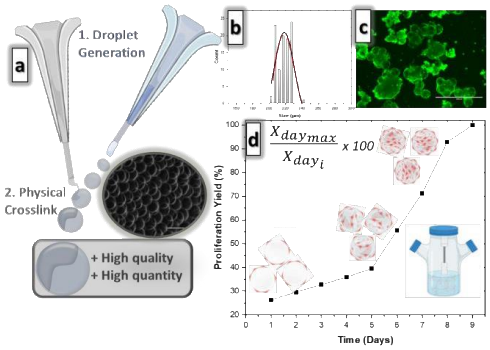

Figure 1. a. Schematic representation of ultra-high throughput production of microgels using in-air microfluidics and physical crosslinking, **b.** Illustrative particle size distribution of alginate microcarriers, **c.** Live/dead image of nine days expanded cell-laden microcarriers, **d.** Fold increase in total cell number was calculated as the ratio $Xday_{max}/Xi$, where X_{max} was the maximum cell density (between day 9 and 1) and Xi is the number of cells microgels were inoculated with.

CONCLUSION

Here, we demonstrate that the use of IAMFs as a promising technology for ultra-high throughput production of microcarriers with adjustable mechanical properties in a manner that allows accurate control over the chemical structure of the surface components of the material.

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Biological Evaluation of an *In Vitro* Biomimetic Platform Based on a Parallel Perfusion Bioreactor Designed to Test and Promote the Osteogenic Commitment of Cells and Biomaterials

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease with detrimental consequences on the functional ability of patients[1]. Recent advances in bone and cartilage tissue engineering and regenerative medicine provide evidence that these strategies offer a promising alternative to conventional surgical treatments. However, mimicking the biomechanical environment of the injured site is one of the hurdles facing translational research[2]. Pulsed electromagnetic field (PEMF) is also being investigated as a safe and noninvasive adjunctive therapy in orthopedics due to its osteogenic and chondrogenic potential, but there is limited research on its use in tissue engineering[3]. Building upon available evidence, an automated, parallel, and tunable perfusion bioreactor, equipped with a PEMF stimulator, was previously developed[4]. In parallel, 3D-printed polylactic acid (PLA) scaffolds mimicking the microarchitecture of trabecular bone were manufactured. This work aimed to perform the biological evaluation of this novel *in vitro* biomimetic platform featuring a perfusion bioreactor and 3D-printed PLA scaffolds as a technological tool for testing and promoting the osteogenic commitment of cells and biomaterials.

EXPERIMENTAL METHODS

Bioreactor. An automated direct perfusion bioreactor, developed for culturing in parallel up to three 3D constructs under tunable uni- or bi-directional perfusion (0.006-24 mL/min) with supplemental PEMF stimulation (1.5 mT, 75 Hz), was adopted.
Scaffolds. Biomimetic PLA scaffolds (total porosity = 60%, average pore size = 600 μm) were 3D printed.
Biological evaluation. The 3D-printed scaffolds were seeded with human mesenchymal stem cells (hMSCs) and exposed to various perfusion conditions (flow rate = 0.3-1 mL/min, uni- and bi-directional perfusion). Controls were PLA scaffolds seeded with hMSCs and cultivated in static condition.
In vitro cytocompatibility was assessed using the live/dead cell imaging assay. Osteogenesis was evaluated via alkaline phosphatase (ALP) release, real-time PCR, multi-omics studies and histological studies. Cell morphology and distribution were assessed via scanning electron microscopy (SEM) and THUNDER imager live cell & 3D assay, Leica.

RESULTS AND DISCUSSION

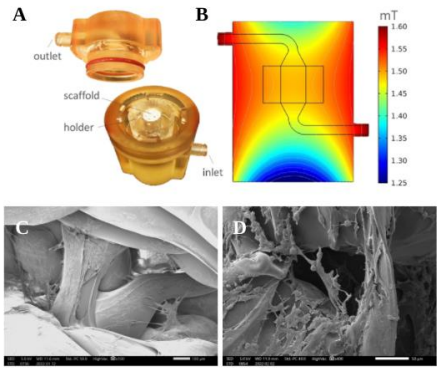

***Figure 1**: (A) Bioreactor culture chamber; (B) Contour plot of the magnetic field; (C) SEM image of hMSCs-seeded scaffold under unidirectional perfusion (bar scale = 100 μm) for 7 days; (D) SEM image of hMSCs-seeded scaffold in static condition (bar scale = 50 μm) for 7 days.*

The preliminary results showed that culturing the cell-seeded scaffolds under direct perfusion improved cell infiltration (Fig. 1C) with respect to static controls (Fig. 1D). The parallel bioreactor system allowed comparing 3D constructs, and automation optimized monitoring, adjusting, and controlling the environmental cues. The evaluation of osteogenic commitment, in addition to experimental setups involving perfusion and PEMF stimulation are ongoing.

CONCLUSION

The proposed *in vitro* biomimetic platform is a powerful tool for testing different combinations of scaffolds and cells designed for bone regeneration, and will aid in unraveling the signaling pathways associated with biophysical stimuli and tissue healing.

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A novel approach for vascularization in 3D *in vitro* adipose tissue models

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INTRODUCTION

3D scaffold-based *in vitro* models are nowadays emerging as innovative tools for a deeper understanding of physiological or pathological tissue maturation or to investigate novel therapeutic treatments. As regards, 3D *in vitro* models required proper materials, which faithfully recapitulated extracellular matrix (ECM) properties, adequate cell lines, and an efficient vascular network; this latter remains still challenging. In this work, UV-based photocrosslinked methacrylated gelatin (GelMA) hydrogels were used to produce a 3D *in vitro* adipose tissue model. Besides the already investigated approaches for scaffold vascularization, we herein proposed the incorporation of decellularized fennel (F), wild fennel (WF) and dill leaves (DL), acting as 3D vascular network with interconnected channels.

EXPERIMENTAL METHODS

GelMA was synthetized following a procedure described elsewhere [1]. F, WF and DL were properly decellularized [2] and morphological analysis of obtained pores and channels was performed by optical stereomicroscope. GelMA hydrogels were obtained by adding Irgacure 2959, as photoinitiator, pouring the polymeric precursor solution in PDMS moulds. Decellularized F, WF and DL were then embedded in the precursor solution prior to UV rays' exposure, and the structures were thus exposed to UV for 150 s to allow GelMA photo-crosslinking. *In vitro* stability tests in distilled water (up to 5 weeks), and mechanical compression tests were performed on hydrogels w/ or w/o decellularized F, WF, and DL. Preadipocytes 3T3-L1 (1 x 10^6 cells mL^{-1}) were encapsulated in the hydrogels w/ or w/o decellularized F, WF and DL, and cell-laden samples were cultured (37 °C, 5% CO_2) up to 14 days. Cell viability was assessed after 1, 3, 7 days of culture, and adipogenic differentiation was also qualitatively investigated by Oil Red O staining after adipogenesis induction [3]. Perfusion tests were performed on 3D hydrogels with decellularized vegetal structures to prove the presence of open channels.

RESULTS AND DISCUSSION

F, WF and DL showed a loss of pigmentation after the decellularization protocol. Stereomicroscope observation detected channels after decellularization whose dimensions comprised in a wide range of µm, ranging from 100-500 µm up to 3 µm; latter value resulted comparable with those of human microcirculation (5-10 µm) [3]. Photocrosslinking process was not affected by the vegetal structures in GelMA hydrogels. In fact, weight variation test, performed on hydrogels w/ or w/o decellularized F, WF, and DL showed a weight loss in the first 96 h, followed by stability plateau up to 5 weeks. Instead, embedding decellularized F, WF and DL into GelMA hydrogels caused a predictable decrease ($p < 0.05$) in mechanical properties (i.e., elastic modulus, stiffness, maximum stress, hysteresis area) compared to GelMA hydrogels.

Anyway, GelMA hydrogels with F, WF and DL showed elastic modulus values (3.55 - 4.27 kPa) comparable with those of physiological adipose tissue (3.25 kPa) [5]. Encapsulated 3T3-L1 preadipocytes showed an increase in the metabolic activity and in viability. This finding suggested vegetal structures did not affect cell adhesion, growth, and proliferation, enlightening how the proposed approach resulted totally biocompatible. Then, GelMA samples in which cells were differentiated, qualitatively exhibited a major accumulation of lipidic droplets 7 days after differentiation with respect to GelMA hydrogels embedding undifferentiated cells, as clearly visible by Red O staining. Herein, we demonstrated decellularized structures did not affect cells functionality as well. Perfusion tests revealed capability of decellularized structures in allowing liquid flows through their internal channels (Figure 1).

Solution was injected in a major hole of the decellularized F, WF and DL, and gradually perfused into the smaller channels.

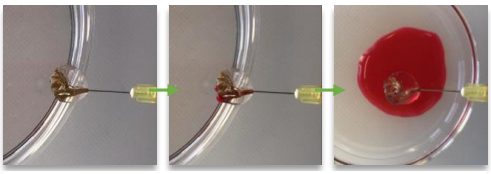

Figure 1 – A first attempt of perfusion tests revealed how vegetal structures' pores allow to solution flow into the open channels, obtained after a proper decellularization.

CONCLUSION

Developed GelMA hydrogels mimicked adipose tissue and the incorporated vegetal structures resulted a prominent solution to ensure an efficient vascular system for a 3D *in vitro* model as possible alternative to fugitive structures. As regards, embedded vegetal structures did not affect photo-crosslinking process, although a predictable decrease in mechanical properties was observed. Anyway, we demonstrated how embedded structures did not influence cell growth, proliferation, and functionality, while first attempts of perfusion tests successfully were carried out.

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Perfusable Microvessel Substitutes Integrated in a Tailored Bioreactor Enable the Investigation of Chemotaxis in vitro

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INTRODUCTION

There is a lack of biomimic in vitro vessel models to enable the investigation of the effect of simultaneous external stimuli on chemotaxis. Such systems can help to investigate and improve angiogenesis, intravasation, extravasation and can function as a drug screening platform. To address this issue, we propose bioprinted microvessel substitutes embedded in fibrin-based extracellular matrix substitutes (ECM) integrated in a tailored bioreactor for that purpose.

EXPERIMENTAL METHODS

The long-term stability of fibrin-collagen and fibrin-gelatin blends was investigated using an in vitro degradation test. To assess the permeability, transwell membranes were covered with hydrogel blends and the diffusion of fluorescein isothiocyanate labelled albumin from bovine serum (FITC-BSA) from the upper well into the PBS-filled lower well was measured using a microplate reader. The release of growth factors typical for inflammation (tumor necrosis factor α (TNF-α), stromal derived factor 1 (SDF-1)) from the hydrogel blends was investigated using an ELISA-kit. Microvessels mimicking arterioles were fabricated using coaxial bioprinting technique by combining an endothelial cell-laden (HUVEC) sacrificial gelatin core with a smooth muscle cell-laden (SMC) fibrin-based shell, all embedded in a fibrin based ECM. For microvessels mimicking venoles, needles used as placeholders were encapsulated in hydrogel prior to crosslinking and removed afterwards, leaving a perfusable microchannel within the ECM. The channels were cultivated using a perfusion pump and a tailored, 3D printed bioreactor system for up to 21 days. The middle part of the bioreactor incorporated the final hydrogel construct of 3 mm thickness. Medium containing cytokines and chemical stimuli was filled in two exterior reservoirs, separated from the hydrogel construct by permeable membranes, and diffused into the hydrogel construct and the perfusable microvessel substitute. The cellular organization of HUVECs and SMCs was investigated using immunostaining and confocal and two-photon microscopy.

RESULTS AND DISCUSSION

All hydrogel blends showed hydrolytic stability for at least 21 days. Swelling and shrinking of fibrin-gelatin blends was tuned by heat pretreatment of the gelatin component. Fibrin-collagen blends initially shrunk while the shrinking was reduced by increasing thrombin concentration and control of pH and temperature. Fibrin-gelatin blends provided twice the permeability of fibrin-collagen blends within the first 10 h. However, both blends levelled at a similar maximum permeability after 48 h. Gels with higher polymer concentration and hence denser microstructure showed lower permeability within the first hours compared to lower concentrated gels. The release of cytokines was distinctly higher from fibrin-gelatin blends (10-20 ng/ml for TNF-α; ~10 ng/ml for SDF-1) compared to fibrin-collagen blends (5 ng/ml for TNF-α; 3 ng/ml for SDF-1) after 24 h. A functional HUVEC monolayer lined the inner lumen of perfusable channels of approx. 500 µm in diameter. The HUVECs were activated by exposure to TNF-α, which diffused into the channels from the exterior reservoirs. The size of the channels was tuned by either the bioprinting settings (flow rate, temperature control) or designs of coaxial adapters and needles and showed similar accuracies as reported previously[1]. SMC showed high viability (< 80%) and characteristic stretching inside the gels.

CONCLUSION

In conclusion, we present a novel and versatile tailored bioreactor system which can be used to investigate the effect of external chemoattractants on the in vitro models. In combination with bioprinted microvessel substitutes, it represents a versatile and easy-to-use approach and can be used for a broad variety of tissue engineering applications.

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Synthesis of aligned hollow polymeric microfibers by coaxial electrospinning for the development of 3D *in vitro* models in perfusion bioreactors

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INTRODUCTION

In recent years, there has been a growing interest in developing novel functional materials to support cell outgrowth in biomedical applications. In this regard, electrospinning techniques are presented as one of the best alternatives to generate scaffolds that mimic the extracellular matrix emulating *in vivo* microenvironment. It has been proven that the alignment of the electrospun fibers provide topographical and biophysical cues that guide cellular morphogenesis[1,2]. Moreover, highly porous electrospun mats could be easily colonized by multiple cell types to reconstruct 3D cell structures. However, non-vascularized 3D cell systems rapidly suffer nutrients (O_2) shortage.

This work proposes the incorporation of aligned hollow microfibers in a perfusion bioreactor to mimic artificial vascularization with simultaneous cellular guidance for 3D cell cultures. This study presents the tune-up and selection of the working variables to prepare biocompatible polyacrylonitrile (PAN) hollow microfibers by electrospinning with adequate alignment, and preliminary cell tests.

EXPERIMENTAL METHODS

Polymer PAN solutions in dimethylformamide (DMF) at concentrations 10, 12, and 13 wt% were prepared. PAN solutions were extruded through a single needle with a syringe pump at different flowrates within 1 to 3 mL h^{-1}, and the distance between the needle and the collector was tried from 7 to 18 cm. The speed of the rotary collector was set at 252, 435 and 1168 rpm. The morphology and diameter of the fibers were analyzed by scanning electron microscopy (SEM).

With the previously selected working conditions (12 wt% of PAN, 2.12 mL h^{-1} of flowrate, 18 cm of distance, 1168 rpm of rotation speed), coaxial electrospinning using a coaxial needle of 0.83 mm ID and 1.83 OD was performed. Polyethylene glycol (PEG) solutions in DMF at concentration of 12 and 18 wt%, and flowrates between 0.71 to 2 mL h^{-1}, were extruded in the core of the fibers. Finally, the effect of extruding the polymer solutions in horizontal or vertical mode on the hollow formation of the microfibers was also evaluated.

Culture tests using C2C12 rat myoblasts were performed and the effect of the influence of the microfiber alignment on the C2C12 myotube differentiation was analyzed.

RESULTS AND DISCUSSION

Figure 1 shows the surface and cross section of the best results of aligned hollow microfiber membranes synthetized by coaxial electrospinning attained at using a 12 wt% of PAN in the polymeric solution and 18 wt% of PEG in the core solution with a flowrate of 0.71 mL h^{-1}, 18 cm of distance, 1168 rpm of collector speed and extruding the polymer solutions in vertical mode.

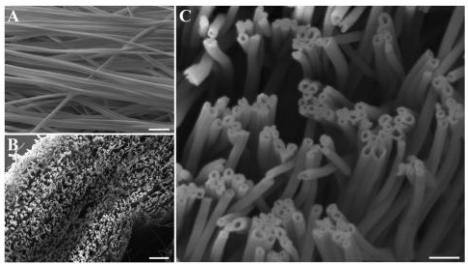

Figure 1. SEM images of aligned hollow microfiber PAN membranes: A) surface, and B, C) cross section. Scale bar: A) 10 µm, B) 50 µm, and C) 5 µm.

These working conditions allowed uniform alignment and centered hollow microfibers, with average external and internal diameters of 1.5 ± 0.1 µm and 0.6 ± 0.1 µm, respectively, and average bulk porosity of 87.3 ± 1%. The complete removal of the PEG, immersing the synthetized membranes in ultrapure water baths, was confirmed by thermogravimetric analysis.

Cell culture results showed C2C12 cells aligned with the hollow microfiber membranes and primary differentiation into myotubes.

CONCLUSION

This study presents the tune-up of different processing variables to attain aligned and centered PAN hollow microfiber membranes synthetized by coaxial electrospinning technique with potential to become 3D structural scaffolds in perfusion bioreactors.

Future work will focus on testing nutrients transport properties of these membranes and co-culture studies e.g., of neuronal and muscle cell lines.

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Photo-crosslinkable gelatin-based bio-inks as strategy towards patient-specific breast reconstruction

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INTRODUCTION

There exists a clear clinical need for adipose tissue reconstruction strategies to repair adipose tissue defects which outperform the currently available approaches, such as breast implants, micro-surgical free tissue transfer and lipofilling. The development of biomimetic materials able to promote cell proliferation and adipogenic differentiation has gained increasing attention in the context of adipose reconstructive purposes. Thiol-norbornene crosslinkable gelatin-based materials were developed and benchmarked to the current commonly applied methacryloyl-modified gelatin (GelMA) with different degrees of substitutions focussing on bottom-up tissue engineering. [1]

EXPERIMENTAL METHODS

Modification of Gelatin. Norbornene-modified gelatin (GelNB) was developed based on a protocol described earlier by Van Hoorick et al. [2] Methacryloyl-modified gelatin (GelMA) was developed according to the protocol described by Van Den Bulcke et al.[3]

Physico-chemical characterization

A rheometer (Physica MCR-301; Anton Paar) was used to evaluate the gelation kinetics of the hydrogels. The gel fraction, mass swelling ratio and enzymatic degradation was assessed on punched-out hydrogel films (8 mm diameter).

In vitro assays

The cytocompatibility of the encapsulated cells was tested in triplicate through a live/dead viability assay using calcein-acetoxymethyl (Ca-AM) and propidium iodide (PI) at day 1, 3, 7 and 14. To quantitively assess the differentiation of the cells into the adipogenic lineage, secretome analysis, a triglyceride assay and Bodipy/DAPI staining were performed at day 7 and 14.

In vivo assays

Scaffolds (both sham scaffolds and scaffolds containing adipose derived stem cells) were implanted sub-mammary in mice and assessed through contrast enhanced µCT imaging, one month post implantation. Following 12 weeks of implantation, ex vivo histology will be performed through a H&E staining.

Statistical analysis

Statistical analysis was performed using a unifactorial analysis of variance (ANOVA). Two values were considered statistically significant when the P-value was <0.05.

RESULTS AND DISCUSSION

The developed hydrogels resulted in similar physico-chemical properties (gel fractions >90% and mass swelling ratio ~13). The mechanical properties of the hydrogels could be tuned by incorporating more or less crosslinkable functionalities or using different crosslinking techniques (i.e. step-growth ~15kPa vs chain-growth ~30kPa). The biocompatibility (viability >85%) as well as differentiation potential of encapsulated adipose tissue-derived stem cells were analysed and showed superior adipogenic differentiation in the thiol-ene constructs. The elasticity of the hydrogels plays a key role in the cell differentiation potential, especially in cell-laden hydrogels and scaffolds. [4] A hydrogel offering an elasticity equivalent to physiological conditions should thus offer a mechanical cue toward the encapsulated ASCs to differentiate into the adipogenic lineage. Indeed, it can be observed that the softest material, namely GelNB55/SH75, resulted in significantly greater differentiation at both time points. This could be observed based on all assays. Initial in vivo data already showed good vascularisation throughout the construct one month post-surgery via contrast-enhanced µCT imaging. Additional in vivo experiments are currently ongoing assessing the differentiation potential and neovascularisation via ex vivo histology of constructs implanted sub-mammary in mice.

CONCLUSION

It can be concluded that the mechanical properties of a biomaterial are of utmost importance with respect to differentiation into the adipogenic lineage. The mechanical cues of GelNB55/SH75 were superior over the other investigated hydrogels. Photo-crosslinkable thiol-ene systems thus offer a promising strategy toward adipose tissue engineering through cell encapsulation compared to the widely used GelMA.

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ACKNOWLEDGMENTS

The authors would like to thank Tim Courtin for his help with recording the ^{1}H NMR spectra. L.V.D. would like to acknowledge the Research Foundation Flanders (FWO) for providing FWO-SB fellowships (1S85120N). Prof. Blondeel and Prof. Van Vlierberghe would also like to thank FWO for providing them with an FWO fellowship (3S039319)

>> 10:30 - 12:30 | SYMP-05 - Gene-activated 3D scaffolds for cartilage and osteochondral repair

Chairpersons: Arlyng Gonzalez Vazquez & Fergal J O'Brien
Location: Room H

10:30 | KL Gene activated - Scaffold-Mediated Viral Gene Delivery for Cartilage and Osteochondral Repair
Magali CUCCHIARINI, Center of Experimental Orthopaedics, Saarland University and Saarland University Medical Center, Homburg/Saar, Germany

11:00 | O1 Gene activated - Are gelatin nanoparticles suitable non-viral vectors for the delivery of mRNA?
Lea ANDRÉE, Department of Dentistry - Regenerative Biomaterials, Radboud Institute for Molecular Life Sciences, Radboudumc, Nijmegen, The Netherlands

11:15 | O2 Gene activated - The activity of fucoidan/dendrimer nanoparticles regarding angiogenesis
Filipe OLIM, CQM-Centro de Química da Madeira, University of Madeira, Campus da Penteada, 9020-105 Funchal, Portugal

11:30 | O3 Gene activated - Toward an innovative cement formulation combining calcium phosphate and lipid-oligonucleotide to address bacterial resistance
Clémentine AUBRY, ARNA/Inserm U1212, CNRS 5320, University of Bordeaux, Bordeaux, France

11:45 | O4 Gene activated - Novel cancer gene-therapy carriers based on the recombinant fusion of polycationic Elastin-Like Polymers and single-chain variable antibody fragments
Sara ESCALERA-ANZOLA, Smart Biodevices for Nanomedicine, University of Valladolid, Edificio LUCIA, Valladolid, Spain

12:00 | FP01 Gene activated - Decellularisation of whole human condyles for osteochondral repair
Hazel FERMOR, School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom

12:05 | FP02 Gene activated - Bone Regeneration with Antibiotic Delivery – A New Approach to Osteomyelitis and Infected Joint Replacements
Gerard INSLEY, Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

Toward an innovative cement formulation combining calcium phosphate and lipid-oligonucleotide to address bacterial resistance

Clémentine Aubry[1*], Christèle Combes[2], Christophe Drouet[2], Corinne Arpin[3], Tina Kauss[1], Philippe Barthélémy[1]
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INTRODUCTION

Calcium phosphate cements (CPC) have been extensively studied for bone applications and showed various advantages such as biocompatibility, bioactivity toward bone cells, ease of shaping, mechanical properties, possible injectability, etc.[1] In link with risks of infection during bone surgery and the increasing challenge of bacterial resistance[2], different strategies have been explored to increase the cement bioactivity, e.g. *via* combinations with ionic species[3] besides antibiotics[4]. However, while their antibacterial effect has been proven[5], few specific studies have been dedicated to bacterial resistance. In response to this antibiotic-resistance threat, lipid oligonucleotides (LONs) have been developed[6]. They exhibited, in resistant bacteria, the ability to decrease the minimum inhibitory concentration of antibiotics such as third-generation cephalosporin like ceftriaxone. Based on this idea, the present work aims at designing a bioactive cement formulation, involving both ceftriaxone and LONs, to deliver the antibiotic molecule while addressing simultaneously the issue of bacterial resistance.

EXPERIMENTAL METHODS

The cement composition based on previous work[4] used a solid phase composed of 50 wt% brushite (dicalcium phosphate dihydrate DCPD, $CaHPO_4.2H_2O$) and 50 wt% vaterite ($CaCO_3$) and a liquid phase composed of deionized water, with a L/S ratio of 0.75. LONs phosphorothioate backbone was used with a modification in 5' with a lipid phosphoramidite[6]. LONs ability to form micelles was followed in calcium-rich solution using a Zetasizer Nano ZS90, Malvern.

Physico-chemical modifications of the cement after introduction of either CFX or LONs. was assessed by kinetic survey at 37°C and under 100% humidity using FTIR spectrometer in ATR mode (Perkin Elmer).

The cement setting time was evaluated using a Texturometer CT3 Brookfield. A release test was made in a 0.9 wt% NaCl solution and titrations were carried out with a UV-Vis spectrophotometer NanoDrop (ThermoScientific).

RESULTS AND DISCUSSION

LONs showed a self-organizing ability in Ca-rich solution, forming large objects (~10000 nm) significantly larger than the typical size of micelles observed in water (~10 nm)[6], showing that Ca^{2+} ions likely interacted with the phosphate backbone of the LONs. In the cement experiments, different behaviors could be identified depending on the added molecule(s). While CFX, like other antibiotics[4], enhanced the chemical transformation of vaterite/brushite into apatite during cement setting (603 cm^{-1}), the presence of LONs seemed to delay this conversion, as an important proportion of brushite was still visible after 24h maturation (525 cm^{-1}) (Fig.1). DNA is known to inhibit apatite crystal growth due to its affinity with its crystal surface[7], and a similar interaction could occur in the case of LONs. Expectedly, we evidenced that such interactions between LONs and the CPC prevented significant LONs release in the tested conditions and induced higher friability of the LONs sample. This could be due to the lower conversion rate, leading to poor particle cohesion, even though the setting time of LONs-bearing cement was shorter than usual. Additional experiments are in progress to optimize the cement formulation to allow both the release of the active agent and the presence of LONs to counter bacterial resistance phenomena.

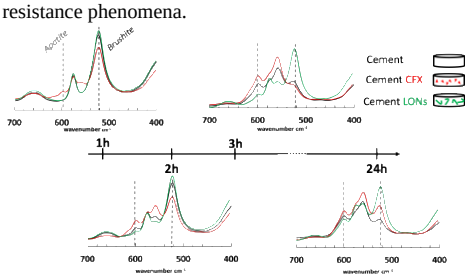

Fig.1 FTIR-ATR measurement of Cement, Cement with CFX and Cement with LONs at 1,2,3 and 24h

CONCLUSION

This work offers a preliminary approach allowing to formulate a new bone cement to tackle antibiotic resistance. It has been shown that LONs impact the usual behavior of CPC cement (maturation, setting time…). Further experiments combining CFX and LONs need to be conducted to adjust different parameters to allow adequate antibiotic release and demonstrate the efficacy on bacterial cultures.

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Scaffold-Mediated Viral Gene Delivery for Cartilage and Osteochondral Repair

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INTRODUCTION

The articular cartilage has a limited ability for self repair[1]. Osteochondral defects that involve both the articular cartilage and the underlying subchondral bone, resulting from trauma and leading to osteoarthritis if left untreated, are serious problems in orthopaedic surgery as they do not fully regenerate an original, hyaline cartilage. Gene therapy is a powerful tool to stably manage these lesions as it allows for a prolonged expression of therapeutic genes in sites of injury, most particularly when delivered via viral vectors in combination with the use of biocompatible materials serving as platforms to control the release of the sequences being carried[2-5].

RESULTS AND DISCUSSION

The goal of this work is to provide the current advances and challenges in the field of scaffold-guided viral gene therapy to heal osteochondral lesions in experimental settings *in vitro*, *in situ*, and *in vivo*. A strong focus is given on the clinically adapted recombinant adeno-associated virus (rAAV) vectors[6] derived from a human non-pathogenic, non-replicative parvovirus broadly employed to treat various human diseases imcluding in the area of regenerative medicine[7-11].

CONCLUSION

Various scaffolds have been employed to guide the delivery of viral gene vectors as an effective and safe tool to treat osteochondral defects. This approach has a strong potential for clinical therapy in human patients in a close future.

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Are gelatin nanoparticles suitable non-viral vectors for the delivery of mRNA?

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INTRODUCTION Growth factors (GFs) are often used in tissue engineering to guide cell differentiation. However, the use of GFs in the clinic has proven difficult due to fast degradation and uncontrolled release. To compensate the fast loss, supraphysiological doses are administrated with the consequence of side effects and, in the worst case, malignancies[1]. Alternatively, messenger RNA (mRNA), as the middleman between genes and proteins, can stimulate cell differentiation via endogenously produced GFs. Thereto, suitable vectors are required for intracellular delivery and endosomal escape. Non-viral vectors such as lipid or polymer nanoparticles are preferred over viral ones due to their better safety profile[2]. Gelatin nanoparticles (GNPs) have been proposed for delivery of small interfering RNA (siRNA)[3]. GNPs are considered biocompatible and biodegradable. Moreover, GNPs can be produced by a simple process and modified through abundant functional groups[4]. Consequently, we have evaluated the suitability of GNPs for intracellular delivery of mRNA and resulting protein expression, aiming to advance the design of GNPs as non-viral vectors for mRNA delivery.

EXPERIMENTAL METHODS GNPs were prepared by coacervation of type A or type B gelatin, and characterized by dynamic light scattering (DLS) and zeta potential measurement. To yield GNPs with a positive surface charge, type A GNPs were surface functionalized with ethylenediamine. mRNA was loaded post-synthesis by adsorption. Loading capacity and release was measured by biochemical assay. The internalization of differently charged, fluorescently-labelled GNPs loaded with fluorescently-labelled mRNA into pre-osteoblastic cells (MC-3T3) was studied using confocal live cell microscopy with staining of lysosomal compartments. Expression of mRNA with or without the addition of the endosomal release agent chloroquine was tested using luciferase mRNA.

RESULTS AND DISCUSSION Spherical GNPs in the range of 300-400 nm and with either positive (19.0 ± 0.8 mV), neutral (2.9 ± 0.3 mV), or negative (-11.6 ± 0.8 mV) zeta potential were synthesized. Loading capacity of mRNA varied between 98.7 ± 0.7 % for positively, 48.8 ± 10.9 % for neutral and 4.0 ± 2.1 % for negatively charged GNPs. mRNA release from positively charged GNPs was low with only 7.3 ± 0.1 % of mRNA released after 48h. Moreover, using heparin-mediated decomplexation, not all mRNA could be retrieved from these GNPs, indicating a strong binding. Although fluorescently-labelled mRNA loaded on GNPs was successfully internalized by cells, no expression of the encoded protein was observed. Visualization of fluorescently-labelled GNPs showed cellular uptake with a predominant lysosomal localization of GNPs after 24h (Fig. 1), indicating a lack of endosomal escape. However, even after the addition of the endosomal release agent chloroquine, no expression of the encoded protein was detected. This suggests that mRNA loaded on GNPs degraded prematurely or did not become available for translation by the ribosome.

CONCLUSION Although GNPs show cellular internalization, they are not yet able to efficiently deliver mRNA to the ribosome. In the case of neutral and negatively charged GNPs, mRNA loading capacity is too low for successful mRNA delivery. In contrast, positively charged GNPs show strong binding of mRNA. However, GNPs do not stimulate endosomal escape, resulting in entrapment of mRNA in lysosomes and lack of protein expression. Our results showcase that internalization of GNPs does not automatically lead to effective mRNA delivery and protein expression. Therefore, further modification and optimization is required to enable therapeutically efficient mRNA delivery from GNPs.

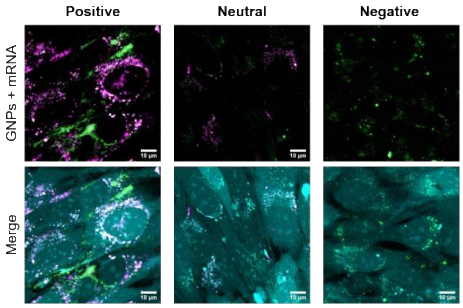

Figure 1 – Internalization of GNPs (green) into pre-osteoblasts (cyan) and their localization in lysosomal compartments (magenta). Scale bar is 10 µm.

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The activity of fucoidan/dendrimer nanoparticles regarding angiogenesis

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INTRODUCTION

Angiogenesis constitutes a physiological process where new blood vessels are formed from pre-existing vascular structures. Due to increased metabolic requirements, tumours are capable of secreting angiogenic factors. The formation of new vasculature in a non-controllable manner contributes to significant changes in the tumour microenvironment, contributing to its ability to invade and metastasize in distant places. As glioblastoma, a severe type of brain cancer, is characterized by high vascular density, strategies targeting angiogenesis may enormously benefit the patient's treatment[1].

Fucoidans are a class of sulfated polysaccharides mainly extracted from brown algae. They have been shown to exert various biological activities, including pro- or anti-angiogenic effects, depending on their structural properties[2]. Thus, their integration in nanoparticles aimed at being used as drug/gene delivery vehicles may endow them with intrinsic anticancer properties.

Dendrimers are monodispersed polymers that grow from a central core, layer by layer, achieving a high number of end groups at their surface. Polyester dendrimers, such those based on 2,2-bis(hydroxymethyl)propionic acid (bis-MPA), are very interesting to be use in nanomedicine due to their degradability at physiological conditions[3].

The main objective of this work was to prepare and characterize fucoidan/bis-MPA-based dendrimer nanoparticles, and further study their behaviour regarding angiogenesis. In the future, we aim to apply these nanoparticles in glioblastoma treatment.

EXPERIMENTAL METHODS

In a first phase, the pro/anti-angiogenic properties of fucoidans from two different species of algae (*Fucus vesiculosus* and *Undaria pinnatifida*), as well as of their respective hydrolysed counterparts, was studied. For this purpose, the effect of the fucoidans in the metabolic activity of endothelial cells (HUVEC and hCDMEM/d3 cell lines were used) was assessed. Then, their effect on the formation of *in vitro* tubular structures formed by the endothelial cells in Geltrex matrixes was evaluated. In a second phase, nanoparticles based on non-hydrolysed fucoidans and bis-MPA-based degradable dendrimers were prepared at different fucoidan/dendrimer ratios, characterized, and their pro-angiogenic activity was evaluated in vivo through the CAM assay.

RESULTS AND DISCUSSION

The two types of fucoidans did not exert considerable effect on the metabolic activity of both endothelial cell lines (they were not cytotoxic in the studied range of concentrations). Regarding the *in vitro* formation of tubular structures, all the polysaccharides (hydrolysed and non-hydrolysed) showed an anti-angiogenic effect. However, the non-hydrolysed polysaccharides presented the best response and were therefore selected for integration in the nanoparticles. By tuning the ratio between fucoidan and bis-MPA dendrimers, it was possible to obtain nanoparticles with different physicochemical properties (such as the Zeta Potential) just based on electrostatic interactions. Regarding the *in vivo* CAM assay, results obtained with the nanoparticles did not evidence a pro-angiogenic response at the tested concentrations.

CONCLUSION

Fucoidan molecules extracted from *Fucus vesiculosus* and *Undaria pinnatifida* show an anti-angiogenic effect in vitro, being the non-hydrolysed ones (with a higher molecular weight) those that present the best anti-angiogenic response. The possibility of manufacturing nanoparticles based on these sulphated polysaccharides and biodegradable dendrimers was also demonstrated. Since these nanoparticles are made of degradable dendrimers, their administration *in vivo* as carriers for drugs/genes should be followed by nanoparticle degradation and fucoidan release, which can then exert its anti-angiogenic properties.

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Novel cancer gene-therapy carriers based on the recombinant fusion of polycationic Elastin-Like Polymers and single-chain variable antibody fragments

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INTRODUCTION

The emerging field of cancer gene therapy allows the selective treatment of malignant cancer cells while keeping the surrounding normal cells alive, which entails considerable benefits when compared to typical treatments such as chemotherapy or radiotherapy. One of the most exciting treatments derived from gene therapy is the transfection of therapeutic genes in the malignant cells and the control of their expression by a tumoral promoter, which gives the system the ability to discriminate between malignant and healthy cells.

Tenascin C (Tn-C) is a complex Extracellular Matrix (ECM) protein that is frequently over-expressed in the stroma of most cancers, and it is associated with poor prognosis, which makes it a great cancer biomarker that can be used for cancer diagnosis and can be exploited for targeted therapies[1]. Tn-C can be specifically targeted using single-chain variable fragments (scFv) that consist on variable regions of heavy and light chains of immunoglobulins joined by a peptide linker.

To facilitate the arrival of the gene therapy to the tumour stroma after intravenous injection, the DNA can be hidden in a polymeric backbone, which protects it from nuclease degradation. Elastin-like polymers (ELPs), biomaterials derived from elastin-mimetic peptide sequences, are promising candidates as carriers for these gene therapies because of their excellent biocompatibility and low toxicity[2]. In this work, we have developed fusion scFv-ELP constructs directed to the tumoral stroma as nanocarriers for gene therapy.

EXPERIMENTAL METHODS

Two ELR-scFv fusion proteins were created using genetic engineering techniques and produced recombinantly in a bacterial host. They were purified by affinity chromatography and characterized by MALDI-TOF, ^{1}H-NMR and SDS-PAGE. Different therapeutic plasmidic DNAs (pDNAs) were also created using genetic engineering techniques. Nanoparticles based on this ELR-scFv and pDNAs were formed by electrostatic interactions between the positively charged lysine-rich backbone of ELRs and negatively charged pDNA. The obtained nanoparticles were characterized by DLS, TEM and AFM and their specificity and affinity against Tn-C was probed by Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) and immunological techniques. After all the physicochemical characterization, their biocompatibility was studied *in vitro* in control non tumorigenic cell lines.

RESULTS AND DISCUSSION

Herein, two ELR-scFv fusion proteins targeting two different isoforms of Tn-C were obtained. The different scFv were selected to target different isoforms of Tn-C and check the most efficient one on their own and their synergistic effect when mixed.

Our nanoparticles presented a Zeta potential of about +30 mV and a size of about 160 nm. Their positive charge allows the interaction with the negatively charged cell membrane, facilitating the pDNA uptake. When loaded with a pDNA that encodes a fluorescent protein, these nanoparticles can be used as imaging technique for diagnosis and when loaded with toxic pDNA, it can be used as therapy (Figure 1). QCM-D and immunological assays were carried out to prove the specificity of the nanoparticles to target Tn-C, showing a much higher specificity than the ELR without scFv.

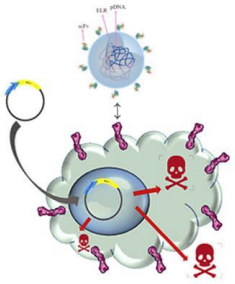

Figure 1. Scheme of the nanodevice

CONCLUSION

These results show the great potential of the developed nanoparticles to target Tn-C, and thus, to deliver therapeutic genes to cancer cells. Further experiments include *in vitro* experiments with Tn-C-expressing tumoral cell lines to prove their efficacy.

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Decellularisation of whole human condyles for osteochondral repair

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INTRODUCTION

Osteoarthritis (OA) of articular cartilage is a progressive and debilitating disease, often necessitating a total joint replacement. Between 2005 and 2015 the worldwide prevalence of OA increased by 32.9 % (from 17.9 to 23.7 million cases)[1]. There is therefore a demand for early stage interventions to prevent or delay joint replacement surgery. Decellularisation technology aims to remove DNA and cellular material from biological tissues to produce collagen rich extracellular matrix scaffolds which are non-immunogenic upon implantation to replace diseased or damaged tissues[2]. These biological scaffolds retain their histoarchitecture, biochemical and biomechanical properties along with functional molecules and are able to facilitate remodeling within the recipient. A potential treatment for OA is a decellularised osteochondral (OC) graft. It is proposed that whole human condyles can be decellularised to produce large, non-immunogenic scaffolds which are then shaped to fit OC lesions.

EXPERIMENTAL METHODS

Whole medial and lateral femoral condyles (FCs) from human donors (N=4) were isolated. The underlying bone was reamed to a total depth of 1 cm. Two medial and two lateral FCs from four separate donors were decellularised and the corresponding lateral and medial FCs retained as cellular controls. The decellularisation method applied utilised low concentration sodium dodecyl sulphate (0.1 % w/v), freeze-thaw cycles, sonication, protease inhibitors and nuclease treatment. Samples were analysed biochemically (quantitative DNA and glycosaminoglycan (GAG) assays) and histologically to stain for the presence of nuclear material and GAGs (haematoxylin and eosin (H&E), 4′,6-diamidino-2-phenylindole (DAPI) and Safranin O staining). Quantitative data was tested for normality with the Shapiro-Wilk normality test. The normally distributed data was analysed using the unpaired Student's t-test. A significance level of 0.05 was set for both tests.

RESULTS AND DISCUSSION

The total DNA content of decellularised bone and cartilage was below the suggested maximum limit of 50 ng.mg^{-1} dry tissue weight[3] at 35.8 (± 19.5) and 4.3 ± 3.7 ng.mg^{-1} (± 95 % confidence interval, CI) respectively. GAG concentrations were not significantly different between cellular and decellularised cartilage (163.5 ± 48.1 and 193.5 ± 50.4 µg.mg^{-1} (± 95 % CI) dry tissue weight respectively). Safranin O staining showed GAG retention in the cartilage over the whole sagittal section of the FCs. H&E and DAPI staining showed a reduction in nuclei in decellularised samples; cartilage and bone were largely devoid of visible nuclei. There were occasional nuclei in the calcified tidemark region between the cartilage and bone and the rare occurrence of nuclei in the bone.

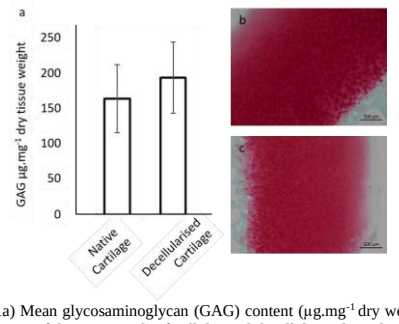

Fig 1a) Mean glycosaminoglycan (GAG) content (µg.mg^{-1} dry weight ± 95 % confidence interval) of cellular and decellularised cartilage (N = 4). No significant difference was found (p = 0.22, unpaired Student's t test). Images of Safranin O-Fast green stained histological sections of cellular (b) and decellularised (c) osteochondral tissue, x 100 mag, 500 µM scale bar.

FCs were adequately decellularised with excellent GAG retention in the cartilage. As cellular bone allografts are routinely and successfully used in orthopaedic surgery, the presence of the occasional nucleus in the decellularised product should not be of detriment to the immunocompatibility of the graft. It is expected that the removal of cells from osteochondral allografts through decellularisation will accelerate bone-bone healing post implantation. Future work will seek to explore if the retention of GAGs is accompanied by a retention of mechanical and functional properties, through indentation and friction testing.

CONCLUSION

Decellularised condyles may provide an easily stored, off-the-shelf alternative to viable cellular allografts for the repair of cartilage defects.

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Bone Regeneration with Antibiotic Delivery – A New Approach to Osteomyelitis and Infected Joint Replacements

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INTRODUCTION

Osteomyelitis and Infected Joint Replacements have been the most challenging problem in Orthopaedics for decades. Whilst there have been huge advances in the development of biomaterial-based bone grafts for orthopaedic applications, healing bone infections remain a major challenge[1]. One approach commonly used by orthopaedic surgeons is the use of calcium sulfate as a biomaterial based delivery platform for local antibiotic release. Calcium sulfate has a relatively rapid reabsorption time, optimal for sustained antibiotic elution. Furthermore, calcium sulfate is biocompatible so its removal via a secondary procedure is not required. However, the rapid rate of resorption outpaces that of bone formation, so whilst it is advantageous as an antibiotic elution platform, it does not have the capacity for functional bone healing[2]. As such, there is a major gap in the market for antibiotic eluting biomaterials to simultaneously treat the infection and facilitate bone regeneration within a defect. We have developed a new class of biomimetic biomaterial call an organo-calcium phosphate bioadhesive (OssStic). This project aims to investigate the ability of this innovative bioactive bone graft as an antibiotic delivery platform.

EXPERIMENTAL METHODS

The potential of OssStic as an antibiotic delivery platform was compared to commercially available calcium sulfate based biomaterial – Osteoset.

Antibiotic loading: OssStic and Osteoset are powder-based materials and antibiotic loading was via mixing in antibiotic solution with the powders. 50 mg/ml vancomycin was mixed with the biomaterial powders. Antibiotic release: Vancomycin release from the biomaterials was detected over 21 days via high performance liquid chromatography. Degradation: OssStic degradation rates was compared to Osteoset as degradation can significantly influence therapeutic release. Both biomaterials were placed in PBS and simulated body fluid (SBF) for 14 days and the % loss in weight was monitored. These two media were chosen as they do not interfere with HPLC detection of antibiotics.

RESULTS AND DISCUSSION

OssStic follows a similar release profile compared to Osteoset over the 21-day period however the release of vancomycin from OssStic was slower. Both initially release a high dose before decreasing gradually over the first 12 hrs. This is due to the antibiotic eluting from the surface of the material initially. As the outer surface degrades, a significant increase is observed again at 24hr that is maintained until day 7 (120hr), after which a significant decrease is observed. Osteoset release drops below the minimum inhibitory concentration (MIC) at day 14 (336 hr) but OssStic continues releasing vancomycin up to day 21 (Fig 1A).

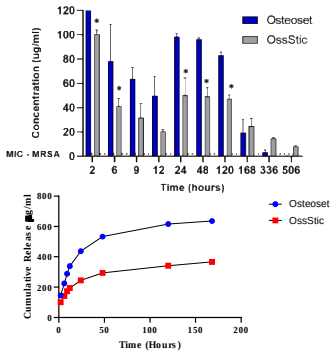

Figure 1: *(A) Vancomycin release from OssStic and Osteoset over 21 days (B) Cumulative release of vancomycin from both biomaterials*

Release data presented as cumulative release shows that OssStic has a lower initial burst release and slower subsequent release compared to Osteoset (Fig. 1B). The OssStic is less susceptible to degradation than the commercially available Osteoset but does have a significant decrease in % mass

CONCLUSION

OssStic proved a significant advance over clinical standard Osteoset, with a more controlled and longer release profile and slower degradation rate that overcomes the challenges associated with current clinical approaches

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ACKNOWLEDGMENTS

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>> 10:30 - 12:30 | SYMP-06 - Electroactive scaffolds in biomaterials

Chairpersons: Markus Rottmar & Sahba Mobini
Location: Room E

10:30 | KL Electroactive - Soft Electronics as Interfaces to Living Tissues
John A. ROGERS, Department of Materials Science and Engineering and Biomedical Engineering, Northwestern University, Evanston, USA

11:00 | O1 Electroactive - Biomimetic Electroconductive PEDOT Nanoparticle Scaffold for Spinal Cord Injury Repair
Aleksandra SERAFIN, Bernal Institute, School of Engineering, University of Limerick, Ireland

11:15 | O2 Electroactive - Dissolution Behaviour and Biocompatibility of Combinatorially Sputtered SiFeCN Coatings for Spinal Implants
Estefanía ECHEVERRI, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden

11:30 | O3 Electroactive - Collagen/Pristine Graphene as an Electroconductive Interface Material for Neuronal Medical Device Applications
Jack MAUGHAN, Chemical Physics of Low Dimensional Nanostructures Group, School of Physics, Trinity College Dublin & Tissue Engineering Research Group, Royal College of Surgeons in Ireland

11:45 | O4 Electroactive - Bioelectronics to study and regenerate bone
Donata IANDOLO, INSERM, U1059 Sainbiose, Université Jean Monnet, Saint-Étienne, FR.

12:00 | FP01 Electroactive - Nutlin-loaded ultrasound-activated piezoelectric nanovectors: Modulation of anti-angiogenic activity
Ozlem SEN, Istituto Italiano di Tecnologia, Smart Bio-Interfaces, Pontedera, Italy

12:05 | FP02 Electroactive - Design of a fully-resorbable electronic device for neural stimulation and monitoring
Isabelle TEXIER, University Grenoble Alpes, CEA, LETI-DTBS, Grenoble, France

12:10 | FP03 Electroactive - A Closed-Loop Soft Robotic Drug Delivery System to Overcome the Foreign Body Response
Lucien H.J. SCHREIBER, Anatomy and Regenerative Medicine Institute (REMEDI), School of Medicine, NUI Galway, Galway, Ireland

Soft Electronics as Interfaces to Living Tissues

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This talk summarizes recent work from our group on electronic and optoelectronic systems that have the ability to maintain stable, long-lived interfaces to living tissues. Examples range from flexible probes, to stretchable sheets, to three dimensional compliant frameworks. Illustrations of uses in areas spanning clinical care and neuroscience research highlight the key features of these biocompatible technologies.

INTRODUCTION

Advanced electronic/optoelectronic systems built using classes of nanomaterials that enable intimate integration with soft biological tissues will accelerate progress in clinical medicine, as diagnostic and therapeutic tools, and in biological research, as instruments for monitoring or modulating natural processes. Specifically, miniaturized electronic elements, light sources, photodetectors, multiplexed sensors, programmable microfluidic networks and other components injected into precise locations of tissues, softly laminated onto their surfaces or spanning volumetric regions as instrumented scaffolds will open up unique and important opportunities. This presentation describes concepts in electronic materials science and assembly processes that underpin these types of technologies through a diverse set of examples. Recent articles summarize key aspects of the current state of the art.[1-5]

EXPERIMENTAL METHODS

The engineering approaches rely on heterogeneous collections of hard and soft materials configured into layouts that approximate the mechanical and geometrical characteristics of targeted sites, surfaces or volumes of tissues of interest. The fabrication methods combine lithographic processes adapted from those used by the semiconductor industry with emerging techniques in microtransfer printing to integrate diverse classes of materials into functional systems. Simulations of the mechanics and the electromagnetic properties guide design choices.

Use cases span minimally invasive interfaces to the human body through the surface of the skin to systems that penetrate to distinct locations in the depths of tissues or that extend throughout three dimensional spaces.

RESULTS AND DISCUSSION

The results presented in this talk focus on optoelectronic and microfluidic interfaces to small-scale tissue constructs derived from human stem cells, ranging from spheroids and organoids to assembloids. Electrophysiological measurements on cortical spheroids and cardiac muscle organoids represent two key examples.

CONCLUSION

Advances in materials science, mechanical engineering and manufacturing methods serve as the foundations for high performance electronic, optoelectronic and microfluidic technologies that can interface intimately with biological systems – thereby blurring the distinction between two. The results have implications in defining the future of healthcare and in establishing opportunities in basic biological research.

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ACKNOWLEDGMENTS

“The authors would like to thank the Querrey-Simpson Institute for Bioelectronics for providing financial support to this project”.

Biomimetic Electroconductive PEDOT Nanoparticle Scaffold for Spinal Cord Injury Repair

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INTRODUCTION

A new trend in the field of tissue engineering (TE) has emerged in the form of conductive biomaterials. It has been known for decades that cells are affected by electrical stimulation [1], especially cells of a conductive nature such as spinal or nerve cells [2]. Therefore, the application of conductive TE scaffolds to spinal cord injury (SCI) models aims at repairing and improving the growth and signalling of damaged spinal cells by facilitating electrical stimulation [1]. In this study, we established a novel synthesis method to produce Poly(3,4-ethylenedioxythiophene) (PEDOT) electroconductive nanoparticles based on the mini-emulsion method. Further incorporation of PEDOT nanoparticles with bioactive components such as gelatin and hyaluronic acid allowed for the development of a novel electroconductive biomaterial. The physico-chemical characterization of this biomaterial includes morphology, compression tests, electroconductivity, and *in-vitro* and *in-vivo* biocompatibility studies.

EXPERIMENTAL METHODS

Structural analysis: The morphology of the developed PEDOT NPs was first analyzed using Scanning Electron Microscopy (SEM). The PEDOT NPs were then incorporated into a hydrogel structure to create NP electroconductive scaffolds. Cross-sectional images of NP scaffolds were analyzed to evaluate the changes in the morphology and porosity of the scaffolds.

Compression Properties: The compression test was carried out on the NP scaffolds using an in-house compression test facility equipped with a 1 kN load cell, with a screw speed of 1 mm/min up to 60% strain. The Young's Modulus of the samples was calculated as the slope in the linear region of a normalized stress vs. strain graph.

Conductivity Properties: The resistivity of the NP electroconductive scaffolds was measured using an Ohm meter. The conductivity was then calculated as follows:

$$\sigma = lRA$$

Where, σ is the conductivity, l is the sample length, A is the cross-sectional area, and R is the resistance.

***In-Vitro* and *In-Vivo* Biocompatibility Assessment:** Scaffolds were seeded with DiI stained Mesenchymal Stem Cells (MSCs) at density of $0.1x10^6$ cells/scaffold and cultured in MEM alpha cell culture medium supplemented with 1% penicillin-streptomycin, 2% L-Glutamine and 20% FBS). After 96 hrs of culturing, the cells were fixed in 4% paraformaldehyde, stained with DAPI and imaged. Implantation of scaffolds into Wistar rats was completed with a complete SCI transection model at T3 level. At 1 month post implementation, the rats were sacrificed, and the tissue of interest excised and processed by means of immunohistochemistry, and imaged.

RESULTS AND DISCUSSION

The development and characterization of the PEDOT NP electroconductive scaffolds were successfully accomplished. The morphology of the PEDOT NPs was round and stable at 187 nm in diameter . The developed NP scaffolds display adequate mechanical strength comparable to that of the native spinal cord, while the conductivity of the scaffolds ranging up to 10^{-6} S/cm. Biocompatibility shows both that the developed NPs, as well as the electroconductive NPs scaffolds, facilitate MSCs cell proliferation and growth. Implantation into rat SCI lesions indicates that the NPs scaffolds are biocompatible and do not promote cytotoxicity in the host (Figure 1).

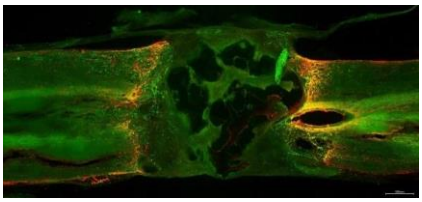

Figure 1. Rat complete transection SCI lesion at T3 level, 1-month post-implantation with PEDOT 1x NP scaffold. NF200 in green and GFAP in red, scale bar 500 µm.

CONCLUSION

From this work, it can be concluded that the developed NPs and the subsequent incorporation of the NPs into electroconductive scaffolds show promise as a TE strategy for SCI repair.

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ACKNOWLEDGMENTS

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Dissolution Behaviour and Biocompatibility of Combinatorially Sputtered SiFeCN Coatings for Spinal Implants

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INTRODUCTION

One of the main limiting factors to the life span of spinal implants is the release of detrimental ions and particles, which are typically produced by wear and corrosion[1,2]. One suggested approach to overcome these issues is the use of silicon nitride-based coatings on metallic implants because of their low wear rates and their ability to slowly dissolve in aqueous solutions into biocompatible ions only, which could be advantageous in terms of limiting the effects of wear debris and ion release[3]. A previous study found that alloying the silicon nitride coating with Fe and C did not have a negative effect on mechanical properties nor biocompatibility in a direct contact *in vitro* test[4]. However, the dissolution behaviour of the coatings remains to be investigated. Furthermore, due to the close proximity to nerve tissues in spinal implants, the effect of the ions released on the neural tissue is a concern. The present study aimed to study the dissolution behaviour and *in vitro* neural cell response of SiFeCN coatings. A combinatorial approach was used for efficient screening of different compositions.

EXPERIMENTAL METHODS

SiFeCN coatings were deposited on CoCr disc substrates by reactive sputtering in an in-house built equipment, allowing for combinatorial processes, using Si, Fe and C solid targets. Nitrogen was supplied as a reactive gas. The coatings were characterized in 9 points using x-ray photoelectron spectroscopy (XPS), vertical scanning interferometry (VSI) and scanning electron microscopy (SEM). The points were placed in a 3x3 grid with 22.5 mm between each point.

The dissolution behaviour was evaluated by exposing the coated samples to cell media for 14 days. The obtained extracts were diluted (1:32, 1:48, 1:64 and 1:80 dilution) and used to measure ion levels with inductively coupled plasma (ICP-OES) and to assess indirect biocompatibility *in vitro* using the MTT assay and glial cells.

RESULTS AND DISCUSSION

The XPS results showed compositional gradients of Si ranging between 36.4-47.3 at.%, Fe 1.4-9.3 at.% and C 4.5-13.9 at.% with average surface roughness, S_a, of 7.4 to 11.1 nm, similar to SiN and CoCr reference materials. SEM after exposure displayed signs of dissolution with visibly increased porosity for the coated samples. The SiN reference also showed substantial changes to the surface. The ICP results (Figure 1) showed a reduction in Co ions from the substrate in the coated samples compared to uncoated. Moreover, the addition of Fe and C decreased the ion release from the coating compared to the SiN reference coating. Extract biocompatibility tests suggested that glial cells tolerated the extracts and their dilutions obtained from the coated samples in a dose-dependent manner and the cell viability was comparable to that of the uncoated CoCr and SiN coating.

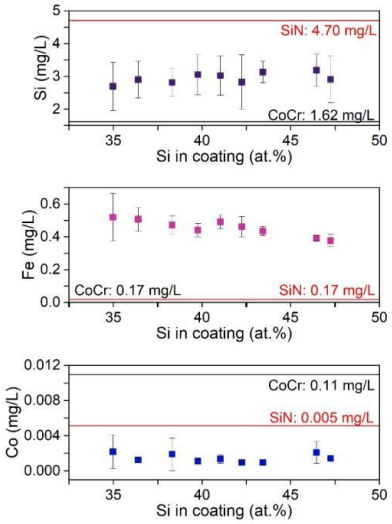

Figure 1. Si, Fe and Co ion release from the coatings. The ion release from the reference materials, CoCr and SiN coating, are marked as lines.

CONCLUSIONS

The findings from this study suggest that using iron and carbon as alloying elements in silicon nitride coatings has the potential to reduce ion release from a metallic substrate and lower the dissolution rate of the coating, while having a comparable cell response to that of the CoCr and SiN control materials. Therefore, SiFeCN coatings merit further investigation as a future option for spinal implants.

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ACKNOWLEDGMENTS

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COLLAGEN/PRISTINE GRAPHENE AS AN ELECTROCONDUCTIVE INTERFACE MATERIAL FOR NEURONAL MEDICAL DEVICE APPLICATIONS

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INTRODUCTION

The growing clinical demand for electrical stimulation-based therapies requires the development of conductive biomaterials that balance conductivity, biocompatibility, and mechanical performance. Traditional conductive materials often induce scarring, due to their stiffness and poor biocompatibility, presenting challenges to their clinical translation. To address these issues, we report the development of a pristine graphene-based (pG) composite material for central nervous system applications, consisting of type I collagen loaded with 60 wt% pG, yielding conductivities (~1 S/m) necessary for efficient electrical stimulation, and with versatile processability.

EXPERIMENTAL METHODS

Pristine graphene (60 wt%) and collagen films (CpG) were synthesised and characterised. Neural cell lines and iPSC-derived neurons were seeded on the surface of the composites, and the metabolic activity, DNA content, cell morphology and release of inflammatory cytokines were assessed. Electrical stimulation was applied to mouse primary cortical neuron isolates to enhance neurite outgrowth and viability. Finally, the CpG composites were fabricated into porous 3D scaffolds, microneedle arrays, and bioelectronics circuits, using freeze drying, dry casting, and 3D printing approaches respectively.

RESULTS

Of all composites tested, CpG 60% exhibited physiologically relevant conductivities (~1 S/m), and robust mechanical properties (~17.8 MPa). To test biocompatibility, four neuronal and glial cell types were grown on composite films, and exhibited robust growth, and glial cells exhibited no change in inflammatory markers IL-6, IL-10, or IL-1β, indicating no significant neuro-inflammatory response. iPSC-derived neurons exhibited typical cellular morphology after 15 days growth on the films, indicating the potential of the material for supporting long-term growth. The achieved conductivity enabled the efficient delivery of electrical stimulation, which was delivered to mouse primary cortical neurons on the composite (200mV/mm, 12Hz, 4h/day, 5 days), enhancing neurite outgrowth, cellular viability and morphology compared to collagen controls. Finally, we demonstrate the versatility and potential applications of the composite using a range of conductive, neural-interfacing structures, including porous scaffolds with aligned internal pores visible under SEM, microneedle arrays (5×5 conical needles, height - 2.5 mm, 625 µm base diameter, tip diameter - 40-80 µm), and 3D-printed working LED circuits for bioelectronics.

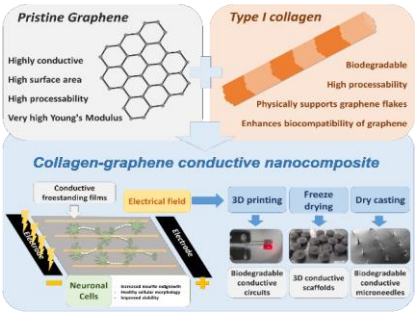

Figure 1 Schematic demonstrating the various properties and applications of the produced CpG 60% composites.

DISCUSSION

These results demonstrate that CpG 60% composites form a versatile neurotrophic platform that balances the need for physiologically relevant conductivity, robust mechanical properties, and excellent biocompatibility. The mechanical properties of the composite give it an advantage over stiffer traditional electrode materials, which can cause scarring due to extreme mechanical mismatch. The composite supported robust neuronal and glial cell growth, with an absence of neuro-inflammatory responses in microglia and astrocytes. In addition, it efficiently delivered electrical stimulation to primary neurons without loss of viability, enhancing neurite outgrowth. Finally, the versatile processing capabilities of CpG demonstrate its potential as a platform for the fabrication of several different neural device types.

CONCLUSION

These results show that CpG composites are versatile, neurotrophic materials, which balance the requirement for physiologically relevant conductivity, robust mechanical properties, and strong biocompatibility, demonstrating the potential for these collagen/pristine graphene composites to be fabricated into next-generation electroconductive medical devices.

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ACKNOWLEDGMENTS

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Bioelectronics to study and regenerate bone

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INTRODUCTION

Due to demographic and lifestyle changes, traumatic injuries have grown to become paramount medical and socio-economic challenges in affluent nations. Despite numerous advances in implant technology, grafts prepared using bone extracted from the patient are still the gold standard. However, the increasing life expectancy calls for innovative and effective approaches to compensate for bone loss.

The knowledge of bone piezoelectricity has inspired the use of physical stimulation together with electroactive materials as smart alternatives for bone tissue engineering. The combination of smart substrates, stem cells and physical stimuli to induce stem cell differentiation is therefore a new avenue in the field.

Herein, I will report on some of the work done on the development of electroactive materials to be used as scaffolds for stem cell culture and differentiation and for the development of biosensors. Electrical stimulation experiments were run to modulate stem cell osteogenic differentiation using a previously developed bioreactor for capacitive coupling.

EXPERIMENTAL METHODS

PEDOT:PSS-based scaffolds were prepared following a method previously described[1]. Briefly, scaffolds with three different compositions were fabricated by the ice-templating technique with increasing collagen type I content. P:P, the sample with no collagen, COLL1 sample with 0.3% (w/w) collagen type I and COLL3, sample with 1.1% (w/w) collagen type I. 400 μm thick slices were obtained using a vibrotome from each scaffold and used for subsequent experiments. Human adipose derived stem cells (hADSCs) were used for all experiments and seeded on previously sterilized scaffolds. Cells seeded on round glass coverslips were used as 2D controls. Electrical stimulation was performed using a set-up previously developed by the group led by Prof. Cartmell[2]. A constant potential of 30V was applied externally for different time periods. Alizarin red S staining and its quantitative evaluation were performed according to a procedure previously described.[3,4]

RESULTS AND DISCUSSION

The developed electroactive scaffolds proved to allow cell penetration and proliferation, as previously demonstrated. Electroactive scaffolds with no collagen led to an increase in mineralized bone matrix under non-osteogenic conditions as revealed by alizarin red staining (fig 1).

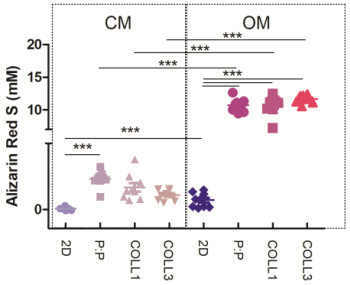

Figure 1. *Alizarin Red staining of samples after 21 days of hADSC culture under normal (CM) and osteogenic (OM) culture conditions.*

The investigated electrical stimulation protocol seems to not affect stem cell osteogenic differentiation as only a slight increase in mineralized matrix was measured after 4 days of continuous ES.

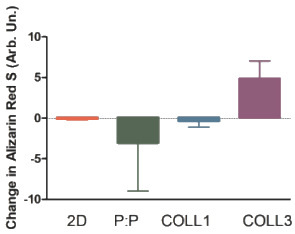

Figure 2. Change in Alizarin red staining after 4 days of continuous capacitive coupling.

CONCLUSION

The electroactive scaffolds confirm to be promising support materials for stem cell culture and differentiation. They do not hamper cell proliferation nor their differentiation. In silico studies will clarify the interactions of the applied electrical stimulation with the electroactive scaffolds,

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Nutlin-loaded ultrasound-activated piezoelectric nanovectors: Modulation of anti-angiogenic activity

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INTRODUCTION

Angiogenesis refers to vessel growth, and plays an essential role in embryonic development, wound healing, and many diseases including cancer metastasis[1]. In conventional therapies, the aim is targeting and blocking the activity of pro-angiogenic factors. Recently, a new strategy has been proposed in cancer nanomedicine, based on nanomaterials that can remotely respond to external stimulation (*e.g.*, ultrasound -US-)[2].

In this work, we show how the angiogenic activity of hCMEC/D3 (human cerebral microvascular endothelial) cells is inhibited by exploiting apolipoprotein E-functionalized nutlin-3a-loaded piezoelectric polymeric nanoparticles (ApoE-Nut-PNPs). that can respond to US remote stimulation (Figure 1)[3].

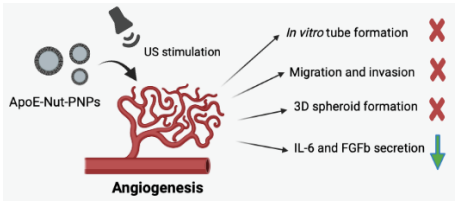

Fig 1. Schematical representation of the study.

EXPERIMENTAL METHODS

Nutlin-3a loaded poly(vinylidene fluoride-co-trifluoroethylene) (P(VDF-TrFE)) nanoparticles (Nut-PNPs) were prepared and functionalized with ApoE peptide, as previously described[4]. After morphological characterization of the nanoparticles, the biocompatibility was tested *via* WST-1 and hemolysis assays. Cellular internalization of the nanovectors was tested by using confocal microscopy. *In vitro* endothelial tube formation assay was performed by considering 8 experimental groups with or without US stimulation. Migration and invasion assays were tested using transwell inserts and a 3D spheroid invasion assay. Angiogenesis-related cytokines production has been assessed using an ELISA kit. Statistical analysis was evaluated by using ANOVA (analysis of variance) and then followed by Fishers *post-hoc* test.

RESULTS AND DISCUSSION

The sizes of the nanoparticles were found to be 66 ± 22 nm, 76 ± 16 nm, 62 ± 20 nm, and 56 ± 12 nm for ApoE-PNPs, ApoE-Nut-PNPs, PNPs, and Nut-PNPs, respectively, while the ζ-potential values are -21.6 ± 0.7 mV, -18.3 ± 0.6 mV, -20.8 ± 0.9 mV, and -18.4 ± 0.8 mV. The release of Nut resulted to be 12.5 ± 0.3% at pH 4.5 upon US stimulation. The experiments were carried out by using a 500 µg/mL of nanoparticles that corresponds to Nut 21.5 µM. As depicted in Figure 2A, the total mesh area significantly decreased in ApoE-Nut-PNPs + US treatment (231463 ± 28310 µm^2, $p < 0.05$). 3D spheroid invasion assay was performed in the presence of glioblastoma cancer cell migration cues on Matrigel matrix. Figure 2B shows fewer cells invaded out of 3D spheroids in ApoE-Nut-PNPs + US stimulated cultures (area = 130067 ± 31247 µm^2, $p < 0.05$), which is in agreement with the transwell invasion assay. We have also profiled 8 angiogenesis-related cytokines, and IL-6 and FGFb resulted significantly down-regulated after ApoE-Nut-PNPs + US stimulation. The decrease of FGFs and IL-6 levels is considered promising to inhibit tumor progression, being a strong connection between angiogenesis and inflammatory processes[5].

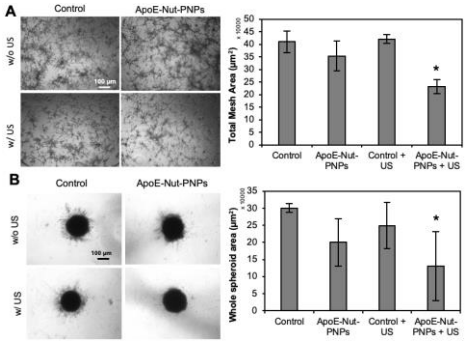

Fig 2. Results of **A)** *in vitro* tube formation and **B)** 3D spheroid invasion assays on Matrigel-coated plate.

CONCLUSION

In this study, ApoE-functionalized nutlin-3a-loaded piezoelectric nanoparticles were synthesized and remotely controlled to inhibit angiogenesis. The obtained findings confirm that ApoE-Nut-PNPs have an *in vitro* anti-angiogenic behavior by inhibiting endothelial cells migration and invasion, vessel formation, and secretion of cytokines related to angiogenesis. In addition, the therapeutic efficacy of the free drug was enhanced by exploiting the prepared piezoelectric nanovectors after US stimulation. Altogether, this study shows that piezoelectric nanoparticles can be exploited as feasible therapeutics to inhibit tumor-induced angiogenesis.

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ACKNOWLEDGMENTS

We would like to acknowledge the financial support of the ERC (European Research Council, grant agreement 70961).

Design of a fully-resorbable electronic device for neural stimulation and monitoring

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INTRODUCTION

At the interface between living tissues and inert electronics, bioelectronic devices require flexible and biocompatible materials. Additionally, the advent of fully-resorbable transient devices would open the way to new medical possibilities for mid-term post-surgical wound monitoring, on-demand electrically controlled drug delivery, or electro-sensitive tissue engineering[1]. With their mixed ionic/electronic conductivity and tunable mechanical, electrical, electrochemical, and biological properties, conductive polymers are materials of choice for the design of the conductive electrodes of such transient devices. Herein, conductive resorbable materials based on sulfated hyaluronan modified by phenyl-boronic moieties (HAS-PBA) as dopants of poly(3,4-ethylenedioxy)thiophene (PEDOT) were designed and characterized. They were further integrated in a fully-resorbable device dedicated to the implantation in the cortex of rats for stimulation and recording.

EXPERIMENTAL METHODS

EDOT was chemically polymerized in the presence of HAS-PBA, obtained in two steps with quantitative yield and minimal polymer chain degradation, to yield the conductive PEDOT:HAS-PBA ink[2]. The bioelectronic device consisting of poly (lactic-co-glycolic acid) (PLGA) films, molybdenum (Mo) conductive tracks and PEDOT:HAS-PBA electrodes for tissue contact was fabricated using usual microfabrication techniques. PLGA films (50% glycolic acid, 20 µm thick) were prepared by drop casting on plasma-treated glass surfaces. The conductive tracks were either laser-cut in a 10 µm-thick molybdenum sheet or obtained by sputtering of a 500 nm thick layer through a negative mask. Therefore, 200 µm width Mo tracks were either pick-and-placed or sputtered on a first PLGA film, then encapsulated within a second film comprising laser-made electrode openings. Finally HAS-PBA:PEDOT material was deposited in the openings to constitute the electrodes (1.2 mm Ø) in contact with biological tissues (Fig. 1).

RESULTS AND DISCUSSION

To achieve suitable PEDOT dopant properties, HA was sulfated (HAS) to increase its acidity and global negative charge, and phenylboronic acid (PBA) moieties were introduced to enhance hydrophobic interactions with PEDOT domains. A synergy was observed between the sulfate and the PBA aromatic groups to enhance the material conductivity that reached 1.57 ± 0.2 S/cm in physiological conditions[2].

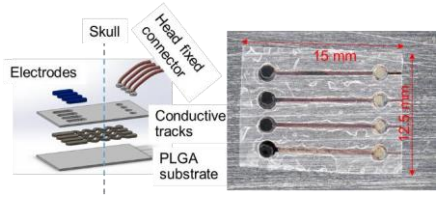

Fig. 1: Left: exploded view of an illustrative device. Right: example of device (15x12.5 mm) with laser-cut Mo tracks.

Mo and PLGA were selected as respectively electrical track and support/encapsulating materials due to their biocompatible and resorbable properties. Electrical impedance spectroscopy (EIS) measurements showed that the PEDOT:HAS-PBA layer reduced the electrode impedance (4 kΩ at 1 Hz), providing a better interface than Mo metal (15 kΩ at 1 Hz).

CONCLUSION

The new conductive PEDOT:HAS-PBA ink appeared as perfectly biocompatible, disintegrable, and exhibited excellent conductive and electrochemical properties. It therefore constitutes a material of choice for contact electrodes in the design of a fully resorbable bioelectronic device. The developed prototype will be implanted in rat cortex for electrical stimulation and recording, and to further explore its resorbability properties.

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ACKNOWLEDGMENTS

This work is part of the STRETCH project founded by the French National Research Agency (ANR) (ANR-18-CE19-0018). LETI-DTBS and CNRS-CERMAV are supported by the ANR in the framework of Arcane Labex (CBH-EUR-GS, ANR-17-EURE-0003) and Glyco@Alps (ANR-15-IDEX-02) programs.

A Closed-Loop Soft Robotic Drug Delivery System to Overcome the Foreign Body Response

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INTRODUCTION

The Foreign Body Response (FBR) is a major obstacle to implantable drug delivery devices[1]. The growth of a fibrous capsule around drug delivery devices can impair function and require the need for early replacement. If the device is occluded, the expected drug regimen can no longer be maintained and causes failure. We developed a soft robotic drug delivery device capable of probing and modulating the FBR. The device is composed of a soft therapeutic reservoir and a fibrosensing porous membrane. The latter makes use of electrochemical impedance spectroscopy to quantify occlusion caused by the FBR. Soft robotic actuations, tuneable by magnitude and frequency, are applied to deliver therapies by overcoming the occluded membrane. The actuation regime is determined by case-based reasoning. This technology has great potential in improving the longevity and function of drug delivery devices.

EXPERIMENTAL METHODS

Fibrosensing devices (Fig. 1A) were manufactured as described in [1], in this case the porous membranes were fabricated of a conductive material. Devices (n=22) were implanted subcutaneously in rodent models for up to 7 days and impedance was measured each day by an external potentiostat. Fibrotic capsule dimensions were measured via microCT and correlated to impedance markers via Pearson. Impedance of 0.15 and 0.3% w/v agarose gels was measured and a k-nearest neighbour classifier was tested to infer gel concentration. The hydraulic conductance of the same gels was calculated using Darcy's law by forcing water through the porous membrane and gel, at 2 psi, and measuring the resulting flow rate. A case-based system was developed by releasing methylene blue dye into agarose gels at different pressures and over multiple actuations, and measuring the area occupied by the dye.

RESULTS AND DISCUSSION

Impedance increased over time in vivo (Fig. 1B) and correlated to the increase in thickness (Fig. 1C) and volume of the fibrotic capsule (Pearson's coefficient 0.83 and 0.81, respectively). Agarose proved to be suitable phantom for drug release studies as it mimicked two consequences of the FBR: (i) significant changes in impedance (p=0.040) and (ii) significant changes in hydraulic conductance of the porous membrane (p=0.027). Gel concentration was inferred by machine learning with 83.3% accuracy. Soft robotic actuations (Fig. 1D) allowed to control the release of methylene blue (Fig. 1E). When given a release to achieve, a case-based reasoning system was capable of finding the most appropriate actuation regime to employ, depending on the gel concentration.

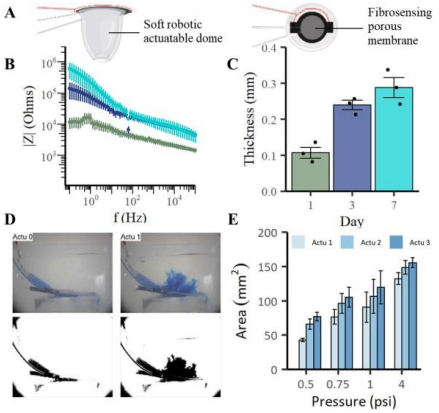

Figure 1 **A.** Schematic of soft robotic reservoir with biosensing membrane. **B.** Impedance and **C.** fibrotic capsule thickness during *in vivo* study. **D.** Release of methylene blue into agarose gels. **E.** Pressure and number of actuation allow to control drug release.

CONCLUSION

Incorporating an impedance sensor into a drug delivery device allows to monitor the FBR and quantify fibrotic capsule formation. Soft robotic actuations can overcome reductions in hydraulic conductance and maintain constant drug release overtime. This self-adapting technology is poised to improve the function and longevity of implantable drug delivery devices.

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ACKNOWLEDGMENTS

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>> 10:30 - 12:30 | SYMP-07 - Biomaterials for organoids and Organ-on-Chips

Chairpersons: Vincent Flacher & Stéphanie Descroix
Location: Room A

10:30 | KL OoO - Biomaterials, Organoids And Microfluidics: A Winning Trio For Organ-On-Chips
Audrey FERRAND, Team 'Environment and Intestinal Epithelium', Institut de Recherche en Santé Digestive (IRSD), Toulouse, France

11:00 | O1 - Using Collagen Microfibers to Unlock in vitro Vascularized Mature Adipose Tissue Regeneration: Applications to Patients-derived Drug-screening Models and Breast Reconstruction
Fiona LOUIS, Joint Research Laboratory (TOPPAN) for Advanced Cell Regulatory Chemistry, Graduate School of Engineering, Osaka University, Japan

11:15 | O2 - 3D Bioprinted Glioblastoma Microenvironment
Jessica SENIOR, University of Huddersfield, UK

11:30 | O3 - Stratified Living Units for Modeling Pancreatic Tumor-Stroma Microenvironment
Maria MONTEIRO, CICECO-Aveiro Institute of Materials, Aveiro University, Aveiro, Portugal

11:45 | O4 - In vitro alveolar-capillary barrier model for the study of particulate matter toxicity
Chiara Emma CAMPIGLIO, Department of Management, Information and Production Engineering, University of Bergamo, Dalmine (BG), Italy

12:00 | FP01 OoO - Cabbage leaves as 3D platform for in vitro adipose tissue model
Lina ALTOMARE, Dipartimento di Chimica, Materiali, Ing. Chimica 'G Natta' Politecnico di Milano, Milan, Italy

12:05 | FP02 OoO - Organ-on-a-chip to evaluate biomaterials
Oscar CASTANO LINARES, University of Barcelona (UB), Barcelona, Spain; Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain; CIBER en Bioingeniería, Biomateriales y Nanomedicina, CIBER-BBN, Madrid, Spain

12:10 | FP03 OoO - Development of optically-tuned bioresins for the rapid volumetric bioprinting of liver organoid-laden metabolic biofactories
Marc FALANDT, Department of Orthopedics, UMC Utrecht, Utrecht, the Netherlands

12:15 | FP04 OoO - sciPULSE ULTRA LOW VOLUME - Dispensing of small droplets in the range of 18 -180 pL
Dietrich KOESTER, SCIENION GmbH, Berlin, Germany

Biomaterials, Organoids And Microfluidics: A Winning Trio For Organ-On-Chips

Audrey Ferrand[1]

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INTRODUCTION

It is now admitted that most animal models fail to predict therapeutic responses in humans, highlighting the need of the development of new models for basic research. Organ-on-chip microfluidic devices plated with patient's cells primocultures in biomaterial scaffold under microfluid flow can recapitulate organ-level physiology and pathophysiology with high fidelity[1]. After a general introduction on organ-on-chips, presenting their three main components (biomaterials, cells/organoids and microfluidic), I will exemplify the development of such a tool with the development of a human colon-mimicking microdevice and its possible applications.

EXPERIMENTAL METHODS

We developed a human colon-like MPS composed of 3D printed U-shaped frame design to accommodate glass coverslips on each side to be compatible with microscopic approach. We filled this chamber with a polyacrylamide (PA) solution to easily tune the scaffold stiffness, here we used a soft (3kPa) or stiff (16kPa) to reproduce either physiological or pathological colon tissue stiffness range. Then, to mimic human colonic crypt topology, we produce and insert into the chamber a 3D printed mold comprising an array of 400µm height and 100µm diameter pillars in order to replicate crypt-like structures at the top surface of the scaffold. To perform epithelial cell culture into the device, the 3D printed-mold is coated with collagen type I to functionalized PA and form a hybrid scaffold. In addition, this U-shaped frame of our device contains fluidic inputs and output localized at the top and inside the hydrogel scaffold allowing dynamic flow control of both luminal and stromal-like compartment.

RESULTS AND DISCUSSION

We demonstrate a simple and efficient method for the fabrication of a MPS combining the topology, the creation of a lumen and basal compartments as well as a microfluidic circuit to control the mass transport into the model. First, we validated that we are able to reproduce crypt-like structure at the top surface of hydrogels either with soft or stiff PA formulation or collagen-coated one. Then, we successfully cultivate epithelial cells on the scaffold during 18 days in order to favor the establishment of an epithelium lining, while preserving the resolution and accuracy in the creation of the human colon-like tissue architecture. After culture, immunostaining show that epithelial cells form a continuous polarized epithelial monolayer on the scaffold based on ZO-1 expression and formation of an actin ring at the apical side of epithelial cells. Finally, we performed diffusion assay to validate that chemical entities injected into the stromal compartment diffuse into the hydrogel. This modality could be useful to generate chemical gradient along the crypt axis.

CONCLUSION

This novel device combining 3D scaffold and microfluidic addressing will allow a better understanding of the tissue (mechanobiology, architecture, functions...) and will be a useful tool to study microbiota, pathogens, and nutrients impacts on the colon epithelium as well as drug screening.

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ACKNOWLEDGMENTS

The human colon-like MPS presented here has been developed with D. Hamel (IRSD/LASS-CNRS, france) and L. Malaquin and J. Foncy (LAAS-CNRS, France). We thank Cancéropole GSO, Plan Cancer, Région Occitanie, Université de Toulouse III, HoliFAB project (European Union's Horizon 2020), MultiFAB (FEDER European Regional Funds) and French RENATECH network.

Using Collagen Microfibers to Unlock *in vitro* Vascularized Mature Adipose Tissue Regeneration: Applications to Patients-derived Drug-screening Models and Breast Reconstruction

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INTRODUCTION

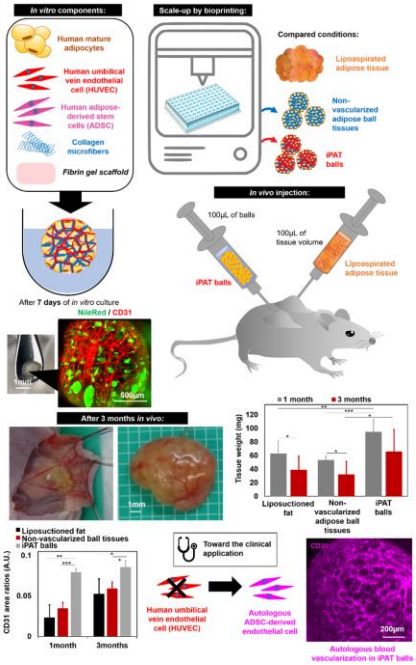
Adipose tissue reconstruction is essential for diverse important applications from breast regeneration, to obesity drug-screening models. However, *in vitro* adipose tissue reconstruction is challenging when it comes to maintain the fragile mature adipocytes state, full of lipids and thus easily breakable, in parallel to the induction of the adipose tissue dense vascular network formation, required for a long-term stable tissue integration after implantation. Also, the current adipose models generally use adipose-derived stem cells (ADSC) differentiated in adipocytes for drug screening assessment. These ADSC-derived adipocytes are still in an immature differentiation state compared to native adipose tissue, differing from obese or diabetic patients specific adipocytes phenotype. To overcome these challenges, collagen microfibers were the key component. Dispersed in fibrin gel, it acts as a protective scaffold for the mature adipocytes[1-2], while guiding the endothelial cells for the blood vasculature formation[3-4].

EXPERIMENTAL METHODS

Porcine type I collagen homogenized in microfibers was mixed with the human cells embedded in a fibrin gel. Mature adipocytes and ADSC were isolated from human adipose tissues and *in vivo* mouse implantation experiments were performed on humanized immune system mice (Kyoto Prefectural University Hospital facilities, n>3 independent experiments). The Bio-X bioprinter (Cellink) allowed the seeding scale-up. For getting ADSC-derived endothelial cells, 7 days in EGM-2 medium (LONZA) incubation was used at passage 0.

RESULTS AND DISCUSSION

Collagen microfiber tissues ensured the maintenance of adipocyte viability and functionality (glucose and fatty acids uptake or release control), also depending on patients' phenotype, with for instance insulin resistance confirmed when using severely obese adipocytes. Non-invasive injectable prevascularized adipose ball tissues were then subcutaneous implanted in mice and the grafts were associated with a higher cell survival and volume maintenance after 3 months (up to x2 heavier than the general lipoaspirate and the non-prevascularized grafts controls), thanks to their greater amount of blood vessels (up to x1.6 times), allowing a balanced host anastomosis (51±1% human/mouse lumens). Finally, the obtention of ADSC-derived endothelial cells allowed to avoid the use of HUVEC for the prevascularization and provided a further step toward future clinical application, enabling



the formation of fully autologous iPAT. The possibility to cryopreserve the iPAT balls was also confirmed with a good long-term viability after 30 days of freezing storage and maintained mature adipocytes functions.

CONCLUSION

The use of CMF in these models was found to maintain the mature adipocytes viability and functionality, while allowing the generation of a high blood vessels vasculature in the drop tissues. Furthermore, with the cryopreservation possibility enabling their later reinjection and thus customized final graft volume, the iPAT technology have the merit to allow non-invasive soft tissue regeneration for long-term outcomes.

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ACKNOWLEDGMENTS

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3D Bioprinted Glioblastoma Microenvironment

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INTRODUCTION

Glioblastomas (GBMs) are highly aggressive tumors that can rapidly migrate to other regions of the brain, accounting for poor prognosis and dismal survival rates of patients. In vivo animal models used to investigate experimental treatments for GBM's usually involve surgical intracranial implantation of tumour cells into mice or rats, with some displaying severe side effects such as stroke post-surgery. Furthermore, there are also doubts regarding the usefulness of such in vivo models. This has been questioned over time where many anti-cancer drugs are shown to be effective in mice but fail to produce the same response in humans[1]. Here we describe the development of an 3D bioprinted model of the GBM physical and chemical environment to produce a more physiologically representative system in which candidate drugs are tested.

EXPERIMENTAL METHODS

Cell lines - Glioma cell lines U87 and U251 derived from human GBM were used in this study. Knockdowns of anti-migratory and pro-migratory genes (ARHGAP12 and ARHGAP29 respectively) were used to examine their roles in the actin polymerization pathway in cancer cell migration. All cells were intrinsically tagged with green fluorescent protein (GFP) for ease or tracking. Cells were grown in DMEM supplemented with 10% foetal calf serum and penicillin/streptomycin in a CO_2 incubator. U87 and U251 wild type and knockdown spheroids were generated in 96-well ultra-low adherence plates (Nunclon®).

3D Migration assay - A non-cell-adhesive agarose hydrogel was fashioned in the shape of a cube containing an empty channel at its core. Single spheroids were then suspended in type I collagen to form a bioink which was subsequently printed within the agarose channel void to form migratory tracts. The distribution of collagen was organised to have low, intermediate, and high-density regions within the construct, replicating the complexity of native GBM. 3D models were then cultured under control conditions (media only) or treated with anti-migratory drugs (CCG-1423, rhosin or combination) **(Figure 1).**

Light-sheet and confocal microscopy - Using light-sheet microscopy, migration was imaged live every hour between time points 0hrs and 24hrs and data was collected in the form of z-stacks and time-lapse videography. For confocal microscopy, constructs were cultured over 48 hours, fixed with 4% PFA overnight and stained for F-actin (phalloidin-TRITC, ECM Biosciences) with a DAPI counterstain.

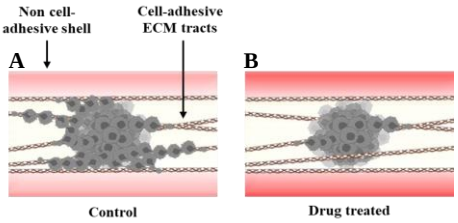

Figure 1 | 3D GBM model consisting of outer non-cell-adhesive shell and inner collagen migratory tracts under **A)** control conditions and **B)** anti-migratory drug treated conditions.

RESULTS AND DISCUSSION

In models where migration is promoted, actin is vastly upregulated and cells assume a migratory mesenchymal phenotype, whereas under anti-migratory drug treatment, cells are amoeboid in shape with a dramatic reduction in actin expression and consequently limited migration velocity **(Figure 2).**

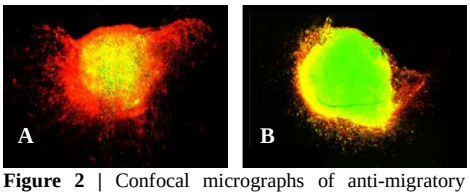

Figure 2 | Confocal micrographs of anti-migratory knockdowns exhibiting **A)** enhanced migration and mesenchymal phenotype under control conditions and **B)** anti-migratory effects under combination drug culture (actin - red, intrinsic cell stain - green).

CONCLUSION

Here, we have demonstrated that it is possible to develop biologically-relevant GBM models that capture the anisotropic nature of the tumour microenvironment using multi-layer biopolymer engineering. The ultimate goal of this research is to develop technology that can help provide personalised treatments for glioblastoma and subsequently improve patient outcomes.

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ACKNOWLEDGMENTS

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Stratified Living Units for Modeling Pancreatic Tumor-Stroma Microenvironment

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a highly deadly and complex neoplasia owing to its unique bioarchitecture in which an abundant juxta-tumoral fibrotic stroma shields cancer cells mass hampering anti-cancer therapeutics delivery.[1,2] A growing evidence has suggested that cancer associated fibroblasts (CAFs), generally localized in periductal/periacinar regions close to the tumor mass, are master players in orchestrating such active desmoplastic tumor microenvironment.[3] Aiming to recapitulate PDAC key hallmarks, herein, stratified pancreatic cancer living units – so termed cancer-on-a-bead models - were generated by using superhydrophobic surfaces. The designed cancer units aim to recapitulate the native stratified and compartmentalized organization of both cancer and stroma compartments where cancer cells are supported by a collagen rich matrix and surrounded by a fibrotic stroma rich in collagen and glycosaminoglycans.

EXPERIMENTAL METHODS

Core-shell tumor-stroma cancer-on-a-bead models were produced via a two-step procedure by using a mechanical electronic repeater and superhydrophobic surfaces.[4] Briefly, for assembling the tumor core template, PANC-1 human pancreatic ductal adenocarcinoma cell line was mixed with gelatin methacrylate (GelMA) and tumor droplets were dispensed onto the superhydrophobic surface, and then photocrosslinked for 40 secs. To generate the tunable fibrotic stroma compartment, human pancreatic CAF-stellate cells were mixed with a GelMA-hyaluronic acid methacrylate (HAMA) solution and then digitally dispensed over the tumor core bead enabling the production of a stratified system. As control, scaffold-free monotypic and heterotypic 3D spheroids were established by the force-floating technique in ultra-low adhesion 96-well plates for 14 days, as described before.[5] The cell viability, F-actin filaments and the secretion of soluble biomolecular markers (human TGF-β1, PDGF-BB, FGF-2 and IL-1β) were analyzed. After 14 days in culture the established 3D models were treated with Gemcitabine at different concentrations (0-100 μM), over a period of 48 h.

RESULTS AND DISCUSSION

Cancer-on-a-bead *in vitro* platforms exhibited biomimetic multi-compartmentalization and tunable integration of cancer associated stromal elements. The assembled living units can be rapidly assembled in-air, exhibited reproducible morphological features, tunable size, and recapitulated spatially stratified tumor-stroma ECM niches. Adding to this, cancer-on-a-bead platforms also demonstrated their versatility owing the ability to manipulate both CAFs cells density and stroma volume in the shell compartment enabling a straightforward fabrication of different fibrotic environments *in vitro* (Fig. 1). Such achievement opens the unique opportunity to rapidly model PDAC tumors with tunable fibrotic components, an important hallmark recognized to play a major role in disease progression and drug resistance. Cancer-on-a-bead models also displayed increased drug resistance to chemotherapeutics when compared to the scaffold-free counterparts, reinforcing the importance to differentially model ECM components inclusion and their spatial stratification as observed *in vivo*.

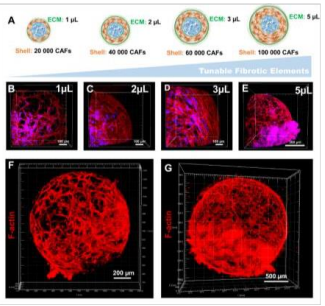

Fig. 1. Cancer-on-a-bead models with differential stromal elements modulation. (A-G) Representative 3D sections of cancer-on-a-bead living units comprising cancer cells surrounded by the stromal CAFs compartment, at day 7 of culture. Red channel: F-actin. Blue channel: DAPI.

CONCLUSION

The established cancer-on-a-bead living units exhibited increased biomolecular markers secretion and drug resistance when compared to their monotypic and heterotypic scaffold-free counterparts, emphasizing the importance of including ECM components and pancreatic cancer-stroma cellular elements similarly to the native environment. Importantly, the ease of assembly, the low-cost, the reproducible features, as well as the possibility to evaluate cellular response by standard imaging methodologies, renders stratified cancer-on-a-bead living units highly valuable platforms for inclusion in static and dynamic high-throughput screening systems while benefiting from an increased biomimicry level.

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ACKNOWLEDGMENTS

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In vitro alveolar-capillary barrier model for the study of particulate matter toxicity

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INTRODUCTION

In vitro air–liquid interface (ALI) cell culture models can potentially be used to assess inhalation toxicology endpoints and are usually considered, in terms of relevancy, between classic (i.e., submerged) *in vitro* models and animal-based models[1].
Although submerged condition is experimentally more simple and widely used, it does not appropriately reflect the *in vivo* process of particulate aerosol depositing onto the lung epithelium where, physiologically, only a thin layer of liquid surfactant should be present. To overcome this limitation and replicate the *in vivo* condition of lung epithelium, we moved to culture lung epithelial adenocarcinoma cells (A549) under ALI condition and develop an aerosol exposure system for the deposition of particulate matter directly onto epithelial cells. This is a more physiological setting than the conventional submerged culture condition, allowing direct exposure of the cells to aerosolized particles (e.g., drugs, pollutants, airborne particulate) and realistically mimicking the *in vivo* exposure conditions during inhalation.
The aim of this study is to validate an *in vitro* cell culture model where the alveolar-capillary barrier can be easily exposed to aerosolized particles to investigate possible toxic effects of airborne pollutants (PM 2.5).

EXPERIMENTAL METHODS

A549 and HMEC (human microvascular endothelial cells) were co-cultured on the two side of a Transwell insert membrane (PET, pore size 0.4 µm) under submerged and ALI conditions. After 14 days, cells morphology was investigated by electron microscopy and the presence of surfactant was analysed by immunofluorescence staining (prosurfactant protein C). In order to verify the formation of alveolar-capillary barrier, a permeability assay (FITC-dextran) was performed at day 3, 7 and 14.
An aerosol exposure system (Figure 1) made of aerosol generator, particle deposition chamber and real-time PM monitoring system, was projected and validated for the test of PM2.5 toxicity. A qualitative and quantitative optimization of the aerosol system was performed in order to provide accuracy, reproducibility and effectiveness in particle deposition.

RESULTS AND DISCUSSION

As previously demonstrated[2], we observed that A549 maintained a round-shape morphology and a multi-layer disposition when they were cultured under ALI conditions. Investigations performed by scanning electron microscopy and immunofluorescence staining confirmed that this type of culture condition support A549 in the maintenance of epithelial Type II phenotype.
The morphology of the two different cells (i.e., A549 and HMEC) seeded on Transwell membrane appeared consistent with the function that each type of cells has to exert (TEM analysis). On the top side of the membrane, epithelial cells showed a multi-layer disposition due to the ALI culture condition. On the bottom of the PET membrane, endothelial cells arranged as single layer forming a tight endothelium.
The complete formation of alveolar-capillary barrier was obtained between 4 and 7 days after seeding. The permeability test performed on the membrane showed how the presence of epithelial and endothelial cells evidently decreases the paracellular transport of FITC-dextran molecules (70 kDa) through the formed barrier.
The aerosol exposure system was validated by the optimization of components geometry, particle solution concentration and aerosol flow rate. An homogeneous deposition of particles was obtained on 3 insert at the same time. By varying the particle solution concentration it is possible to increase/decrease the quantity of particle that were nebulized onto the epithelial cells.

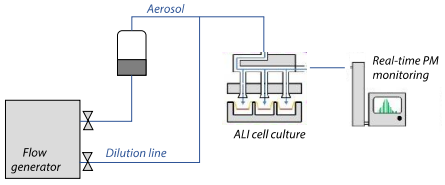

Figure 1 – Schematic representation of the aerosol exposure system.

CONCLUSION

The proposed *in vitro* model composed by an alveolar-capillary barrier and an aerosol particle exposure system allowed to test different airborne pollutants and evaluated possible toxic effects mimicking the in vivo exposure during inhalation. Further investigations are under studying to evaluate the effects of particulate matter on microvascular endothelium.

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The authors would like to thank TRC Tecora Company for providing aerosol system components.

Cabbage leaves as 3D platform for in vitro adipose tissue model

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INTRODUCTION

Adipose tissue is histologically characterized as a type of loose connective tissue that, normally, constitutes about 10% of the total body mass. It is highly vascularised and it is the only tissue with unlimited growth throughout adulthood[1]. Until now, different scaffold-based 3D *in vitro* models have been investigated for adipogenesis and adipocyte differentiation, studying both biopolymers, synthetic polymers and their combinations[2]. In recent years, plants have been identified as an alternative source of decellularized scaffolds for their ability to offer a broad range of potential architectures and surface topographies to support mammalian cell growth[3]. In the present study, savoy cabbage (S) and black cabbage (B) were selected and tested as potential scaffolds for scaffold-based 3D in vitro models to study adipose tissue. Their 3D structure, characterized by cavities and their intrinsic vascular structure, could promote cell adhesion, growth and adipocytes differentiation[4].

EXPERIMENTAL METHODS

Different decellularization protocols were performed on black and savoy cabbage leaves, combining mechanical agitation and ultra-sonication on square-based samples (10 x 10 mm^2). SDS solution was used as a decellularizing agent, followed by washing with aqueous $CaCl_2$ solution (100 mM, 4 x 20 mL) washes in $CaCl_2$ solution and water (4 x 20 mL) were performed to remove detergent residues.

Morphological characteristics of samples were investigated by means of stereomicroscope and scanning electron microscopy (SEM). Physical and mechanical characteristics, such as swelling ratio, contact angle and tensile properties, were also evaluated.

Indirect cytotoxicity test was performed according to the ISO 10992: 3T3-L1 murine preadipocytes were seeded on eluate at 1, 3 and 7 days. Cell viability was evaluated by means of Alamar Blue assay.

3T3-L1 preadipocytes were seeded on both savoy and black cabbage ($2x10^5$ cells per sample) and, after 7 days, adipogenic differentiation was induced through immersion in differentiation culture medium[5]. Adhesion and proliferation were evaluated by Alamar blue assay and SEM images, while differentiation was evaluated by Oil Red O test and Nile Red test combined with Hoechst 33258.

RESULTS AND DISCUSSION

Image at stereomicroscope showed that after optimization of decellularization protocol both S and B leaves were clean with no detergent residues, moreover the morphology of the plants is unmodified. SEM images (Figure 1) showed a rough regular geometrical grid structure with stomata without their cells, removed by decellularization process.

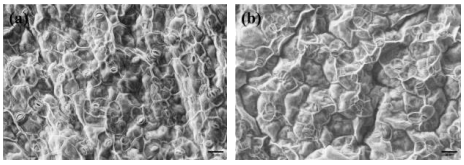

Figure 1 SEM images of S (a) and B (b) at 1000x magnification

Water uptake was measured up to 7 weeks, samples reached a plateau (1581% for S, 2388% for B) of water absorption after 4 and 3 weeks, respectively.

For all time point considered, S and B cabbage leaves showed no residual cytotoxicity, and cells showed more than the 85% of viability.

The SEM imaging, carried out 3 and 7 days after seeding, revealed good cell adhesion to the S and B scaffolds. Metabolic activity measured by Alamar blue assay, increased up to 14 days indicating that both leaves can support cell growth. Finally, Oil Red O staining (Figure 2), performed 14 days after seeding, showed a strong intracellular lipid accumulation typical of adipocytes.

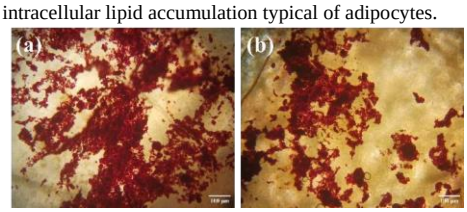

Figure 2 Oil Red O staining of lipid droplets on savoy cabbage (a) and black cabbage (b)

The presence of lipid droplets is confirmed by Nile Red staining, moreover Hoechst staining allowed to highlight numerous nuclei on S and B surfaces.

CONCLUSION

Decellularization protocols have been optimized on cabbage leaves, maintaining the original morphology and the obtained specimens showed no residual cytotoxicity. The obtained scaffold was successfully employed for promote preadipocytes adhesion and their differentiation. Further studies will allow to understand the possibility of exploiting leaves branches as vessels for nutrient diffusion paving the way to the development of a mature tissue.

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Organ-on-a-chip to evaluate biomaterials

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INTRODUCTION

The limited knowledge in the field of tissue engineering about biokinetic conditions and biochemical signaling in traditional in vitro protocols often leads to poor interpretation of the results due to a lack of quality data. This implies carrying out a large number of experiments in animals in order to obtain results that have to be directly extrapolated to the human case. In the last decade, Organ-on-a-chip (OoCs) technologies have become an essential research tool as it has boosted many aspects of biological research domains in broad fields such as medicine and Engineering. With these, it is possible to reproduce, in a controlled manner, the cellular microenvironments of organs or tissues and thus investigate the effect of different stimuli, signals, and drugs. In this work we show three different OoCs devoted to evaluating i) proangiogenic materials; ii) cardiac tissue scaffolds; iii) brain neuron tissue biomaterials.

EXPERIMENTAL METHODS

Platforms were designed CAD software and soft lithography in PDMS after *in silico* optimization by finite elements simulations of flows, species diffusion, a gradient of signaling, and electrical field generation. Computational models were then experimentally validated. Materials to be validated were produced by electrospinning using polylactic acid as the main component and adding calcium releasing nanoparticles for proangiogenic materials; and pure PLA with a high content of D-isomer for cardiac and neural platforms. *In vitro* tests were performed evaluating the migration of rat endothelial progenitor cells (rEPCs) in the proangiogenic devices[1]; polarization and differentiation of primary cardiomyocytes (PCM) in the cardiac platform[2]; and differentiation of rat progenitor neurons (rPNs) in the neural chip. In all the chips, fibrin or methacrylated gelatin-based gels were used as 3D extracellular matrix (ECM) Immunohistochemistry was used to light cells structures and quantify the level of the material efficiency and feasibility through confocal imaging.

RESULTS AND DISCUSSION

Results evidenced that the proangiogenic platform was useful to differentiate a proangiogenic material from its control efficiently and it was reproducible. In the proangiogenic tests, rEPCs were able to migrate longer distances following the cell tip which was following the direction of the gradient. Chemotaxis and collective cell migration could also be inferred and modeled by in silico phase-field theory. This behavior was especially interesting with the creation of calcium ions gradients able to stimulate mesenchymal stem cells to create the angiogenic cocktail able to mobilize rEPCs (Fig. 1).

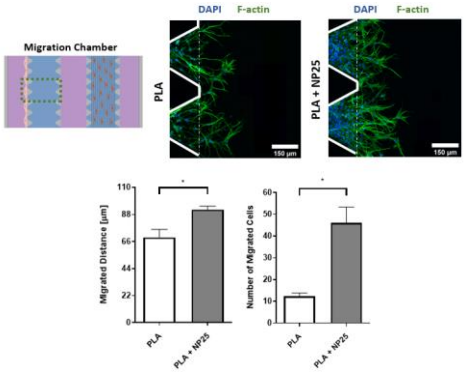

***Figure 1**. Evaluation of the calcium releasing biomaterial effects on rEPC and BM-rMSC in 3D microfluidic assay.*

On the other hand, we were able to polarize PMCs using pure PLA electrospun fibers and the application of an electric field parallel to the direction of the fibers allows them to differentiate and be functional. Finally, we could differentiate and make rPNs viable thanks to the low stiffness ECM applied.

CONCLUSION

These results demonstrate that the gap between *in vitro* characterization techniques and in vivo assays is getting narrower. OoCs represent a serious alternative to in vivo models even able to improve outcomes and versatility.

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Development of optically-tuned bioresins for the rapid volumetric bioprinting of liver organoid-laden metabolic biofactories

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INTRODUCTION: Developing advanced *in vitro* platforms for biomedical research is a key challenge in tissue engineering, which bioprinting promises to tackle allowing the precise patterning of cell-laden biomaterials into hierarchical architectures. Volumetric bioprinting (VBP) is a novel light-based biofabrication technique that overcomes challenges posed by conventional bioprinting approaches, through the layerless biofabrication of viable and complex cell-laden structures at high printing speeds[1]. Given the requirement of high cell densities to create functional tissue mimics, in light-based printing, strategies to overcome the light-scattering effect of intracellular organelles are needed to ensure high-resolution prints. In this study, an optically engineered bioresin was developed to pattern morphologically undisrupted organoids into complex centimeter-scale assemblies. Patient-derived human liver organoid-laden structures were printed to create advanced *in vitro* models that recapitulate liver functions involved in systemic homeostasis and detoxification.

EXPERIMENTAL METHODS: In VBP, visible light back-filtered projections of a 3D object are directed on a volume of cell-laden bioresin (gelatin methacryloyl with visible-light photoinitiator lithium phenyl-2,4,6-trimethylbenzoyl-phosphinate). The photosensitive resin is then crosslinked in a spatially controlled fashion. Resolution in the presence of a hepatic cell line and patient-derived liver organoids was enhanced via supplementation of iodixanol, a refractive index-matching compound. Optically-tuned resins were used to print high hepatic organoid densities (>10^7cells/mL). Organoid viability and hepatic differentiation post-VBP were tested and compared to extrusion bioprinted (EB) constructs. Organoid-laden, mathematically-derived architectures with different structural properties were printed at high resolution and cultured under dynamic perfusion to evaluate ammonia metabolism.

RESULTS AND DISCUSSION: VBP-printed 3D constructs were fabricated in tens of seconds, achieving the highest resolutions reported to date (41.5±2.9µm positive and 104.0±5.5µm negative features). Iodixanol concentration was optimized to match the refractive index of intracellular components of single cells and organoids, ensuring comparable resolution between cell-free and cell-laden prints. Compared to EB-printed structures, where shear forces resulted in organoid fragmentation and lower viability (73.2±1.2%), VBP-printed organoids showed high viability (93.3±1.4%), undisrupted morphology and displayed apicobasal polarity post-printing. Complex gyroid-like structures with different pore architectures were printed within 16-20s and showed tunable permeability and surface-area-to-volume ratio. When cultured in a dynamic perfusion system, these structures exhibited enhanced rates of ammonia metabolism (33.5±5.8-24.3±1.4nmol $mg_{total\ protein}^{-1}$) compared to static controls (12.7±0.3nmol $mg_{total\ protein}^{-1}$), as well as shape-dependent changes in the metabolic rate of the embedded organoids.

CONCLUSION: This study demonstrated the contactless bioprinting of complex and mechanically fragile biological structures (liver organoids) via VBP. Via refractive index-matching, an optically-tuned gelMA resin enabled high-resolution printing of cell-laden structures, obtaining previously unachievable feature sizes. Organoids displayed high viability and metabolism, as well as hepatic differentiation capacity post-printing. The dynamic culture of convoluted VBP-printed structures was demonstrated through architectures that could modulate organoid function in a printed shape-dependent fashion. The combination of organoid technology with the ultra-fast printing times and freedom of design offered by VBP shows promise for the development of new predictive platforms for *in vitro* disease modeling and drug screening research.

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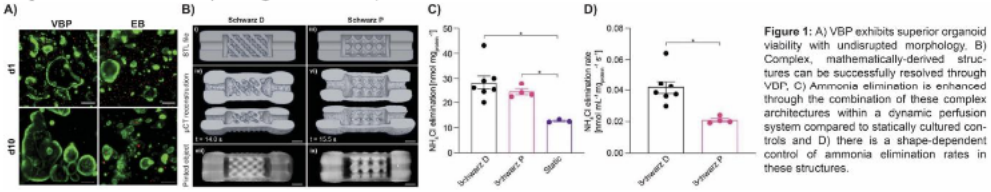

Figure 1: A) VBP exhibits superior organoid viability with undisrupted morphology. B) Complex, mathematically-derived structures can be successfully resolved through VBP. C) Ammonia elimination is enhanced through the combination of these complex architectures within a dynamic perfusion system compared to statically cultured controls and D) there is a shape-dependent control of ammonia elimination rates in these structures.

>> 10:30 - 12:30 | SYMP-08 - Bio-inspired supramolecular scaffolds as cell niches for biomedical applications

Chairpersons: Chris Sammon & Dammy Olayanju
Location: Room B

10:30 | KL1 Supramolecular - b-sheet peptide based supramolecular scaffolds: from design to application
Alberto SAIANI, Department of Materials, University of Manchester, Manchester, United Kingdom

11:00 | O1 Supramolecular - Heparin-Guided Binding of VEGF to Supramolecular Biomaterial Surfaces to Create Dual-Functionalized Hemocompatible Devices
Dina IBRAHIM, Eindhoven University of Technology, Eindhoven, The Netherlands

11:15 | O2 Supramolecular - Biodegradable dendrimers for mRNA therapeutics: crossing the BBB of the ischemic brain
Marília TORRADO, i3S/INEB - Institute for Research and Innovation in Health/Institute of Biomedical Engineering, University of Porto, Porto, Portugal; ICBAS - Abel Salazar Biomedical Sciences Institute, University of Porto, Porto, Portugal

11:30 | O3 Supramolecular - Hybrid supramolecular and photoresponsive gelatin hydrogels as dynamic cell culture matrices and volumetrically printable bioresins
Marc FALANDT, Dept. of Clinical Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

11:45 | O4 Supramolecular - Corneal endothelial tissue engineering using smart, multi-layered polymer sheets
Jasper DELAEY, Polymer Chemistry & Biomaterials group, Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent (Belgium)

12:00 | FP01 Supramolecular - Bioinspired Composite Paste-type Inks For 3D Printing Scaffolds with Bone Regeneration Potential
Izabela-Cristina STANCU, Advanced Polymer Materials Group, University Politehnica of Bucharest, Bucharest, Romania

12:05 | FP02 Supramolecular - 3D Bioinspired Hydrogels with Molecularly Imprinted Nanoparticles Sequester Endogenous Growth Factors and Synergistically Direct Stem Cell Fate Commitment
Simão P. B. TEIXEIRA, 3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Braga/Guimarães, Portugal

12:10 | FP03 Supramolecular - Dual-crosslinked degradable elastomer with self-healing properties
Mathilde GROSJEAN, Department of Polymers for Health and Biomaterials, IBMM, University of Montpellier, Montpellier, France

b-sheet peptide based supramolecular scaffolds: from design to application

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INTRODUCTION

The use of non-covalent self-assembly to construct materials has become a prominent strategy in biomaterials science offering practical routes for the construction of increasingly functional materials for a variety of applications ranging from cell culture and tissue engineering to in-vivo cell and drug delivery.[1] A variety of molecular building blocks can be used for this purpose, one such block that has attracted considerable attention in the last 20 years is *de-novo* designed peptides.[3] Peptides offer a number of advantages to the biomaterial scientists. The library of 20 natural amino acids offers the ability to play with the intrinsic properties of the peptide such as structure, hydrophobicity, charge and functionality allowing the design of materials with a wide range of properties. Synthetic peptides are chemically fully defined and easy to purify through standard processes. Being build form natural amino acids they result usually in low toxicity and low immune response when used in-vivo and can be degraded and metabolised by the body.

EXPERIMENTAL METHODS

In the past 15 years our group has focussed on the development of a technological platform for the design of novel bio-functional materials, in particular hydrogels, exploiting the self-assembly of β-sheet forming peptides based on Zhang original design. The β-sheet motif is of increasing interest as short peptides can be designed to form β-sheet rich fibres that entangle and consequently form very stable hydrogels.[2] These hydrogels can be easily functionalised using specific biological signals and can also be made responsive through the use of enzymatic catalysis and/or conjugation with responsive polymers.

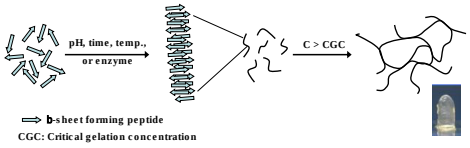

Fig 1.: Schematic representation of the self-assembling pathway of b-sheet forming peptides.

RESULTS AND DISCUSSION

One particular feature of this peptide design is that when self-assembled into an antiparallel b-sheet all hydrophobic residue side groups are located on one face of the b-sheet while all the hydrophilic residue side groups are located on the opposite face. As a result, it is thought that two b-sheets come together to bury their hydrophobic faces and form the "elemental" fibres of the network. The fibres formed are usually twisted and have a rectangular cross-section with a width ranging from 3 to 10 nm depending on the length of the peptide used and a thickness of ~ 1.5 nm. There are three remarkable structural features in these peptide fibres (Figure 1B): fibre core which contains all the hydrophobic residues and controls the fibre cohesion and morphology; fibre surface which contains all the hydrophilic residues and controls the fibre solubility and fibre-fibre associative interactions and fibre edges where the hydrophobic residues can be exposed to water. The final properties of the hydrogels will depend on three key factors: the intrinsic properties of the fibres, the network topology formed and the fibres interaction with the media (saline solution).[3]

The ability to tailor the properties of the hydrogel by manipulating the peptide design has allowed us to formulate hydrogels for a range of applications. Most recently we developed injectable hydrogels for the delivery of cardiac progenitor cells in the heart[4] as well as hydrogels with tailored interactions with Doxorubicin for its controlled delivery.[5]

CONCLUSION

Through the fundamental understanding of the self-assembly and gelation of these peptides across length scales we have been able to design hydrogels with tailored properties for a range of applications including for the culture of a variety of cells, injectable and sprayable hydrogels for cell and drug delivery as well as shear thinning hydrogel for 3D bio-printing.

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Heparin-Guided Binding of VEGF to Supramolecular Biomaterial Surfaces to Create Dual-Functionalized Hemocompatible Devices

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INTRODUCTION

A major challenge for cardiovascular biomaterials is creating an endothelial lining at the blood-material interface to prevent thrombus formation. A translationally attractive approach to achieve this is *in situ* endothelialization *via* recruitment of endogenous cells.[1,2] Biofunctionalization of the material with angiogenic growth factors, such as vascular endothelial growth factor (VEGF), can enhance this process. VEGF is a strong mitogen for endothelial cells and regulates their survival, migration and proliferation, especially when immobilized.[3] Heparin can bind to VEGF via its heparin-binding domain. We hypothesize that heparin-guided VEGF immobilization will enable the *in situ* endothelialization via binding of $VEGFR^+$ endothelial precursor cells, such as angiogenic monocytes.

In this study we aimed to create a hemocompatible and regenerative biomaterial by dual functionalization with anti-thrombogenic heparin and pro-angiogenic VEGF using supramolecular chemistry. To achieve this, we combined a heparin-binding-peptide (HBP) coupled to Ureidopyrimidinone (UPy), which is supramolecularly coupled to a UPy-modified PCL (PCLdiUPy) base polymer.

Materials were functionalized with (1) a pre-complex strategy, in which heparin and $VEGF_{165}$ were complexed before functionalization, and (2) a two-step strategy, in which materials were first functionalized with heparin and next with $VEGF_{165}$. By varying the functionalization strategy and the molecular ratio of heparin:$VEGF_{165}$, a detailed understanding of the molecule adsorption on the surface and their influence on cell behavior was obtained.

METHODS

PCLdiUPy and UPy-HBP were synthesized by SyMO-Chem BV. The materials were characterized with atomic force microscopy (AFM), water contact angle (WCA) measurements, and photoelectron spectroscopy (XPS) to establish the successful integration of the HBP at the material surface. The adsorption behavior of both heparin and VEGF was assessed with quartz crystal microbalance with dissipation energy (QCM-D). In order to probe the biological activity of the surface-bound VEGF, we assessed the materials' capacity to support endothelial monolayer formation using human umbilical vein endothelial cells (HUVECs) as a model cell. To that end, HUVECS were cultured on drop-cast films of the different material groups in starvation medium for up to 5 days. HUVEC surface coverage was quantified using fluorescent staining.

RESULTS AND DISCUSSION

PCLdiUPy surfaces showed a fibrous morphology in AFM phase images. Phase separated domains were observed upon addition of UPy-HBP, with a decrease in WCA compared to PCLdiUPy. XPS measurements showed UPy-HBP addition increased surface nitrogen content, further demonstrating incorporation of UPy-HBP into PCLdiUPy. QCM-D results showed a clear frequency shift upon heparin addition on UPy-HBP surfaces, depending on mol% of UPy-HBP, for both the two-step and the pre-complex functionalization approach. For the pre-complex approach, non-specific adsorption of the $VEGF_{165}$ to the pristine PCLdiUPy surfaces was observed, while no significant shift was seen upon flow of heparin.

HUVECs were cultured on the differently functionalized surfaces to probe functionality of the VEGF presentation, and whether heparin-guided VEGF adsorption alters the cellular response when compared to non-specific VEGF adsorption. On UPy-HBP-presenting surfaces, HUVECs displayed a 7-9 fold higher cell coverage compared to heparin-only functionalized surfaces with a more favorable behavior on surfaces functionalized with the two-step approach**.** Also, On the UPy-HBP surfaces, the HUVECs displayed an enhanced surface coverage in response to heparin-VEGF functionalization when compared to the materials without the peptide, both for the one- and the two-step approach. This shows that heparin-VEGF functionalization via the UPy-HBP leads to a favorable cell response in comparison to the non-specific binding of VEGF or the heparin-VEGF complex.

CONCLUSIONS and OUTLOOK

These findings confirm the efficient dual-functionalization of our material with a heparin-VEGF complex via a supramolecular HBP, and the bioactivity of the VEGF using HUVECs as a model cell. Current investigations are focused on establishing the ability of the materials to specifically recruit endothelial precursor cells directly from circulation. This study forms the basis for a supramolecular biomaterial platform to enable modular functionalization with growth factor cocktails.

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Biodegradable dendrimers for mRNA therapeutics: crossing the BBB of the ischemic brain

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INTRODUCTION

According to the WHO, stroke is the 2nd cause of death worldwide and the 1st in Europe. Ischemic stroke, representing the majority of the cases, occurs when blood supply to the brain is occluded, resulting in a cascade of events that leads to neuronal death. Yet, the only FDA approved therapy (tissue plasminogen activator) is not applicable to most patients (<10% of cases), and it only mitigates the effects of occlusion by restoring the blood flow, lacking neuroprotective properties[1].

BDNF has been put forward as a promising neuroprotective approach. However, its reduced plasma half-life and the poor blood-brain-barrier (BBB) permeation have limited its therapeutic administration. Additionally, the uncontrollable delivery of BDNF can lead to deleterious side effects[2].

Gene therapy has been explored as a solution for effective BDNF expression at the lesion site. Nevertheless, a biocompatible and safe vector, capable of protecting the neuroprotective nucleic acid from endonuclease degradation, crossing the BBB and reaching the ischemic brain in an efficient way, remains an unmet challenge.

Herein, we propose the use of biodegradable dendrimers as delivery vectors of mRNA encoding for BDNF (BDNFmRNA) as a novel neuroprotective strategy. These dendrimers, recently developed and patented in our group[3], can complex mRNA, shielding it after intravenous administration. Also, their nanosize, fully-biodegradability, multivalency and the possibility of fine-tuning their properties make them promising candidates to a successful therapy.

EXPERIMENTAL METHODS

The explored dendrimers rely on a gallic acid-triethylene glycol-ester (GATGE) repeating unit and a poly(ethylene glycol) (PEG) chain. Dendriplexes with BDNFmRNA were prepared at different N/P ratios (moles of dendrimer amines/moles of mRNA phosphate groups) and characterized in terms of size and polydispersity index (PdI) by dynamic light scattering, and zeta-potential by laser doppler electrophoresis. mRNA complexation was evaluated by gel retardation and SYBRGold exclusion assays. Dendriplex internalization and cytotoxicity were assessed in mice cortical neuronal cultures, using Lipofectamine-2000 (L2k) as control. To evaluate safety for i.v. administration, hemolysis and clotting assays were performed, incubating dendriplexes with purified human red blood cells and re-calcified human plasma, respectively. Finally, nanoparticle biodistribution and BDNF expression was evaluated in a mice model of stroke (pMCAO), after systemic administration of Cy5.5-labeled dendriplexes (n=3) or vehicle (n=3). Two groups (n=6) were used as controls: no surgery and SHAM (i.e. surgery with no artery occlusion).

RESULTS AND DISCUSSION

Dendriplexes showed appropriate characteristics, as they present sizes <100 nm, essential for BBB permeation, PdI values <0.3, positive net charges, and mRNA complexation efficiencies >90%. The internalization studies revealed that our dendriplexes were present in virtually all neuronal cells in culture, with higher accumulation in perinuclear regions, contrary to L2k, which internalization was reduced. Also, no cytotoxicity was observed with our nanoparticles at the used N/Ps, whereas only less than 50% of cells were viable with L2k. Hemolytic studies revealed that dendriplexes are non-thrombogenic and coagulation assays further confirmed the hemocompatibility of our system.

In vivo, we were able to observe our nanoparticles in the brain and in the specific case of the stroke animals, an increased accumulation of dendriplexes was detected in the ipsilateral side, expectedly due to the stroke-compromised BBB. Importantly, an increased BDNF expression was verified in the brains of the animals injected with dendriplexes, with a significantly higher amount in the stroke area. This demonstrates the great ability of the dendrimer to protect and effectively deliver the BDNFmRNA to the ischemic area after a systemic administration, where it is expected to enhance neuronal survival. The neuroprotective effect of BDNF is now being evaluated either by histological analysis or by animal functional tests, constituting the proof-of-principle of our strategy.

CONCLUSION

Together, these results point to a novel neuroprotective therapy in the context of stroke, using a biocompatible tool that is able to cross the BBB and reach the injury site, delivering the effector nucleic acids in a safe and controllable way. Also, with the emerging field of mRNA therapeutics, these vectors represent a valuable asset to be explored to further applications.

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Hybrid supramolecular and photoresponsive gelatin hydrogels as dynamic cell culture matrices and volumetrically printable bioresins

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INTRODUCTION: Three-dimensional bioprinting is providing new opportunities in tissue engineering for the production of geometrically complex structures containing living cells. Among bioprinting techniques, volumetric bioprinting (VBP) is an emerging method able to shape cell-laden photoresponsive hydrogels, also termed bioresins, into centimetre-scale constructs in a matter of seconds with resolution in the range of tens of micrometers.[1] The design of bioresins is especially challenging when embedding cells that thrive in soft microenvironments (of which the elastic modulus can be as low as ~100 Pa). Most available photocrosslinkable hydrogels usually form highly elastic, stiff covalent networks, while gels with low mechanical properties easily undergo deformation and loss of the printed shape. Conversely, supramolecular interactions tend to be much weaker than covalent bonds and are reversible, meaning that these bonds can reform once broken due to, i.e., cell-driven mechanical stresses.[2] However, a hydrogel relying only on supramolecular bonds might display limited stability for long-term tissue culture.[3] In this study, we developed a novel photocrosslinkable material, combining covalently photocrosslinkable moieties and supramolecular host-guest interactions, with β-cyclodextrin (β-CD) and adamantane (ADA), on a gelatin backbone. This new gelatin-based hydrogel can maintain geometrical fidelity post-printing, while creating a soft micro-environment, compliant with cell-scale mechanics (by reversible disruption of the supramolecular bonds, driven by cell-expressed forces).

EXPERIMENTAL METHODS: Gelatin with varying degrees of methacryloyl substitution (GelMA, with a range in Degree of Functionalization (DoF) of 40% to 80%) was synthesized using carbonate-buffer (~pH 9) with varying amounts of methacrylic anhydride at 50°C. The GelMA backbone was subsequently functionalized with supramolecular moieties using DMSO with adamantane isothiocyanate at 60°C (GelMA-ADA). All materials were dialyzed, sterile filtered, and lyophilized to yield the gelatin materials. β-CD was functionalized with an acrylate group. The resulting DoF was measured using a 2,4,6-trinitrobenzenesulfonic acid (TNBSA) assay and ^{1}H-NMR. GelMA-ADA (5% w/v in PBS) was photocrosslinked in the presence of LAP (0.1 w/v%) and acrylated β-CD to prepare the bioresins used for VBP. A Dynamic Mechanical Analyzer (DMA) was used to determine the compression modulus, stress relaxation kinetics, and elasticity ratio of the crosslinked hydrogels. Photorheological analysis was applied to study the covalent crosslinking kinetics and to find the storage and loss modulus of samples of different bioresin concentrations. Samples for mechanical testing and geometrically complex 3D architectures were obtained via volumetric printing (with either a custom-built device or via a Tomolite printer, Readily3D SA). Cell viability and metabolic activity assays were performed with a cell density of ~10^6 cells/mL (human mesenchymal stromal cells, MSCs, and endothelial colony forming cells, ECFCs), Matrigel and GelMA (multiple DoF) without any host-guest crosslinking groups were used as controls.

RESULTS AND DISCUSSION: The compression modulus of the supramolecular materials was significantly higher than that of the respective GelMA controls, at the same degree of methacryloyl substitution (i.e., 4.68±0.15 kPa and 0.94±0.09 kPa for 5%w/v, 60% GelMA-ADA and GelMA, respectively). As such, the supramolecular gels showed a better shape fidelity post-printing. Photorheology showed that the supramolecular materials had faster reaction kinetics and higher storage modulus than the GelMA controls. Alamar blue assay indicated that the metabolic activity of embedded cells in the supramolecular gels was significantly higher than in GelMA controls and comparable to Matrigel controls. This is especially noteworthy, as stiffer gels normally result in a lower metabolic activity of the cells, this is likely due to the ability of the material to be remodelled by cells via disruption of the supramolecular host-guest interactions. Cell viability was comparable to GelMA controls. Both MSCs and ECFCs showed a spread morphology, forming cell-cell connections, only in the supramolecular gels, which showed sign of deformation and contraction from day 3, in response to cellular traction forces. Furthermore, volumetric printing with the hybrid materials was possible with a variety of supramolecular bond ratios in a versatile manner (such as hollow discs, sharp points, gyroids, channels, and more).

CONCLUSION: We developed a versatile and scalable method to synthesize hybrid photo- and host-guest responsive material for the bioprinting of complex 3D objects. The ease in tuning the mechanical properties by changing the supramolecular to covalent bond ratio provides many possibilities, especially for printing with cells that require a different microenvironment to function optimally. The results show that these materials allow de-coupling the bulk stiffness from the microscale, cell-level behaviour. Furthermore, the development of the new shape stable gels that do not restrict cell bioactivity, migration, and collective remodelling of the microenvironment and tissue morphogenesis provides new opportunities for the biofabrication of soft tissues.

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Corneal endothelial tissue engineering using smart, multi-layered polymer sheets

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INTRODUCTION

Corneal endothelial damage and diseases are major contributors to blindness or severe visual impairment worldwide[1]. The corneal endothelium is the innermost cell layer of the cornea and consists of a cellular monolayer that maintains the stroma in a dehydrated state through a "pump-and-leak" mechanism. A critical loss of cells due to damage, disease or aging will result in opacification of the cornea[2]. Currently, the only treatment consists of a corneal transplantation from healthy cadaveric donor tissue. Unfortunately, only 1 donor is available for every 70 patients[3]. Additionally, the most advanced transplantation technique (DMEK) has proven to be difficult to master and requires, providing a barrier to bring the best possible techniques to the patient. To tackle the shortage and to facilitate the difficult transplantation process, the present work focusses on the development of transparent (>90%), thin (≤5µm), multilayered sheets constituting a poly(D,L-lactide) (PDLLA)-based shape-memory polymer (SMP)to provide structural rigidity and an automatic unscrolling behavior after implantation. Additionally, a crosslinkable gelatin-based hydrogel acts as an extracellular matrix (ECM) mimic. These sheets provide a substrate for corneal endothelial cells (CECs) to enable subsequent ocular implantation thereby restoring the damaged endothelium and the patient's vision.[4]

EXPERIMENTAL METHODS

SMP was synthesized starting from PDLLA (PDLLA), which was produced through conventional ring-opening polymerization. The PDLLA was reacted with the reaction product of isophorone diisocyanate and Bisomer PEA, resulting in the SMP precursor. Multi-layered sheets (ø=12mm) were developed through successive spincoating steps. A sacrificial gelatin layer (H_2O, 10w/v%) was spincoated onto a glass plate followed by a SMP layer (THF, 4w/w%). Next, a layer of gelatin-methacrylamide-amino-ethylmethacrylate (Gel-MA-AEMA)[5], was spincoated (H_2O, 10w/v%) as final layer, after applying an argon plasma treatment (0.8mbar, 30s). Finally, Gel-MA-AEMA and the SMP were crosslinked using UV-A (6 mW/cm^2, 30 min) irradiation. Isolation of the sheets occurred by dissolving the sacrificial layer (H_2O, 40°C). Sheets were characterized for their transparency (UV-VIS/NIR, 390-700 nm), thickness (white light interferometry) and glucose permeability (side-by-side diffusion setup,) as well as for their surface composition (XPS) and compatibility with CECs using B4G12 cells. Finally, the SMP was characterized for its shape-memory properties (DMA).

RESULTS AND DISCUSSION

Multi-layered sheets were successfully produced. The sheet thicknesses ranged between 0.8 and 1.5µm, thinner than the natural Descemet's membrane (10-20µm). All produced sheets showed a transparency of >98% (wet state, 380-700nm). The sheets were sufficiently permeable (>2.36*10^{-3} cm/s) towards glucose, both in the presence and absence of the gelatin-derived coating. Upon seeding the sheets with B4G12 CECs, the cells developed their characteristic shape. Immunocytochemical staining confirmed the presence of Na^+/K^+ ATPase pumps and tight junctions (ZO-1), indicating good cellular phenotype and proliferation. Additionally, the SMP proved to be effectively triggered at body temperature and exhibited excellent shape-memory (R_f ,R_r >98%) and processing capabilities.

CONCLUSION

Transparent (>90%), thin (<10 µm), sheets were successfully produced and were sufficiently permeable for glucose. Additionally, the sheets were able to support the proliferation of B4G12 CECs. Additionally, a novel SMP might improve the implantability of the sheets over even natural transplants.

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Dual-crosslinked degradable elastomer with self-healing properties

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INTRODUCTION

A wide array of chemically crosslinked degradable elastomers based on synthetic polyesters have been investigated for tissue engineering applications in the past years[1]. These elastomers present various advantages such as a rubber-like behavior matching with those of the soft tissues, and an ability to preserve 3D structure over their degradation[2]. Among the various chemical strategies available to develop 3D networks, photo-crosslinking is an efficient strategy due to its fast reaction time, easy implementation and low thermal energy production[3]. However, one major drawback is the inability to be healed after damage or breaking. This can be overcome by supramolecular networks whose polymer chains are linked via dynamic and/or reversible non-covalent bonds, that act as crosslinks which bring self-healing properties to the material. Hydrogen bonds are the most widely studied reversible non-covalent bonds due to their ability to quickly create bonds with high strength and excellent reversibility[4]. In this study, our objective was to combine the high mechanical properties and degradation properties of chemically photo-crosslinked elastomers, with the self-healing properties of hydrogen-bonds supramolecular networks. To this aim, a degradable 8-arm star-shaped PEG-*b*-PLA block copolymer was designed and functionalized with either methacrylic and/or catechol groups. Various degradable dual-crosslinked elastomers were then designed and the mechanical properties, self-healing efficiency, and biodegradability were evaluated.

EXPERIMENTAL METHODS

1. Preparation of elastomers

Star-shaped copolymers were synthesized by ROP before being functionalized with methacrylate and/or catechol groups. Elastomer films were prepared by solvent evaporation of polymer solutions containing defined amounts of methacrylate and/or catechol copolymers and exposed to UV light for 5 minutes per side.

2. Mechanical properties

The mechanical properties were assessed through tensile test at room temperature on dog-bone strips (10x2mm) with an Instron 3344 testing machine equipped with a 500 N load cell at a deformation rate of 10 mm.min^{-1}.

3. Self-healing study

The samples were cut in two pieces thanks to a razor blade. Then, the pieces were put together, pressed for 5 seconds and heated at 37°C in an oven for different times.

RESULTS AND DISCUSSION

The influence of the methacrylate/catechol (MC/CT) ratio on the tensile properties was investigated. It was demonstrated that the amount of catechol improved the elongation but decreased the stress at break. Indeed, elongation and stress reached 539% and 10.7 MPa for MC/CT 100/0 respectively, against 932% and 1.8 MPa for MC/CT 0/100. MC/CT 75/25 was selected as the best compromise with intermediate values between MC/CT 100/0 and MC/CT 0/100 (798% and 3.4 MPa) and interesting self-healing properties. The self-healing abilities were studied at 37°C. The samples were cut and put together for different times. The self-healing efficiency (SHE) was calculated by comparing the values of elongation and stress at break of the healed samples to the original ones. SHE increased with time and MC/CT 75/25 was totally recovered after 60 minutes (Figure 1). SHE reached 100% of the elongation at break and 120% for the stress, which reflects the properties improvement through the dynamic reorganization of the network.

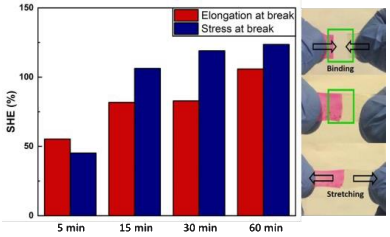

Figure 1. Evolution of SHE with time of MC/CT 75/25 and images of self-healing of a cutting film

CONCLUSION

In this work, we designed dual-crosslinked elastomers thanks to methacrylate and catechol functionalized star-shaped PEG-PLA copolymers. The resulting elastomers exhibit mechanical properties that are compatible with soft tissue applications and self-healing properties at 37°C. Moreover, the elastomers showed to be degradable *in vitro*. Therefore, we demonstrated that our strategy is an elegant approach to design, from a single copolymer type, degradable biomaterials combining both rubber-like behavior and self-healing properties.

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>> 10:30 - 11:30 | Tech S3 - Characterization of biomaterials: in situ monitoring

Chairpersons: Erik Nilebäck & Philippe Lavalle
Location: Room F

10:30 | KL in situ monitoring - QCM-D as a powerful tool to design and evaluate Biomaterials
Erik NILEBÄCK, Biolin Scientific AB, Västra Frölunda, Sweden

10:45 | O1 In situ monitoring - Real-Time Monitoring and Optimization of Hydrogel Photocrosslinking in Volumetric Printing
Sammy FLORCZAK, Department of Orthopedics, UMC Utrecht, Utrecht, the Netherlands

11:00 | O2 In situ monitoring - Sono-responsive Hybrid TiO2/Polymer Nanomaterials for Actively Targeted Sonodynamic Therapy of Cancer
Ivan ZLOTVER, Department of Material Science and Engineering, Technion – Israel Institute of Technology, Haifa, Israel

11:15 | O3 In situ monitoring - Bio-impedance and Microscopy Monitoring of Spheroid Inside a Microfluidic Device for Electro-Chemotherapy Applications
Pauline BREGIGEON, Univ Lyon, Ecole Centrale de Lyon, INSA Lyon, Université Claude Bernard Lyon 1, CNRS, Ampère, UMR5005, 69130 Ecully, France

QCM-D as a powerful tool to design and evaluate Biomaterials

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Quartz Crystal Microbalance with Dissipation monitoring, QCM-D, is a well-established surface sensitive technology which has been used for nanoscale tracking of biomolecular[1,2]-, lipid[3]-, and polymer[4,5] interactions at surfaces and interfaces for more than two decades. Via time-resolved information on mass, thickness and viscoelastic properties of surface adhering layers, the method can be used to analyze molecular adsorption, monitor layer build-up, characterize layer properties, and measure molecular interaction with thin films formed at the surface.

In contrast to optical technologies such as ellipsometry and SPR, the acoustic QCM-D technology senses hydrated mass, i.e., the mass of both the molecules studied and the coupled solvent. This feature enables detection of conformational and structural changes of the adlayers. QCM-D analysis reveals for example the dynamics of lipid bilayer formation via vesicle rupture and fusion, and it detects the transition between hydrated and dehydrated states of thin polymer layers. The ability to analyze hydrated structures and their dynamic behavior makes QCM-D a powerful tool when working with materials where layer conformation and degree of hydration are important aspects to obtain desired interfacial properties. By varying conditions such as substrate material, temperature, pH, salt concentration or salt type, the properties and dynamic behavior of the designed structure can be characterized and tailored for target applications to achieve for example biocompatibility, protein repellency, antifouling properties, and drug delivery capabilities.

Despite its long track record in molecular interaction analysis and biointerfacial science, QCM-D has untapped utility in the steadily growing area of biomaterials development. Here we give an overview of the technology and the information it provides. We present case examples that demonstrate how the method can help throughout the entire biomaterial development process; from the build-up and exploration of suitable material designs to the characterization of film properties, and the tailoring of stimuli-responsive behavior to promote certain interaction with the surrounding environment.

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Real-Time Monitoring and Optimization of Hydrogel Photocrosslinking in Volumetric Printing

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INTRODUCTION

Volumetric bioprinting (VBP) holds much promise for future endeavors in the field of Biofabrication. This high-speed, light-based approach to additive manufacturing enables for the fabrication of structures of clinically-relevant sizes within tens of seconds, and it does so using photocurable resins without the need for any extrusion through nozzles or layer-by-layer deposition. There are, however, new challenges yet to be tackled for the successful application of VBP, most notably, the difficulty in monitoring the kinetics of the process in real time. To date, there are no available set-ups for measuring physic-chemical properties of the bioresin during printing, and the only information that can be easily collected in a contactless fashion is based on imaging. However, water-rich hydrogels, typically needed for tissue culture, display minimal changes in their refractive indexes during crosslinking, meaning prints are nearly indistinguishable from the unreacted material. Here, we propose a new imaging and data analysis strategy to monitor minute changes in the refractive index of gelatin methacrylol (gelMA) during crosslinking. Entropy-based image processing was also used to quantify, in real-time, the progress of the print. The process allows for non-invasive assessment of the printing process, increasing automation in 3D printing and maximizing shape fidelity.

EXPERIMENTAL METHODS

A home-built volumetric printer was used to sequentially fabricate pairs of cylindrical structures comprising of gelatin methacryloyl (GelMA) with a visible-light photoinitiator (lithium phenyl-2,4,6 -trimethylbenzoyl - phosphinate). One cylindrical structure was used for physical measurement to verify diametrical accuracy. The printing of the second cylinder was imaged under a classical optical Schlieren method, by which a collimated pinhole light source was used to illuminate the samples during printing. This was then captured by a CCD sensor and recorded. The data from these analyses was used to generate both a curve of statistical entropy over time, and a point in time corresponding to optimal geometrical accuracy. This allowed us to infer and predict the point at which dosage of light was sufficient, such as to produce a structure with an optimal geometry. A photorheological data-driven approach was then used to normalize light-dosages along the z-axis in order to improve accuracy and homogenize curing times for more complex models perpendicular to the tomographic plane. Conical gelMA structures were printed to demonstrate this, with the geometric accuracy of these being verified through microCT scanning.

RESULTS AND DISCUSSION

Through a combination of Schlieren imaging and entropy-based analysis, a relationship was established between the entropy curves of the printed structures over time, and the point of optimal cure. In particular, we observed that the cylindrical GelMA structures reached a point of optimal cure (and hence optimal diametric accuracy) just prior to reaching the global maxima of statistical entropy. In addition to the imaging data of the hydrogels during printing, this data can be used as a real-time indicator of print doneness with little to no computational overhead. The prints demonstrated similar entropy curve profiles between trials and different models. This indicated that when combined with the storage modulus-based homogenization technique, the various regions of the geometry crosslinked within a close-enough period relative to each other such as to elicit a similar image entropy response, thus indicating the more general applicability of the technique to arbitrary geometries.

CONCLUSION

This study demonstrated a versatile imaging-based quantitative approach to determine the progress of a print in real time, and, more in general, allows monitoring of the hydrogel crosslinking kinetics through observing changes in its optical properties. This can then be used to predict when sufficient light dosage has been delivered to the resin. By adjusting the relative intensities between the layers, this technique can be applied to most arbitrary geometries, and shows potential for the fabrication of more accurate and complex hydrogel-based tissue engineering scaffolds.

ACKNOWLEDGMENTS

This project received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 949806, VOLUME-BIO)

Sono-responsive Hybrid TiO_2/Polymer Nanomaterials for Actively Targeted Sonodynamic Therapy of Cancer

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INTRODUCTION

Sonodynamic therapy of cancer relies on the production of reactive oxygen species (ROS) by activation of a sono-responsive molecule or particle that accumulates in the tumor with ultrasound (US). We developed a new family of sono-responsive hybrid nanomaterials made of amorphous titanium dioxide (TiO_2) and amphiphilic poly(ethylene oxide)-*b*-poly(propylene oxide) (PEO-PPO) block copolymers that exhibit several integrated features – (i) size that can be tailored between a few tens to several hundreds of nanometers, (ii) potential accumulation in solid tumors by the enhanced permeation and retention effect, (iii) active targeting by surface glycosylation and (iv) sono-responsiveness. These nanomaterials are synthesized by a simple, reproducible, and scalable sol-gel method.[1] Nanoparticles (NPs) have shown good compatibility in cancer cells *in vitro*, and upon irradiation with the therapeutic US, they produce ROS and trigger cancer cell apoptosis.[2] In this work, we investigated the surface-modification of these nanoparticles with glucose derivatives to actively target solid tumors that overexpress glucose transporters and, by doing so, to increase their accumulation in the tumor and the SDT efficacy while reducing toxicity in off-target body sites.

EXPERIMENTAL METHODS

Ti(IV) oxo-organo complexes were synthesized by a sol-gel process in which Ti(IV) isopropoxide is dissolved in dry acetone and aged for different times (1 to 50 days). This complex was mixed with a solution of the PEO-PPO copolymer Tetronic-1107 that was glucosylated by the conjugation of gluconolactone using a microwave-assisted ring-opening reaction in the presence of tin(II) 2-ethylhexanoate (catalyst) and nanoprecipitated in water to obtain the nanoparticles. Nanoparticles were characterized by dynamic light scattering (size), differential scanning calorimetry, thermogravimetric analysis, X-ray photoelectron spectroscopy, and electron microscopies. For in vitro studies, the nanoparticles were fluorescently labeled with fluorescein isothiocyanate. To assess cell compatibility, uptake, and sonodynamic efficacy, the rhabdomyosarcoma cell line Rh30 was grown in 2D and 3D cultures, exposed to the nanoparticles, and analyzed by imaging flow cytometry and light-sheet microscopy.

RESULTS AND DISCUSSION

We synthesized and characterized glucosylated hybrid TiO_2/polymer nanoparticles with sizes in the 20-200 nm range. Their rounded morphology and smooth surface were visualized by high-resolution-scanning electron microscopy (**Fig. 1a**). Then, we investigated the relationship between the nanoparticle size and surface glucosylation on cellular uptake and viability of 2D cultures of the Rh30 cell line that overexpresses glucose transporters. Results showed that the cell remained alive upon exposure to the glucosylated nanoparticles without US treatment, while significant cell death after US irradiation *in vitro* (data not shown). These findings indicated that there is a contribution of cellular uptake to the therapeutic efficacy of the glucosylated sono-responsive nanoparticles. Next, we developed a 3D Rh30 cell model that is a more preclinically relevant model to test the uptake, toxicity, and sonodynamic efficacy of the nanoparticles. We demonstrated that the nanoparticles (green fluorescence) are up-taken by the cells (nuclei stained in blue, **Fig. 1b**). Most nanoparticles could be visualized in the outer cell layers of the spheroid and a small number in the inner ones.

Further, we investigated the ability of US-irradiated nanoparticles to reduce the viability and proliferation capacity of Rh30 cells in the spheroid. Without US irradiation, the glucosylated nanoparticles are cytocompatible. Conversely, a significant decrease in the total cell viability was observed upon US irradiation (**Fig. 1c**). These results highlight the promise of this platform for the SDT of solid tumors.

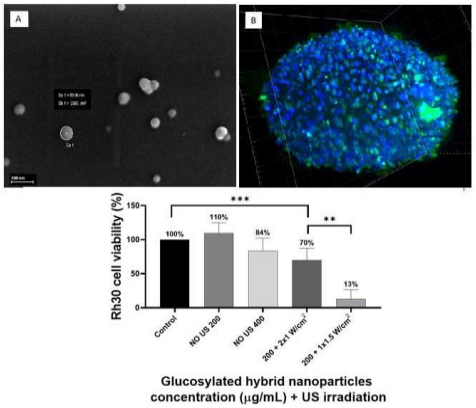

Fig. 1. (a) HR-SEM micrograph of glucosylated nanoparticles, (b) light-sheet microscopy image of a Rh30 spheroid exposed to the nanoparticles (green). Cell nuclei are stained in blue. (c) Viability assay of 3D Rh30 cell cultures exposed to the nanoparticles and irradiated under different US conditions. The size of the nanoparticles in b and c was 100 nm.

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Acknowledgments

This research was funded by the Israel Science Foundation (ISF Grant #327/19). A.S. thanks the support of the Tamara and Harry Handelsman Academic Chair.

Bio-impedance and Microscopy Monitoring of Spheroid Inside a Microfluidic Device for Electro-Chemotherapy Applications

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INTRODUCTION

In the search for a safer and more efficient treatment than classic chemotherapy, electro-chemotherapy (ECT) has emerged as a solid alternative[1]. This method is based on a phenomenon known as electroporation (EPN), occurring when pulsed electric fields (PEF) are applied to cells, and resulting in an increase of cell permeability to drugs. As the interest for such treatment is growing, the need for reliable tumor models to study the effect of PEF on cells is increasing. Spheroids have been identified as a good tumor model[2], able to reproduce cell-cell interaction, to try to have a better understanding of EPN mechanisms on in vivo tissues. To perform such studies, we have developed a microfluidic device (Figure 1a) allowing spheroid culture inside agarose hydrogel microwells, and their monitoring with both bio-impedance and high-content microscopy after EPN. HT29 colorectal cancer cell spheroids were treated with ECT and monitored inside the device, using sine burst electroporation[3] in presence of the anti-cancer agent bleomycin.

EXPERIMENTAL METHODS

A 2% agarose hydrogel is molded and grafted[4] on an amine-functionalized ITO-coated glass slide, used as an electrode for EPN and bio-impedance measurement. HT29 colorectal cancer cells are seeded in the microwells to form spheroids of reproducible size and location (Figure 1a). A silicone seal is placed around the hydrogel to form a microfluidic chamber (Figure 1b), which is closed with another ITO coated glass slide, and mounted inside a sealing device enabling water tightness and electrical contact. EPN Hepes buffer supplemented with the anticancer drug bleomycin (20 µg/mL), is then injected inside the microfluidic chamber, to change the medium inside the chamber from culture to EPN buffer, with calibrated time and volume (data not shown). EPN is performed by applying 2 sine bursts (300 V_{pp}, 10 kHz, 5 ms) of previously determined parameters (data not shown). Growth is monitored by microscope imaging and bio-impedance measurement, which principle is illustrated on Figure 1c, and proliferation is characterized thanks to a commercial kit (Click-iT EdU Alexa546, Invitrogen) and confocal microscope imaging.

RESULTS AND DISCUSSION

EPN parameters used here led to a reversible EPN as similar proliferation layers can be seen in yellow for non-treated and EPN only spheroids (Figure 1d). Moreover, ECT (delivery of bleomycin in spheroids by EPN) is efficient as there are nearly no proliferative cells for treated spheroids. Figure 1d also shows that, as expected, for spheroids in contact with bleomycin but not submitted to EPN, cell proliferation is not affected. Spheroid growth was also quantified by bright field imaging and led to the same conclusion. Interestingly, preliminary results also showed that it is possible to monitor spheroid growth by measuring the evolution of bio-impedance value with time at a given frequency when structured coplanar electrodes (interdigitated pattern) are used to improve the sensibility of the impedance measurement.

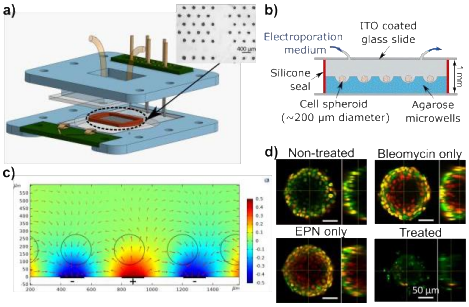

Figure 1: (a) Schematic of the microsystem, with 2.5X bright field image of HT29 spheroids. (b) Schematic drawing of the microfluidic chamber (side view). (c) COMSOL modeling of the principle of bio-impedance monitoring of spheroids with interdigitated electrodes. Color range = Electric potential (V), Arrow = Electric Field. (d) Orthogonal views of confocal images of spheroids 72h after the experiment. Green = cell nuclei, Red = proliferative cell, Yellow = both.

CONCLUSION

These results highlight the functionality of our device as an in vitro platform to test and monitor anti-cancer drug uptake in spheroids by EPN. After this first application, we intend to complexify the tumor model by producing co-culture spheroids with both cancerous cells and fibroblasts, and to optimize the electrode design to improve bio-impedance monitoring.

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ACKNOWLEDGMENTS

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>> 11:30 - 12:30 | Tech S4 - Production methods
Chairpersons: Elzbieta Pamula & Jasper Van Hoorick
Location: Room F

11:30 | KL1 Production methods - Influence of Endotoxin on Cellular Activity
Jos OLIJVE, Rousselot Biomedical, Gent, Belgium

12:00 | KL2 Production methods - Gelatin: From Biomaterial to BIO INK
Jasper VAN HOORICK, BIO INX, Ghent, Belgium

12:15 | O1 Production methods - Cellularized MPL-produced microscaffolds as building blocks for cartilage defects repair.
Olivier GUILLAUME, 3D Printing and Biofabrication Group, Institute of Materials Science and Technology, TU Wien, Vienna, Austria

Cellularized MPL-produced microscaffolds as building blocks for cartilage defects repair.

Olivier Guillaume*1,2, Oliver Kopinski-Grünwald1,2, Sandra Van Vlierberghe3 and Aleksandr Ovsianikov1,2

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INTRODUCTION Current approaches in the field of tissue engineering are roughly represented by two options, either scaffold-based or scaffold-free. Amongst the limitations of those techniques, we can point-out the difficulty to obtain a homogenous cell seeding, and the lack of mechanically competent support [1]. We propose here to utilize a new strategy which enables the fabrication of millimeter-size tissue constructs by self-assembly of multiple tissue units (TUs) based on human adipose derived stem cells (hASCs) spheroids encaged within single microscaffolds. The fabrication of multiple of those microscaffolds with high porosity and thin struts is permitted thanks to the high resolution capability of multiphoton lithography (MPL) that we have developed in our group.

EXPERIMENTAL METHODS Commercially available BIO INX resin (from Xpect-INX) is based on a multifunctional acrylate-endcapped urethane-based poly(caprolactone) [2] dissolved in THF with M2CMK at 10mM as photo-initiator. Fullerene-shape microscaffolds, called buckyballs (BB), were MPL-printed, with a femtosecond pulsed laser at 800nm, 10x microscope objective, at intensity of 85 mW. After washing the remaining non-polymerized resin, the BB were individually deposited into multi-well plates using BioSorter (from UnionBio). Each well was then seeded using 4000 hASC expanded in fully supplemented EGM-2 with 10% serum. After formation of the spheroid-loaded BB (48hrs), the plates were incubated in chondrogenic (CM) or control media (for 5 weeks both). Morphology and viability of the cells growing inside the tissue units (TUs) were assessed using scanning electron microscopy (SEM) and Live/dead staining. Chondrogenesis was assessed using biochemical assay, gene expression and histology. Finally, the ability of those TUs to self-assemble and to engineer a millimetre-size cartilage tissue was tested by incubating in a cylindrical agarose mold several hundreds of those TUs.

RESULTS AND DISCUSSION Our *in vitro* data showed that the capability of the hASCs to form spheroids was not impacted by the presence of the printed BB and within 48 hrs, cells agglomerated to create TU spheroids. The TUs maintained high viability and preserved their chondrogenic potential. The fusiogenicity of multiple TUs was maintained, to a similar degree than normal spheroids, but with the advantage that the volume of those TUs after fusion was stable over-time (Figure 1A) [3]. Multiple chondrosphere-TUs could be engineered and could merge into a larger tissue, which offers great perspective to fill up cartilage defects (Figure 1B).

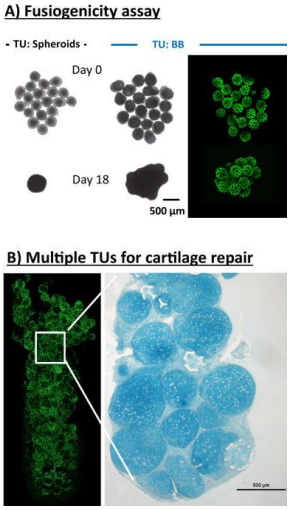

Figure 1. *Fusiogenicity of 20 TUs based on spheroids (left) versus cellularized BB (right), and comparison of their size after 18 days of culture. The BB structures are visible using fluorescent microscopy (A). Multiple chondrosphere-BB can self-assemble into a large-size, stable tissue (Alcian Blue histological staining for sGAG), offering great perspective for cartilage defects repair.*

CONCLUSION The fabrication of cellularized microscaffolds to be used as building blocks for cartilage tissue engineering offers great perspective. Those TUs have the advantages that they can be injected into a defect, rapidly self-assemble and that the volume of the resulting engineered tissue is stable over time due to the absence of compaction, as usually seen using conventional spheroids.

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ACKNOWLEDGMENTS This work was financially supported by the European Research Council (Consolidator Grant 772464 A.O.)

Influence of Endotoxin on Cellular activity

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INTRODUCTION

Endotoxins or lipopolysaccharides (LPS) are found in the outer membrane of gram-negative bacteria and have profound *in vivo* responses. Toll-like receptor 4 (TLR4) is the lipid A inflammatory signal transducer responsible for the TLR4 signal pathway induction [1].They can trigger strong immune responses and therefore are unwanted contaminants in (bio)materials and their activity must be as low as possible. Hence, FDA defined 2.15-20 EU/medical device or 0.06-0.5 EU/ml as limits depending on the type of application.

However, LPS receives significantly less attention during *in vitro* testing. Apart of the well-known effects on immune cells, the effects on various cellular activities have been reported [2]. Levels as low as 0.005ng/ml selectively silences mesoderm induction during directed differentiation of human embryonic cells [3]. Traces as low as 0.002ng/ml, significantly alter proliferative response of hematopoietic stem cells [4].

EXPERIMENTAL METHODS

In our studies we investigated the influence of LPS on cellular activity in different gelatin based systems. Besides Mesenchymal Stem Cells (MSC) differentiation and Peripheral Blood Mononuclear Cell (PBMC) cytokine release in gelatin-methacryloyl (GelMA) based hydrogels we also investigated the influence of endotoxin in gelatin coatings on the proliferation of seeded Adipose Derived Stem Cells (ADSC)

For these experiments we used different type A gelatins with different MW (Mw from 6.5 to 145 kDa) and different endotoxin contamination levels (<4 to 20000 EU/g). The LPS content was analyzed using the Endozyme recombinant factor C method.

RESULTS AND DISCUSSION

An increase in endotoxin in the GelMA based hydrogel gave lower MSC and ADSC differentiation. PBMCs in GelMA hydrogels containing high endotoxin levels lead to a significant increase in cytokine production indicating an inflammation response. ADSC seeded on gelatin coatings with high endotoxin levels lead to a decrease in proliferation. The results clearly indicate a negative effect of endotoxin on cell activities in our experimental set-up.

CONCLUSION

Endotoxin contamination is important and needs to be taken into account also during *in-vitro* culturing Endotoxins impact both immune and non-immunogenic cell systems and can lead to results misinterpretation and wrong *in-vivo* translations.

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Gelatin: From Biomaterial to BIO INK

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INTRODUCTION

Gelatin has proven to be one of the most popular materials in the field of tissue engineering and regenerative medicine due to its high cell interactivity, structural resemblance to the natural extra cellular matrix and overall abundance[1].
However, while gelatin is very promising from a biological perspective, it can be very difficult to process, especially for 3D bioprinting applications. To this end, several strategies have emerged including chemical modification of the material to make it photo-crosslinkable and therefore stable at physiological conditions. In this respect, the most popular modification of gelatin is the introduction of methacryloyl functionalities via the modification of the primary amines in gelatin with methacrylic anhydride[2]. However, while chemical modification of gelatin can overcome some of the limitations associated to the use of gelatin for tissue engineering and regenerative medicine, these modifications alone are often insufficient for straightforward processing via 3D bioprinting. Therefore, in the present lecture, an overview is presented of gelatin-based formulations tailored for efficient processing via different biofabrication technologies.

EXPERIMENTAL METHODS

Printability of gelatin-based formulations for extrusion-based bioprinting was optimized by adding shear-thinning agents. This optimized bio(material)ink formulation was then used in combination with a sacrificial ink (SUPPORT INX) to result in the formation of channeled structures for blood-vessel formation. For Digital light projection technologies, a gelatin based formulation was finetuned by the addition of photo-initiators and photoblockers, resulting in a bioink with intermediate resolution.
Finally, multiple gelatin-based formulations were optimized for high resolution multiphoton lithography applications in the presence of cells.

RESULTS AND DISCUSSION

For extrusion-based printing, it is of importance that the bio(material)ink presents shear-thinning behaviour. This means that the material will have a high viscosity at rest but becomes liquid under the exertion of external forces. After removal of these external forces, it is important that the material recovers its high viscosity fast in order to maintain its shape after exiting the printing nozzle. In this respect, we managed to produce the world's first gelatin-based bio(material)ink which enables printing at 37°C. As a proof-of-concept experiment, this material was combined with a sacrificial SUPPORT INX in order to generate channeled structures for blood vessel formation. After washing away of the sacrificial inks, these channels were seeded with endothelial cells (HUVECS).

Besides optimisation for extrusion-based printing, also preliminary experiments were performed towards the generation of a gelatin-based ink for DLP-based 3D printing. Here, the formulation was optimised by varying photoinitiator/photoblocker ratio until printing of channeled structures was feasible.

Finally, several gelatin-based formulations were optimised for high resolution, two-photon polymerisation both in the presence and absence of cells. In this respect, high resolution printing was possible with over 70% cell viability. The development of high-resolution gelatin-based inks can have significant importance for the development of organ-on-chip applications.

CONCLUSION

Clever chemical modifications in combination with formulation strategies allow optimal use of gelatin as a raw material for 3D bioprinting applications. As a consequence, the application potential of the biofabrication technology can be stretched even further.

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>> 15:30 - 16:30 | PSOP-08 - Antimicrobial biomaterials
Chairpersons: Lia Rimondini & Philippe Lavalle
Location: Room C

15:30 | O1 Antimicrobial - Chitosan-DEAE Nanoparticles for the Development of Antipneumococcal Therapeutics
María Rosa AGUILAR DE ARMAS, Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC), Madrid, Spain; Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales, y Nanomedicina (CIBER-BBN), Madrid, Spain

15:45 | O2 Antimicrobial - Evaluation of the Staphylococcus Aureus Bacteriophages Lytic Activity Depending on the Biopolymer Carrier
Liga STIPNIECE, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre, Riga Technical University, Riga, Latvia; Baltic Biomaterials Centre of Excellence, Headquarters at Riga Technical University, Riga, Latvia

16:00 | O3 Antimicrobial - Antibiotic-free Collagen-Hydroxyapatite Scaffolds Reinforced with 3D Printing to Treat Infection and Support Bone Regeneration in Load-Bearing Defects
Katelyn GENOUD, Tissue Engineering Research Group (TERG), RCSI University of Medicine and Health Sciences, Dublin, Ireland

16:15 | O4 Antimicrobial - Biological characterization of antibacterial Ag-doped calcium titanate layer on titanium implants
David PIÑERA AVELLANEDA, Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering, Technical University of Catalonia (UPC), Barcelona East School of Engineering (EEBE), 08019, Barcelona, Spain

Chitosan-DEAE Nanoparticles for the Development of Antipneumococcal Therapeutics

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INTRODUCTION

One of the most urgent challenges humanity will have to tackle in the years to come is the increasing antimicrobial resistance of bacterial pathogens. *Streptococcus pneumoniae* is an important human pathogen, responsible for most of the bacterial lower respiratory tract infections, which are the first cause of death among infectious diseases, at least before the COVID-19 pandemic. Due to insufficient vaccine coverage of all pneumococcal serotypes and the high percentage of antibiotic-resistant strains, the search for alternative treatments of *S. pneumoniae* infections is a priority[1]. Advanced biomaterials provide an interesting and versatile platform to implement new and more effective strategies to fight bacterial infections.

Here we synthesized nanoparticles of chitosan derivatized with dimethylaminoethyl groups (ChiDENPs) to emulate the choline residues in the pneumococcal cell wall and act as ligands for choline-binding proteins (CBPs). CBPs have emerged as sources or targets for novel anti-pneumococcal molecules design[2]. The union of ChiDENPs to these CBPs inhibits daughter cell separation and promote pneumococcal chain formation that could be better removed by phagocytosis[3].

EXPERIMENTAL METHODS

The chitosan-diethylaminoethyl conjugate (ChiDE) was synthesized by reaction of chitosan with dimethylaminoethyl chloride. Purified ChiDE was used to obtain ChiDENPs and ChiNPs-711 (ChiDE-based NPs that encapsulated Cpl-711) by ionotropic gelation using tripolyphosphate (TPP) as crosslinker.

The ability of ChiDEP to sequester CBC present on the bacterial surface was studied by fluorescence microscopy visualizing the ability to form chains. Cpl-711 release from ChiNPs-711 was studied by UV spectroscopy (λ=280 nm). Antibacterial activity was studied using *S. pneumonia R6*. After staining with BacLight LIVE/DEAD kit, samples were observed at a 63×

RESULTS AND DISCUSSION

ChiDENPs mimic pneumococcal cell wall ability to bind CBPs. Such ability has been shown to interfere with normal pneumococcal physiology, most probably by hijacking the CBPs that participate in cell wall remodelling, and thus causing a chained phenotype that can be useful to improve the immune system recognition and clearance of pneumococci. Besides, the CBP binding capacity of ChiDENPs has been exploited for the preparation of an encapsulated choline-binding enzybiotic (Cpl-711) with interesting therapeutic potential. Such a system was superior in stability to the NPs produced with non-derivatized chitosan since the specific interaction between the DEAE moieties and the CBM of Cpl-711 was proved to prevent aggregation effects. This nanoparticulate enzybiotic preparation released the enzyme in 2–3 h time, putatively providing a longer therapeutic window for the in vivo administration of Cpl-711 since in vivo half-life of enzybiotics has previously been reported to be shorter than 1 h. The released enzyme retained anti-pneumococcal activity, although the presence of DEAE residues within the system made such activity dependent on the relative abundance of DEAE and pneumococcal cells.

CONCLUSION

In this presentation evidence on the potential of ChiDENPs to be used as an antimicrobial itself or as a scaffold for the production of advanced antimicrobial products, based on its structural resemblance to pneumococcal cell walls will be provided.

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Evaluation of the *Staphylococcus Aureus* Bacteriophages Lytic Activity Depending on the Biopolymer Carrier

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INTRODUCTION

Bacteriophages are viruses that infect bacteria and use bacterial cells as sources for their replication.[1] Bacteriophages are functionally divided into two groups: lysogenic and lytic. Lysogenic bacteriophages infect bacteria and live in them without any disturbance to the bacterial cell. Lytic bacteriophages are viruses that infect and kill bacteria, including antibiotic-resistant ones. In the bacteriophage therapy it is important that the bacteriophage is active for a sufficient period of time. To start lysis, the amount of bacteriophages in the environment must be sufficient. The administration of the bacteriophages is usually performed by intravenous administrations of high initial doses (10^8 PFU/mL) at the first 6 h after surgery. *Staphylococcus aureus* (*S.aureus*) is a major cause of various infections in humans and animals. *S.aureus* causes infections of the skin, soft tissues, bones and joints. Lytic bacteriophages of *S.aureus* have been used in the development of antibacterial materials.[2] In this study, the stability (*i.e.* maintenance of lytic activity) of *S.aureus* bacteriophages was assessed. As the overall goal is to obtain systems that could be used as lytic *S.aureus* bacteriophages' carriers in the form of coatings, the main focus here was on the lytic activity of bacteriophages during the processing steps required for coatings' development.

EXPERIMENTAL METHODS

Commercial bacteriophage cocktails (*Pyo* and *Staphylococcal*, Eliava BioPreparations Ltd.) were used to obtain *S.aureus* specific bacteriophages. 0.5 wt% chitosan and 1 wt% Na-alginate solution with propagated lytic *S.aureus* bacteriophage solution (ratio 1:1) was prepared. Phage titer (PFU/mL) was determined by a plaque assay using *S.aureus* reference strain ATCC 25923. In addition, the bacteriophages' titer in the TRIS-HCl after incubation of the bacteriophages containing matrices was determined using the plaque assay.

RESULTS AND DISCUSSION

The results showed that addition of the bacteriophages in the 0.5 wt% chitosan resulted in an immediate and complete loss of titer. This was most likely due to the acidic nature (pH 4.07) of the solution, which was shown to be critical for the bacteriophages used in the study. In turn, *S.aureus* bacteriophages, namely, *Pyo* phage and *Staph* phage, showed good stability in Na-alginate solution with the titer reduction less than 1 log unit. As Na-alginate needs to be dried to form a coating and to store and handle the product, the phage stability (*i.e. S.aureus* bacteriophages' titer changes) during drying of the Na-alginate films was assessed. It was observed that titer of the bacteriophages decreased during incubation at 40°C. No lytic zones were detected for the samples of completely dried Na-alginate. Glycerol was added to Na-alginate as a protective additive that would optimize drying and increase stability of the bacteriophage during drying. However, its effect was insignificant. Given that (i) acidic medium of the chitosan solution proved to be lethal for the bacteriophages and (ii) drying of the Na-alginate proved to be critical for loss of lytic activity of the bacteriophages, the Ca-alginate hydrogels for embedding the lytic *S.aureus* bacteriophages (*Pyo*) was evaluated. The titer reduction after cross-linking Na-alginate with $CaCl_2$ was below 1 log unit. In addition, titer of the bacteriophages embedded in Ca-alginate matrices remained reasonably close to the planned or nominal value $(2.1\pm0.2)\cdot10^8$ PFU/mL. After 72 h, only 0.29±0.02% of encapsulated bacteriophages were released from the Ca-alginate matrices.

CONCLUSION

Parameters and components for the development of polymer matrices containing lytic *S.aureus* bacteriophages were screened. The *S.aureus* bacteriophages retain their lytic activity in the Na-alginate solution. Drying of the Na-alginate matrices proved to be critical for loss of lytic activity of the bacteriophages. It was concluded that the most optimal option is to use the Ca-alginate matrices, where the embedded bacteriophages retain their lytic activity and a long-term local supply of bacteriophages at the implant site could be ensured.

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Antibiotic-free Collagen-Hydroxyapatite Scaffolds Reinforced with 3D Printing to Treat Infection and Support Bone Regeneration in Load-Bearing Defects

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INTRODUCTION

Treatment of osteomyelitis, a severe bone infection caused predominately by *Staphylococcus aureus* bacteria, requires surgical debridement of the infection site and long-term systematic antibiotics which can limit bone regeneration and cause the development of resistant bacteria[1]. Highly porous collagen-hydroxyapatite (CHA) scaffolds developed in our lab have shown success in bone healing *in vivo* and capability for bacterial inhibition *in vitro* when loaded with metal-based antimicrobial (MBA) doped-bioactive glass[1,2,3]. However, the capacity of these materials to be used in load-bearing situations is limited due to their insufficient mechanical properties. As such, the aim of this study was to develop a biomaterial to delivery non-antibiotic antimicrobials while providing a suitable bioactive environment for bone regeneration with appropriate structures in place for load-bearing applications.

EXPERIMENTAL METHODS

A range of MBA-doped hydroxyapatite (MBA-HA) concentrations were fabricated and characterized (formulations not disclosed due to IP restrictions), which were blended within a collagen hydroxyapatite (CHA) slurry prior to freeze drying to form collagen-MBA-HA (C-MBA-HA) scaffolds. To enhance the mechanical properties of these scaffolds, a 3D printed framework was used to reinforce the scaffolds by incorporation into the CHA slurry prior to freeze drying. Following *in vitro* evaluation of both osteogenesis with rat mesenchymal stromal cells and antibacterial properties with *S. aureus*, the scaffolds are also assessed *in vivo* in a 5mm rat femoral defect.

RESULTS AND DISCUSSION

CHA scaffolds were successfully functionalized with optimised MBA-HA doses and concentrations to achieve antimicrobial and osteogenic properties. In brief, the C-MBA-HA scaffolds supported equivalent calcium production to the CHA scaffolds at 28 days and revealed homogenous alizarin red staining. Additionally, the C-MBA-HA scaffolds caused a 50% reduction in *S. aureus* and developed zones of inhibition on *S. aureus* plates after 24 hours. The mechanical properties of the C-MBA-HA scaffolds were significantly enhanced with the integration of the 3D printed polymer framework to better mimic cancellous bone without compromising the hight porosity and interconnected porous microarchitecture required to support cellular infiltration and bone formation. Initial results from the *in vivo* study suggest equivalent bone formation in both the reinforced CHA scaffold and reinforced C-MBA-HA scaffold demonstrating the potential of this treatment for bone infection.

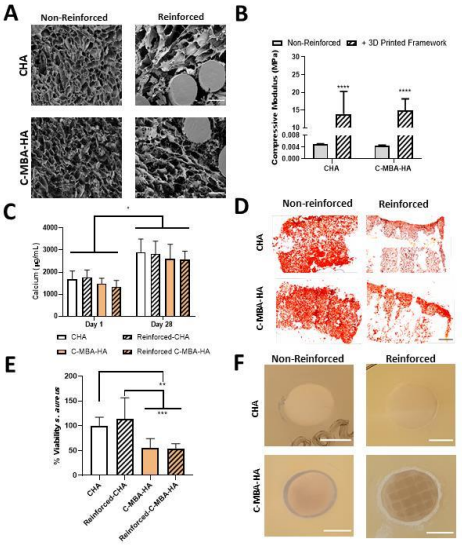

Figure 1: (A) SEM images of reinforced and non-reinforced CHA and C-MBA-HA scaffolds, Scale = 200µm (B) Compressive modulus of reinforced CHA and C-MBA-HA scaffolds, (C) Calcium deposition over 28 days, (D) Alizarin red staining representative of calcium deposition at 28 days. Scale =200µm. (E) *S. aureus* viability after 24 hours, (F) Visible zone of inhibition of *S. aureus* in C-MBA-HA groups on agar plates. Scale = 5mm * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

CONCLUSION

The successful development of a non-antibiotic antimicrobial and osteoconductive scaffold with enhanced mechanical properties for treatment of load-bearing segmental defects has the potential to be a one-step local treatment for osteomyelitis as well as in a prophylactic in cases with high infection risk factors.

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Biological characterization of antibacterial Ag-doped calcium titanate layer on titanium implants

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INTRODUCTION

Titanium (Ti) implants are widely used for orthopedic applications due to suitable bone-bonding ability[1]. However, bacterial infections can hamper the process. Bacteria can adhere to the surface of the implant, proliferate and further form a biofilm. Under normal conditions, the presence of opportunistic pathogens in the tissue is quickly fought by the host's immune system, but the implant generates a niche of immune depression where bacteria are able to induce an infection[2]. Once the implant is placed, host cells and bacteria begin a competition to adhere to the implant surface. Therefore, the rapid integration in the bone tissue and the prevention of bacterial infection are key to the success of the implant. With this aim, a silver (Ag)-doped calcium titanate layer on Ti implants has been developed[3] and the surface competition between cell and bacteria on the Ag-doped calcium titanate layer has been evaluated.

EXPERIMENTAL METHODS

Commercially pure titanium grade 2 (Ti) was thermochemically treated in order to obtain calcium titanate (Ti-Ca) and silver-doped calcium titanate (Ti-CaAg)[3]. Human Osteoblast-like cells (SaOS-2) adhesion and morphology were assessed by immunofluorescence and metabolic activity assays. Crystal violet staining of *Staphylococcus aureus* and *Staphylococcus epidermidis* was used to study the biofilm formation on the samples. Finally, the competition between cells and bacteria was studied in order to mimic a pre- or post-implantation infection.

RESULTS AND DISCUSSION

The addition of silver in the calcium titanate layer slightly reduced the number of adhered cells in the first 24 hours by 20%. However, after 72h the number of cells was the same in all conditions, showing the typical morphology of SaOS-2 and the presence of focal adhesions. Regarding the study of biofilm formation, the Ag-doped calcium titanate layer achieved to reduce the biofilm formation by 90%. In the co-culture studies (Figure 1), the number of cells was reduced in the case of the controls, but on the samples that incorporated Ag, the number of cells was maintained even in the presence of bacteria (Figure 2).

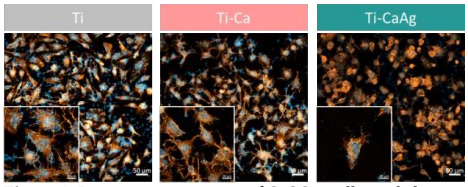

Figure 1. Representative images of SaOS-2 cells seeded in co-culture with *S. aureus* by using fluorescence staining. Scale bar: 50 µm (10x) and 20 µm (40x).

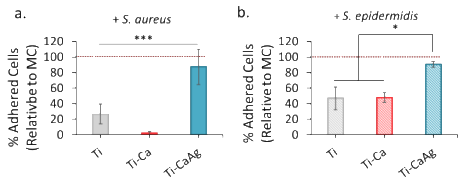

Figure 2. (**a**) % adhered cells in co-culture with *S. aureus* relative to free-bacteria SaOS-2 monoculture (MC) (**b**) % adhered cells in co-culture with *S. epidermidis* relative to free-bacteria SaOS-2 MC

The obtained results suggest that Ag-doped calcium titanate layer is able to inhibit bacterial biofilm formation without affecting SaOS-2 adhesion. Moreover, the Ag-doping surface is capable of preventing and protecting bone bacterial infection.

CONCLUSION

Ag-doped calcium titanate layer on Ti is able to inhibit bacterial attachment without damaging human osteoblast-like cells. Moreover, this layer is capable of protecting the surface of titanium implant from the growth of opportunistic pathogens while enabling osteointegration by fighting potential bacterial infections.

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>> 15:30 - 16:30 | PSOP-09 - Nanobiomaterials
Chairpersons: Gianni Ciofani & Maria Pau Ginebra
Location: Room A

15:30 | O1 Nanobiomaterials - 3D-printed Plasmonic Scaffolds for SERS Sensing and Imaging of Cancer Models
Clara GARCIA-ASTRAIN, CIC biomaGUNE, Basque Research and Technology Alliance (BRTA), 20014 Donostia - San Sebastián, Spain; Centro de Investigación Biomédica en Red de Bioingeniería Biomateriales, y Nanomedicina (CIBER-BBN), 20014 Donostia-San Sebastián, Spain

15:45 | O2 Nanobiomaterials - Surface-functionalized nanomedicines with FcRn-targeted ligands for intestinal delivery of semaglutide
Soraia PINTO, Instituto de Investigação e Inovação em Saúde (i3S), University of Porto, Porto, Portugal; Instituto de Ciências Biomédicas Abel Salazar (ICBAS), University of Porto, Porto, Portugal

16:00 | O3 Nanobiomaterials - General features of metal sulfide biomineralization in microorganisms and particularity in intracellular biomineralization of copper sulfide
Yeseul PARK, Aix-Marseille Université, CEA, CNRS, BIAM, Saint Paul lez Durance, France

16:15 | O4 Nanobiomaterials - Development of Virus-Like Particle platform for the control of cell behavior
Hasna MAAYOUF, Université de Haute-Alsace, IS2M-CNRS 7361, Mulhouse, France

3D-printed Plasmonic Scaffolds for SERS Sensing and Imaging of Cancer Models

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INTRODUCTION

Bioprinting has become a useful tool for the rapid fabrication of scaffolds to support tissue or tumor growth[1]. However, there is still a lack of detection and imaging tools able to monitor cell behavior within these complex 3D cell models over long periods of time[2].
In this work, we propose the use of surface-enhanced Raman scattering (SERS) for in situ biosensing and bioimaging of 3D cell cultures. For that purpose, different SERS active 3D-printed scaffolds have been designed for the detection of relevant cancer biomarkers and to support the growth of cells labelled with SERS tags. SERS takes advantage of the remarkable optical properties of noble metal nanoparticles (NPs) due to their Localized Surface Plasmon Resonances (LSPR) that result in strong absorption and scattering of light at specific wavelengths, creating high local electric fields at the surface[3]. These electric fields enhance the Raman scattering of the molecules adsorbed on the metal surface and allow for extremely low detection limits. Furthermore, the excitation wavelength can be tuned within the near infrared range (NIR) matching the so-called biological transparency window (650-950 nm) and, thus, improving light penetration in tissues. The 3D-printed plasmonic scaffolds allow for the simultaneous *in vitro* imaging of different types of cells as well as for *in situ* detection of targeted biomarkers.

EXPERIMENTAL METHODS

Different types of gold nanoparticles, gold nanostars (AuNSs) and gold nanorods (AuNRs), were prepared following previously reported methods[4] Plasmonic inks were prepared by dissolving different polymer mixtures in nanoparticles solutions of varying concentrations. Scaffolds were printed using a multi-headed 3D Discovery bioprinter (RegenHU, Switzerland)[5]. AuNSs were also labelled with SERS tags for cell-labelling. For this study, cells from a hormone receptor positive breast cancer cell line (MCF7) and human dermal fibroblasts (HDF) were labelled with AuNSs encoded with biphenyl-4-thiol (BPT) or benzenethiol (BT) SERS tags.

RESULTS AND DISCUSSION

SERS active scaffolds were prepared using different polymer mixtures, for example, methacrylated hyaluronic acid, alginate, κ-carrageenan or polyethylene glycol diacrylate, all of which confirm suitable rheological properties for 3D printing. These hydrogel-based inks incorporate plasmonic nanoparticles, such as AuNRs, which can be used for the detection of biomarkers in situ. Biocompatibility tests confirmed their non-cytotoxic nature, and via SEM we could confirm the 3D printing fidelity. SERS measurements were conducted using scaffolds with varying concentrations of AuNRs and using model molecules such as mercaptobenzoic acid or adenosine with detection limits close to 10 μM. Finally, SERS imaging of SERS tag labelled cells confirmed the potential of the scaffolds for cell bioimaging in real time.

CONCLUSION

In this work we demonstrated the potential of 3D-printed plasmonic scaffolds for SERS bioimaging and biosensing. The fabricated plasmonic scaffolds proved their efficiency for the detection of potential biomarkers. For imaging studies, the scaffolds supported the growth of MCF7 cancer cells and HDF labelled with suitable Raman tags.

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Surface-functionalized nanomedicines with FcRn-targeted ligands for intestinal delivery of semaglutide

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INTRODUCTION

Type 2 diabetes *mellitus* (T2DM) is a metabolic disorder that occurs when the body cannot respond fully to insulin, causing hyperglycemia[1]. GLP-1 analogs have been used as a promising strategy for the treatment of T2DM, providing an effective glycemic control due to their resistance to the degradation by dipeptidyl peptidase-4. However, these antidiabetic peptides are mainly administered by subcutaneous route, which is associated to a poor patient compliance[2,3]. Recently, a GLP-1 analog, semaglutide, was approved for oral delivery, but still presents low bioavailability[4]. In this sense, the main goal of this work is to improve the oral delivery of semaglutide by encapsulating into mucodiffusive polymeric nanoparticles (NPs), surface-functionalized with ligands that target the intestinal neonatal Fc receptor (FcRn), responsible for the transcytosis of nanosystems through the intestinal epithelial cells.

EXPERIMENTAL METHODS

Surface Plasmon Resonance (SPR) Analysis: The pH-dependent binding of FcRn-targeted ligands to the human FcRn (hFcRn) was tested by SPR. Further, the affinity of FcRn-functionalized NPs to the receptor was also evaluated.

PLGA-PEG-MAL Functionalization: PLGA-PEG-MAL was chemically linked via MAL-thiol chemistry to FcRn-targeted ligands. The conjugation efficiency (CE) was determined by Micro-BCA assay. XPS was also performed to evaluate the successful conjugation of PLGA-PEG-MAL and FcRn-targeted ligands.

Production of nanoparticles: Functionalized semaglutide-loaded PLGA-PEG NPs were produced by double emulsion technique and characterized, in terms of mean particle size, polydispersity index, zeta potential, association efficiency (AE) and drug loading (DL).

***In vitro* release assay:** The release profile of semaglutide was tested for 8 hours by incubating semaglutide-loaded NPs with medium that mimic the gastric and intestinal environment.

***In vitro* uptake studies:** To evaluate the interaction of functionalized NPs with intestinal cells that endogenously express the hFcRn (Caco-2 cells), an *in vitro* uptake study was conducted.

RESULTS AND DISCUSSION

The mucodiffusive semaglutide-loaded NPs produced by double emulsion technique had a mean particle size close to 187 nm, a neutral surface charge, and an AE and DL of approximately 54% and 1%, respectively. A sustained release profile for semaglutide was obtained in medium that mimic the gastrointestinal environment. By SPR analysis, it was showed the affibody ZFcRn16 and the peptide CQRFVTGHFGGLYPANG had the best pH-dependent binding to hFcRn, binding weakly at pH 7.4 than pH 6.0. Both ligands were chemically linked to PLGA-PEG-MAL through MAL-thiol chemistry, resulting in a CE for the peptide and the affibody of around 79% and 16%, respectively. Likewise, the successful conjugation of PLGA-PEG-MAL and the FcRn-targeted ligands was proved by XPS, where sulfur was detected and an increase in nitrogen was observed. Afterwards, both functionalized polymers were used to produce polymeric NPs, resulting in an increase in the mean particle size, comparatively with non-functionalized NPs. It was demonstrated by preliminary SPR studies that the FcRn-targeted NPs had a similar pH-dependent binding to hFcRn as the FcRn-targeted ligands alone. Additionally, *in vitro* uptake studies showed the functionalized NPs had a higher interaction with the Caco-2 cells than non-functionalized NPs.

CONCLUSION

In this work, monodisperse functionalized semaglutide-loaded NPs were produced with a loading around 1% and a peptide and an affibody CE of 79% and 16%, respectively. Moreover, the NPs functionalized with both FcRn-targeted ligands bound to hFcRn in a similar pH-dependent manner as the unconjugated ligands. It was also showed that the NPs conjugated with these ligands had a higher interaction with Caco-2 cells than non-conjugated NPs. Further, the intestinal permeability of both functionalized nanosystems will be tested using an *in vitro* 3D intestinal model, and the functionalized NPs with higher intestinal permeability will be studied in *in vivo* models to validate their potential in enhancing the oral bioavailability of semaglutide.

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General features of metal sulfide biomineralization in microorganisms and particularity in intracellular biomineralization of copper sulfide

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INTRODUCTION

Metal sulfides are one of the most commonly discovered mineral phases in nature and often produced by biomineralization processes initiated by microorganisms as well.[1] A few metal sulfide biominerals are found in the form of intracellular biominerals in microorganisms and often appear as a response to metal ion toxicity in the environment.[2] Intracellular biomineralization of metal sulfides occurs in various forms and degrees of biological control[1], and one of the tightest biological control is representatively found in greigite (Fe_3S_4) produced by magnetotactic bacteria.[3] In this context, we discovered a new type of intracellular biomineral composed of copper sulfide in a sulfate-reducing magnetotactic bacterium *Desulfamplus magnetovallimortis* sp. BW-1.[4] The bacterium could biomineralize spherical nanoparticles composed of copper sulfide in their periplasmic space at an enhanced copper ion concentration.

EXPERIMENTAL METHODS

Cell culture Pre-cultured cells were centrifuged down and inoculated to a culture medium of Cu(II) 13.9 µM and Fe(II) 20 µM. The cell culture was kept in the dark at 28 °C for 20 hours to produce copper sulfide particles.

TEM Particles were imaged on Ni grids using a Tecnai G2 BioTWIN (FEI Company) at 100 kV.

HRTEM, STEM-HAADF, STEM-EDS We used a ThermoFisher Talos F200X G2 scanning transmission electron microscope at 200 kV. Bright-field and HRTEM images, SAED patterns and EDS maps (10 µs dwell time) were obtained.

Cu K-edge XAS SXFM measurements were conducted using an incident photon energy of 9 keV for X-ray fluorescence (XRF) mapping with a dwell time of 20 ms. Cu K-edge XAS and Cu Kα XRF were collected at 8.8-9.2 keV.

Cryo-ET Cell samples and 10 nm gold beads were applied to glow-discharged holey carbon R2/1 Cu 200 mesh grids, which were blotted and vitrified. Cryo-ET data was collected on a Titan Krios TEM G3i. Data sets were collected at 300 kV.

NanoLC-tandem MS Triplicated cell samples were prepared with reference samples. Proteins were extracted and subjected to a SDS-PAGE electrophoresis. The whole proteome was subjected to in-gel trypsin proteolysis and the resulting peptides were characterized by MS/MS performed with a Q-Exactive HF instrument (Thermo) coupled to a nanoUPLC.

RESULTS AND DISCUSIONS

The nanoparticles produced by BW-1 in the enhanced concentration of copper ions are about 70 nm size. Remarkably, each particle is composed of 1-2 nm nanocrystallites that are based on copper sulfide with the hexagonal crystal system. Based on EDS and SAED measurement results, the particles have a crystal structure similar to blue-remaining covellites. However, the Cu K-edge XANES shows rather a similar property to Cu_2S having only Cu^+. Combining both results, we concluded that the nanoparticles stay in a thermodynamically unstable 'primitive state'.[5]

Moreover, the nanoparticles do not easily aggregate with each other, which is not common in other periplasmic biominerals discovered in microorganisms or in extracellular particles found in the BW-1 culture. We found organic matrices surrounding the particles, which tentatively make the particles to stay separated. We further investigated on associated proteins using differential proteomics, of which the result suggests that two periplasmic proteins, a DegP-like protein and a heavy metal-binding protein, are possibly attributed to the biomineralization of an unknown mechanism for intracellular biomineralization.

CONCLUSION

We discovered a novel intracellular biomineral composed of copper sulfide having a peculiar crystal structure within BW-1. The particles were located in the periplasmic space surrounded by an organic substance. In general, the crystal structure and the organization show a tight biological control based on biogenic macromolecules. Our study also suggests a new mechanism of intracellular biomineralization based on our proteomics data.

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Development of Virus-Like Particle platform for the control of cell behavior

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INTRODUCTION

Protein self-assemblies derived from nature are of growing interest in medicine and nanotechnology fields[1]. Organized from repeated association of monomers, they are able to spontaneously form well-defined molecular architectures. This is the case of virus-like particles (VLPs)[2]. These are non-infectious nano-scale self-assemblies that can be genetically modified and produced through recombinant DNA techniques, giving them great versatility for applications in vaccinology and immunology. However, the field of biomaterials still makes a little use of these tools. Here we show that VLPs can be used to develop cell-signaling nanoscaffolds presenting bioactive peptides on their surface for the control cell behavior.

EXPERIMENTAL METHODS

The AP205 VLPs we worked with are formed from 180 copies of the CP3 coat protein. We modified by molecular cloning the N- and C-termini of CP3 in order to add different peptides to the particle surface (Fig. 1A). The resulting constructs were produced in bacteria, purified by affinity through their 6 histidine purification tags and size exclusion chromatography then analyzed by SDS-PAGE and protein assay.

The adhesion of C2C12 mouse cells (5000 cells/cm²) on AP205 particles adsorbed on PDMS was assessed by confocal microscopy. Cell spreading was quantified after image segmentation using ImageJ. Statistical analyses were performed using Two-way ANOVA test.

RESULTS AND DISCUSSION

We were able to produce particles expressing adhesive (RGD) and osteogenic (BMP2) peptides at their surface that are known to trigger similar cell responses as their native proteins (Fig. 1A). The RGD peptide is derived from the fibronectin RGD site. The BMP2 peptide is derived from the epitope of the human bone morphogenetic protein 2 (73 to 92 amino acids). We showed that AP205-RGD particles stimulate adhesion and cell spreading of mouse cells C2C12 with a similar efficacy as native fibronectin whereas AP205-BMP2 particles do not (Fig. 1C). With similar methods, we were able to produce heteromeric particles co-expressing RGD and BMP2 peptides (Fig. 1A). The presence of RGD on our multifunctional particles can promote cell adhesion similarly to AP205-RGD particles suggesting that this system can be used as a platform to promote cell adhesion together with other cell functions (Fig.1C). We are currently studying the synergetic effect of the heteromeric particles on C2C12 differentiation into osteoblasts.

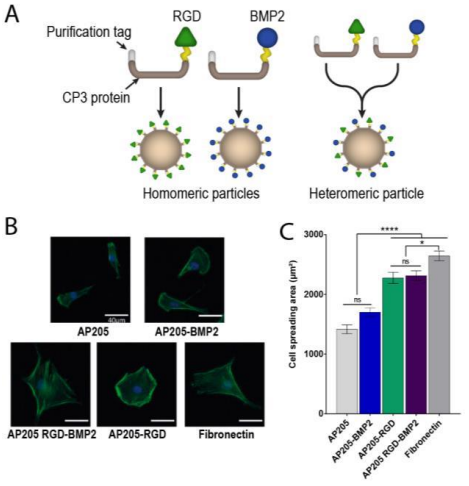

Fig. 1: A Virus-Like Particle platform for the control of cell behavior. Schematic representation of AP205 VLPs constructs (A). Immunostaining of C2C12 cells after 6h of incubation on PDMS surfaces treated with various AP205 particles or fibronectin at the concentrations of 200 µg/mL. Scale bar: 40 µm (B). Quantification of cell spreading area in µm² measured on each condition. Data is presented as mean ± SEM. *P* values were obtained by using Two-way ANOVA test. $*P < 0.05$, ****, $P < 0.0001$ versus the group control (C).

CONCLUSION

These results show that it is possible to combine bioactive peptides on the AP205 particle surface in order to create signaling nanoscaffolds that control different facets of cell biology. We are currently developing a modular VLP-based system on which we can tune the stoichiometry and concentration of multiple signaling peptides.

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ACKNOWLEDGMENTS

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>> 15:30 - 16:30 | PSOP-10 - Neural tissue engineering

Chairpersons: Zaida Alvarez & Abhay Pandit

Location: Room F

15:30 | O1 Neural tissue - Design of a Fibre-based Neuronal Guidance Scaffold for the Inner Ear

Peter BEHRENS, Institute of Inorganic Chemistry, Leibniz University Hannover, Hannover, Germany; Department of Otorhinolaryngology, Head and Neck Surgery, Hannover Medical School, Hannover, Germany; Cluster of Excellence Hearing4all, Hannover, Germany

15:45 | O2 Neural Tissue - Anisotropic Interfacial Complexes of Collagen and Glycosaminoglycans as Mimics of Neural Tissues

Rui R. COSTA, 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Avepark – Parque de Ciência e Tecnologia, Zona Industrial da Gandra, 4805-017 Barco, Guimarães, Portugal; ICVS/3B's – PT Government Associate Laboratory, Braga/Guimarães, Portugal

16:00 | O3 Neural Tissue - Fabrication of Living Electrodes Using Multiphoton Induced Degradation in a Photoresponsive Hydrogels for Neural Interface Applications

Jasper Van Hoorick, CÚRAM SFI Research Centre for Medical Devices, National University of Ireland Galway, Galway, Ireland

16:15 | O4 Neural Tissue - The mechanical side of the brain: a tissue engineered model to explore the role of mechanical properties alterations in CNS demyelination

Eva Daniela CARVALHO, i3S – Instituto for Health and Research in Innovation, University of Porto, Porto, Portugal / INEB – Institute of Biomedical Engineering, University of Porto, Porto, Portugal / FEUP – Faculty of Engineering, University of Porto, Porto, Portugal

Design of a Fibre-based Neuronal Guidance Scaffold for the Inner Ear

Peter Behrens[1,3*], Monika Seegers[1,3*], Inga Wille[1,3], Jennifer Harre[2,3], Thomas Lenarz[2,3], Athanasia Warnecke[2,3]

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INTRODUCTION

Loss of hearing is a major health problem of today's society, bearing not only direct implications for acoustical communication, but also affecting social integration. When hearing loss occurs due to malfunction of the sensory hair cells in the inner ear (sensorineural hearing loss), the most effective therapy is the implantation of a cochlear implant (CI) which electrically stimulates the spiral ganglion neurons (SGNs). However, in the usual situation, the distance between the cochlea electrode and the spiral ganglion neurons is large (Fig. 1 top) which leads to the necessity to apply rather strong electrical stimuli; also, due to the spreading of the electric field over the large distance involved, several SGNs are stimulated simultaneously, leading to a loss in frequency resolution of the perceived acoustic signal. A closer electrode-nerve contact could improve this situation. Whereas SGNs are known to form new neurites under favourable conditions, for example in presence of the growth factor BDNF ((brain-derived neurotrophic factor), these do not grow towards the cochlea electrode. For example, a BDNF-releasing hydrogel placed in the scala tympani offered a favourable surrounding for the continued growth of neurites, however, without effecting direct guidance of the neurites towards the electrode contacts.[1] To achieve this, a directional guidance clue should be offered. Therefore, we set out to construct a neuronal guidance scaffold (NGS) and drug delivery system based on fibers.[2] The design of this scaffold (Fig.1) is described here.

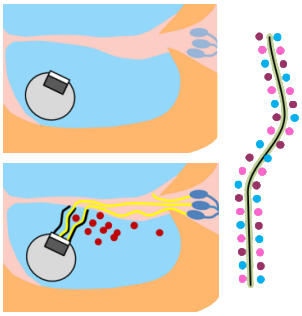

Fig. 1. Top: Placement of the cochlea electrode within the scala tympani, illustrating the large distance to the SGNs (light blue) to be stimulated. Bottom: Sketch of the fiber-based NGS which releases growth factors and provides a favourable environment for neurite growth. Right: Sketch of an NGS fiber coated with HS (green) and different biomedical agents (laminin, BDNF, NT-3).

EXPERIMENTAL METHODS

Degradable fibres (Glycolon®, Resorba Medical GmbH) were used as the basis for the construction of the NGS. Pristine as well as aminolysed fibers were employed. The aminolysis used ethylenediamine and standard coupling agents (*N*-hydroxy succinimide or *N,N'*-dicyclocarbo-diimide). Heparan sulphate (HS) and laminin were bound covalently on the surface of the fibers. Growth factors BDNF and NT-3 (neurotrophin-3) were adsorbed onto the fibers from phosphate-buffered saline (PBS) solutions and their release was studied. Functional cell culture studies were performed with spiral ganglion cells or pieces of spiral ganglion strands isolated from neonatal rats.[2]

RESULTS AND DISCUSSION

Our concept for an NGS is based on fibers used as suture materials which degrade within a timescale of several months. The fibers are coated with HS as a molecule representative of the extracellular matrix which is also known to reversibly bind and deliver growth factors.[2] Laminin is bound to the fibers to provide anchor points for neurites when they grow along them. Experiments with laminin-decorated fibers and spiral ganglion strands show strong adhesion of spiral ganglion cells to the fiber and pronounced growth of SGN neurites along the fiber. BDNF is adsorbed onto the fibers and can be released to exert a neuroprotective action.[2] In cell culture studies with supernatants from BDNF release studies, the survival of SGNs is significantly enhanced. NT-3 shall be employed to stimulate the growth of neurites from SGNs. Gradients in NT-3 concentration are known to direct the processes growing out from neurons.[3]

CONCLUSION

Several major components of the designed NGS have been established. Further work shall concentrate on the combination of the different biomolecules and the spatiotemporal control of the released concentrations.

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ACKNOWLEDGMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – Hearing4all – EXC 2177/1 – Project ID 390895286.

Anisotropic Interfacial Complexes of Collagen and Glycosaminoglycans as Mimics of Neural Tissues

Rui R. Costa[1,2*], David Caballero[1,2], Diana Soares da Costa[1,2], Subhas C. Kundu[1,2], Rui L. Reis[1,2], Iva Pashkuleva[1,2]

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INTRODUCTION

Lesions in neural tissue disrupt the cell orientation essential to propagate electrical signals and action potentials along nerve fibers. The regeneration of this tissue is impaired by glial scars, which cause aberrant cellular functions and inhibit the regrowth of axons[1]. Developing new biomimetic materials that promote neuronal cell alignment has been challenging since several physicochemical cues like composition, anisotropy and stiffness are essential for the healing process. We hypothesize that fibrillar structures that mimic the neural tissue's extracellular matrix (ECM) can be obtained by interfacial polyelectrolyte complexation (IPC)[2] and will aid the regeneration.

EXPERIMENTAL METHODS

We used ECM biomolecules for the IPC: collagen type I (Col) as polycation, and glycosaminoglycans (GAGs) heparin (Hep) or chondroitin sulfate (CS) as polyanion. A flow-focusing microfluidic system was fabricated in-house using PDMS-based soft lithography to continuously assemble Col/Hep and Col/CS. Aqueous solutions of Col and GAG were injected at 5 µL min^{-1} into the chip. An interfacial complex was formed in the channels upon contact between the two solutions. The complex was pulled in the air at the microchip exit and coalesced as handable microfibers.

We used different microscopies (SEM, brightfield and phase-contrast microscopy) to characterize the assembled fibers. The tensile strength was assessed according to a modified ASTM D3379 standard for single filament materials. The composition and polyelectrolyte distribution were visualized by confocal microscopy after immunostaining (anti-Col(I) antibody-488 for Col and wheat germ agglutinin-633 for the GAG). A PC12 neuronal cell line was used to assess the biofunctionality of the fibers for up to 7 days.

RESULTS AND DISCUSSION

Phase-contrast microscopy confirmed the formation of interfacial complexes: we observed an emergent physical boundary between the biopolymers' streams. The obtained dry microfibers (≈30 µm in diameter) were tough and flexible, which allowed their knitting without damage (Fig. 1A). The swelling of the microfibers in water depended on the GAG charge - Col/Hep fibers (Hep is a strong polyelectrolyte) had an average diameter of 200 µm. In comparison, Col/CS (CS is a weak polyelectrolyte) microfibers exhibited diameters of about 370 µm. These results showed a more compact structure for the Col/Hep complexes than the Col/CS ones and agreed with previous results for these two GAGs[3]. Moreover, we observed different polyelectrolyte distributions: in Col/CS, both components were homogeneously distributed within the fiber, whereas a core/shell structure with a shell made of Col and a Hep core was visible for Col/Hep fibers (Fig. 1B).

Col/Hep microfibers had a tensile strength of about 5 kPa, which is six-fold higher than the strength of Col/CS microfibers (0.8 kPa). Because these IPC microfibers are made of ECM biomolecules, we anticipated a favorable cell adhesion along with the microfiber. Indeed, we observed fast attachment (24h of incubation) and spreading of PC12 neuronal cells onto the microfibers. After 3 days of culture, a monolayer of cells attached along the fiber was visible (Fig. 1C).

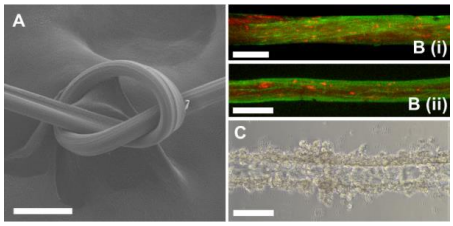

Fig. 1: (A) Knot tied on a Col/GAG microfiber. (B) Distribution of Col (green) and GAG (red; (i) CS, (ii) Hep). (C) PC12 neuronal cells adhered to a Col/GAG microfiber after 3 days. All scale bars are 100 µm.

CONCLUSION

We demonstrated that microfluidics combined with the IPC is a feasible approach for processing microfibers from ECM components, *i.e.* biomaterials that mimic key aspects of native nerve tissue: composition, anisotropy, and mechanical properties, accompanied by a controlled adhesion of neuronal cells. We confirmed the important and differentiated structural role of Hep and CS in these constructs. Further studies will pinpoint the specific biochemical signaling induced by each GAG and its effect on cell behavior.

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ACKNOWLEDGMENTS

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Fabrication of Living Electrodes Using Multiphoton Induced Degradation in a Photoresponsive Hydrogels for Neural Interface Applications

Augusto Loffredo[1*], Lana Van Damme[2], Jasper Van Hoorick[2], Sandra Van Vlierberghe[2], Abhay Pandit[1], Manus Biggs[1]

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INTRODUCTION

Peri-implant 'living interface" approach has recently been used to fabricate neural interface constructs termed living electrodes which consist of a scaffold encapsulating a neural population and which allows biologically mimetic neuromodulation and greatly facilitates electrode-tissue integration, with an aim to eliminate peri-electrode gliosis[1]. Although these strategies to 'living electrode' design have shown efficacy, there is a critical need to advance these interfacing systems to facilitate hierarchical neural integration and in particular, to modulate neural morphological polarization and axonal orientation to ensure bi-directional electrode communication. The objective of this work was to fabricate a living scaffold construct through a novel system of multiphoton 3D printing of a photoresponsive hydrogel. We hypothesize that this system is capable of inducing linear polarized axonal outgrowth from a microelectrode site to a target neural tissue to recapitulate the spatial control of neural tissues.

EXPERIMENTAL METHODS

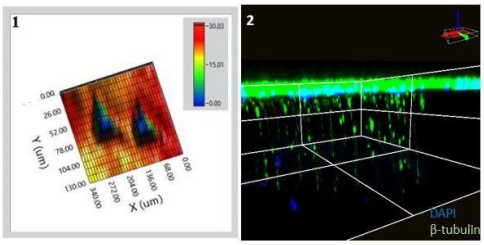
Methacrylamide-functionalised gelatine (GelMA) was synthesised as previously described[2] and a 39% degree of substitution was obtained as confirmed by ^{1}H-NMR spectroscopy. The photoresponsive hydrogel system was prepared by diluting GelMA in PBS at 5% w/v concentration containing 0.1% w/v of LAP as photoinitiator, subsequently exposing the solution to UV-A light. Using a commercially available two-photon lithography system (Nanoscribe© Photonic Professional GT), localised microregions of degradation were formed by exposing the hydrogel to a near infrared laser in presence of a biocompatible two-photon initiator (DAS)[3]. Nanoindentation was used to confirm the intended topography and analyse the mechanical properties of laser-exposed and nearby regions.

To fabricate the living scaffolds constructs, an array of 10 µm diameter micro-tunnels was developed into GelMA hydrogel through free-radical-induced degradation as described above. Primary cortical neural population was isolated from E17 *Sprague dawley* pups and seeded into the morphologically modified hydrogels for evaluation of its ability in guiding and polarising neurons. This capability, as well as cell viability, was checked using immunofluorescent imaging and via LIVE/DEAD assay respectively.

RESULTS AND DISCUSSION

Two-photon excitation of the hydrogel was hypothesised to induce backbone degradation resulting from the formation of a high number of radicals, resulting in the cleavage of amide bonds and generating observable localised scaffold degradation[4]. This phenomenon was confirmed through nanoindentation analysis of surface patterned GelMA hydrogels, resulting in a topographical and mechanical map of two-photon induced gel degradation (Figure 1).

Furthermore, confocal microscopy of cortical neural cultures seeded onto GelMA hydrogels patterned with vertical microchannels of 500 µm indicated that neurites infiltrated the hydrogel matrix exclusively through the degraded micro-tunnels, with neurite processes present in all degraded regions and somata restricted to the hydrogel periphery (Figure 2).



CONCLUSION

By patterning the proposed hydrogel system with an array of parallel micro-channels, we demonstrated that hierarchical tissue engineered constructs with controlled neural polarity and spatial distribution can be fabricated. The approach proposed here can be integrated with a micro-electrode array, producing a "living electrode" construct with an aim to enhancing electrode tissue integration and chronic electrode functionality.

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The mechanical side of the brain: a tissue engineered model to explore the role of mechanical properties alterations in CNS demyelination

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INTRODUCTION

With over 2.5 million people affected worldwide, multiple sclerosis (MS) represents a serious health, economic and social burden with no long-term suitable treatment. Although in the brain oligodendrocyte precursor cells (OPCs) are capable of differentiating into oligodendrocytes (OLs) and producing myelin sheaths in denuded axons, the process of remyelination fails with disease progression leading to irreversible functional failure. Growing evidences suggest that matrix rigidity plays a crucial rule throughout OPC differentiation and OL myelination by unbalancing the intra/extracellular forces[1]. In MS, the astrocytic change of phenotype (astrogliosis) and the consequent extensive extracellular matrix (ECM) remodeling with the formation of a glial scar contribute to a complete alteration of the microenvironment surrounding OLs. We hypothesized that OLs respond to dynamic biomechanical changes altering their differentiation capacity. By tuning mechanosensing mediated pathways one expects to be able to promote remyelination.
Here we propose a 3D tissue-engineered model to study the impact of mechanical properties changes on OL differentiation and functionality. The platform consists of an array of polydimethylsiloxane micropillars designed to act as surrogate axons embed within an alginate (ALG) matrix.

EXPERIMENTAL METHODS

PDMS microstructures were fabricated through replica molding. ALG hydrogels were produced by combining modified ALG formulations containing the cell adhesive peptide RGD (GGGGRGDSP) or the matrix metalloproteinase sensitive peptide PVGLIG (GGYGPVG↓LIGGK).

RESULTS AND DISCUSSION

The micropillar array promotes the differentiation and maturation of OPCs in OLs. When cultured in ALG hydrogels OPCs are viable, and differentiation status is favoured in matrices with high PVGLIG content. The impact of the mechanical properties' changes on OL differentiation was assessed by culturing OPCs in increased ALG content hydrogels (1%, 2% and 3% wt/v). OL differentiation was preferred in softer matrices (shear modulus, G*~100 Pa) in comparison with matrices with G* values around 350 Pa and 1300 Pa. The genetic expression of specific OPC transcription factors and other differentiation markers (Sox10, Nkx2.2., CNP and PLP) was decreased for stiffer matrices. Impaired OL differentiation was also verified in hydrogels with similar stiffness values but with increased stress-relaxation times, which indicates an enhanced cellular behaviour in matrices with augmented capability of dissipating cell-induced forces.
Due to the pivotal role of the glial scar in altering the mechanical properties of the demyelinating tissue, the astrocytic reactive phenotype was also mimicked within 3D matrices. Astrocytes were activated either through biochemical (LPS/IFN-γ) or mechanical (external crosslinking) stimuli. The biochemical stimulus induced an astrogliosis-like phenotype with increased expression of *Lcn2* and *Aqp4* and no significant changes in cellular viability and morphology. Mechanical stimulus was achieved through an external crosslinking of barium, leading to a six-fold increase in the stiffness of the hydrogels. A dramatic alteration in cellular morphology was observed, along with an increased expression of astrogliosis (*Gfap* and *Aqp4*) and mechanosensing (*Piezo1*) related genes.
By combining the culture of OLs and activated astrocytes within the tissue-engineered model we are expecting to understand the crosstalk between these cells and investigate the effects of matrix alterations provoked by dynamic astrogliosis processes on OL differentiation.

CONCLUSION

In this work we have established an *in vitro* platform that can function as a toolbox to dissect (de)myelination processes occurring under differential mechanical conditions. OLs respond to ALG matrices mechanical properties alterations (stiffness and stress-relaxation properties) by changing their differentiation pattern. Additionally, an astrogliosis-like phenotype was achieved in astrocytes cultured in ALG matrices either through a biochemical or a mechanical stimulus.

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ACKNOWLEDGMENTS

The authors acknowledge the funding from projects UTAPEXPL/NTec/0057/2017 (FCT - UT Austin Portugal Program) and GRANT13074566 (Air Force Office of Scientific Research, USA). ED Carvalho Ph.D. fellowship (FCT; SFRH/BD/140363/2018).

>> 15:30 - 16:30 | CS1 - Clinical Session I: Cardiovascular clinical applications

Chairpersons: Laurence Bordenave & Aldo Boccaccini
Location: Room B

15:30 | KL Clinical cardiovascular - Biomaterials in Vascular Surgery: Clinical Issues and (Endo)Vascular perspectives
Caroline CARADU, Bordeaux University Hospital, Bordeaux, France

15:45 | O1 Clinical cardiovascular - Preclinical Evaluation of a Novel Nanofibrous Resorbable Synthetic Vascular Stent up to 12 Months in Rabbits
Anthal SMITS, Eindhoven University of Technology, Eindhoven, The Netherlands

16:00 | O2 Clinical cardiovascular - CHARACTERIZATION OF A CELL-ASSEMBLED EXTRACELLULAR MATRIX (CAM) SUTURE MATERIAL
Paul BORCHIELLINI , Univ. Bordeaux, Inserm, BioTis, UMR1026, Bordeaux, France ; CHU de Bordeaux, Bordeaux, France

16:15 | O3 Clinical cardiovascular - Extent of Calcification in Heart Valve Tissue Engineering; a Systematic Review and Meta-Analysis of Large-Animal Studies of Pulmonary Valve Replacement.
Dewy VAN DER VALK, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands

Biomaterials in Vascular Surgery: Clinical Issues and (Endo)Vascular perspectives

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In light of the advanced state of vascular surgery today, it is hard to realize that the technique of vascular suturing is barely 100 years old. It was the first step toward vascular reconstruction and before World War I, Carrel developed nearly all the technical manoeuvres used in open vascular repairs today. The ideal suturing material is yet to come and should be easy to handle, react minimally in tissues, inhibit bacterial growth, hold securely when knotted, resist shrinking, absorb with minimal reaction after the tissue has healed, adapt to vessel growth, be nonallergenic, noncarcinogenic, and nonferromagnetic.

Venous grafting was the first option for diseased arterial vessel replacement. However, the limited availability of healthy autologous vessels for bypass grafting procedures has led to the fabrication of prosthetic vascular conduits using expanded polytetrafluoroethylene (ePTFE), polyethylene terephthalate (Dacron®) and polyurethane.[1] Nonetheless, only 45% of standard ePTFE grafts are patent as femoropopliteal bypass grafts at 5 years, while autologous vein grafts display a 60–80% patency.[2] In standard ePTFE grafts, neither transanastomotic nor transmural endothelialisation occurs to any significant extent. Knitted Dacron grafts, most commonly used for aortic replacement, incorporate a velour finish, thereby enhancing the anchorage of fibrin and cells to promote tissue integration. They are often crimped longitudinally to increase flexibility, elasticity and kink resistance. However, these properties are lost soon after implantation, as a consequence of tissue ingrowth.

Elements that define an ideal biomaterial necessary to the design of a vascular graft are bio-compatibility, infection resistance, suturability, off-the-shelf availability, tensile strength and viscoelasticity of the blood vessel, a fatigue-resistant tissue with long-term durability with a thromboresistant barrier between blood and the surrounding tissue, controllable vessel tone, platelet activation and leukocyte adhesion. Since then, strategies to create a suitable material for a vascular graft have focused on three areas of research: coatings and surface chemical modifications of synthetic materials, biodegradable scaffolds and biopolymers.

Coatings, chemical and protein modifications on otherwise inert materials have been employed to improve endothelialization, inhibit inflammation, limit graft porosity (gelatine), thrombogenicity (heparin coating, carbon deposition) and improve antiseptic properties (silver and/or triclosan coating). Several investigators have endeavoured to endothelialize the luminal surfaces of synthetic vascular grafts to mimic the biologic responsiveness of the native vasculature.[3] However, the success of cell transplantation was limited because of difficulties in cell sourcing and attachment, retention during pulsatile flow conditions, and more importantly the absence of off-the-shelf availability. Indeed, with the advent of endovascular surgery, an endovascular first strategy has been adopted in a majority of patients with the use of (drug-coated) balloons, stents, endografts, aiming toward biomimetic and increasingly lower-profile devices. Nowadays, bypasses are mainly reserved for patients with critical limb-threatening ischemia who can't bear a delay in care of several weeks to obtain an endothelialized substitute.

Biodegradable polymers have then been used as scaffolds on which layers of cells are grown for the development of a functional vascular graft. The scaffold degrades and is replaced and remodelled by the extracellular matrix secreted by the cells. However, the challenges of cell sourcing are compounded by long culture periods that range between 2 and 6 months, and the proliferative capacity of cells isolated from elderly patients is limited. The mechanical strength of the materials may be comparable to that of native vessels, but compliance mismatch limits long-term patency.

The functional importance of normal physiologic responses of the vascular wall has guided attempts to closely mimic the native arterial wall in the design of a new generation of vascular grafts. Living and autologous blood vessels constructed by rolling sheets of cell-assembled extracellular matrix (CAM) were the first tissue-engineered vascular grafts to be implanted in the human arterial circulation[4] which opened the door to an "off-the-shelf" approach for vascular tissue engineering. The US Combat Casualty Care Research Program (Humacyte) has also worked on an off-the-shelf conduit bioengineered human acellular vessel that is resistant to infection and incorporates well into native tissues.[5]

Indeed, in recent decades, the extensive use of endovascular repairs and the increase in immunocompromised patients at higher risk for infection have made vascular (endo)graft infection an important topic for research[6] and planning of optimal management and biological substitutes are in high demand in tertiary referral centres such as Bordeaux University Hospital.

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CHARACTERIZATION OF A CELL-ASSEMBLED EXTRACELLULAR MATRIX (CAM) SUTURE MATERIAL

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INTRODUCTION

Suture materials are still the gold standard for most surgical closing and repair processes, such as attaching prostheses or approximating tissues. The successful outcome of the suture relies on the behavior of its composing material within a biological environment. This depends on the intrinsic properties of the starting material[1]. In addition, the ideal suture material must provide superior mechanical properties and favorable handling characteristics. It should be accepted by the patient without rejection or chronic inflammation, resist infection, and be integrated and remodeled with the patient's tissue. Since CATGUT suture material use has been forbidden in France and many European countries, there is no biological suture material available in the market for human surgical procedures.

Our research group has developed biological material synthesized by fibroblasts in the laboratory and called Cell-Assembled extracellular Matrix (CAM)[2]. This CAM was successfully implanted in Humans[3]. This study aims to develop an innovative, non-chemically denatured, non-living, and completely biological suture material using CAM for cardiovascular surgical applications.

EXPERIMENTAL METHODS

For ovine and human sheets production, dermal fibroblasts were seeded in 225 cm^2 flasks and cultured for 16 weeks without antibiotics. Eight-week-old CAM sheets were produced in parallel and used to evaluate the histological and mechanical changes of long-term culture. For the thread fabrication, CAM sheets were cut using surgical scissors following a joined double spiral pattern to create 2-, 3- and 5-mm-wide CAM ribbons. It is necessary to obtain a comprehensive understanding of the *in vitro* properties of this CAM-based thread (e.g., mechanical features, histological properties, etc.) to establish the best design for our thread and needle. So, tensile tests were performed and tensile breaking strength, elongation, modulus of elasticity, coefficient of friction, and knot slippage and security were evaluated. Results were compared to the US and European pharmacopeia standards for non-synthetic suture materials (CATGUT).

Then, attention was focused on the surgical needle selection and the damage caused through the tissue by the needle. Macroscopic and scanning electron microscopy evaluations of the defect generated through a CAM tissue by the passage of reverse cutting, taper cutting, and taper point needles were analyzed. Next, a CAM-based thread was mounted on each needle using a pneumatic attaching machine. Attachment strength between the needles and the threads was measured using a uniaxial tensile test. The ideal needle was determined by the best ratio between the size of the damage generated through the tissue and the failure force at the needle/thread attachment and by the most suitable needle shape for use in vascular surgery.

Finally, we realized a proof-of-concept for using our suture material in conditions mimicking a surgical carotid anastomosis on sheep (n=3). We chose the best design of our 16-week-old ovine CAM-based thread for this short-term evaluation to validate our suture material: blood leaks, histological evaluation, etc.

RESULTS AND DISCUSSION

Most of the mechanical properties of 16-weeks-old CAM-based threads, as expected, were superior to the ones of 8-weeks-old CAM-based threads but inferior to the US and European standards for natural suture material (CATGUT) as enacted in the respective Pharmacopeias. The ideal model of CAM-based suture material with suitable mechanical properties was a three-mm-wide ribbon crimped in a 17-millimeter-long and ½ curved taper needles. In addition, carotid anastomosis using our innovative suture material was successfully realized without any complication. This suture material was easily manipulable, and no blood leakage was observed.

CONCLUSION

We demonstrated that it is possible to design an innovative and completely biological CAM-based suture material with the appropriate mechanical strengths for many cardiovascular surgical applications.

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ACKNOWLEDGMENTS

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Preclinical Evaluation of a Novel Nanofibrous Resorbable Synthetic Vascular Stent up to 12 Months in Rabbits

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INTRODUCTION

Approximately 5 million patients in the US and Europe suffer from critical limb ischemia (CLI), which is characterized by hampered blood flow to the lower legs due to blockage of the arteries. CLI can be treated using medical stent devices, but the current stents evoke an adverse response in the body, causing 70% of these implants to reocclude within 2 years. To overcome this, we developed a regenerative stent that stimulates functional tissue restoration directly *in situ*. In contrast to other (resorbable) stents that consist of relatively large struts, the regenerative stent is composed of electrospun resorbable synthetic nanofibers that allow opening of an occluded artery, while promoting tissue regeneration via a favorable inflammation-driven response[1]. We recently demonstrated the proof-of-concept for this stenting technology when implanted in the abdominal aorta in rats[2]. In that study we observed the rapid colonization of the fibrous stenting material with endogenous cells, including an influx of α-SMA+ smooth muscle-like cells and a mild and transient inflammatory response, characterized by the influx of macrophages with a predominant M2 phenotype. Endogenous neotissue was formed with a remarkable *de novo* formation of mature elastic fibers[2].

Building on these encouraging findings, the goal of the current study was to evaluate the long-term functionality and regenerative capacity of our stent in a rabbit model with up to 12 months follow-up.

EXPERIMENTAL METHODS

The animal experiments were conducted conform the guidelines for the use of laboratory animals, as formulated by Dutch law on animal experimentation. Nanofibrous stents were fabricated using electrospinning as previously reported[2]. The stents were implanted in the rabbit iliac artery (total n=9 rabbits) using transcatheter positioning (5 french introducer sheath), followed by balloon expansion in the artery (2.0 mm stent diameter). Bare metal stents were implanted in the opposing leg as golden standard controls. Animals were followed up until 12 months of implantation with angiography performed at 0, 3, 6, 9, and 12 months to assess stent patency. In addition, Optical Coherence Tomography (OCT) and ultrasound were performed at 3, 6 and 12 months to characterize stent/tissue composition and compliance, respectively. Animals were sacrificed at 3, 6, and 12 months (n=3 per time point) in order to analyze stent resorption, cellularization and tissue formation using immunohistochemistry.

RESULTS AND DISCUSSION

Our analysis so far has revealed that the stents remained patent throughout the 12-month follow-up, with a stable inner diameter from 3 months on. No signs of adverse inflammation were observed throughout the follow-up time. At 3 and 6 months, abundant cell influx into the porous stent fibrillar structure was observed, as well as a thin luminal neotissue layer covered with endothelial cells. Scaffold resorption appeared to be minimal at those time points. However, at 12 months, scaffold resorption was largely complete as qualitatively evaluated from histological analysis, leading to flexible arterial tissue. The synthetic scaffold material was replaced by collagenous tissue, which displayed progressive maturation with follow-up time from a more collagen 3- to a more collagen 1-dominated tissue. Importantly, regeneration of endogenous elastic fibers was observed. More in-depth analyses are currently ongoing to characterize stent compliance, tissue composition and phenotypes of infiltrating cells in more detail.

CONCLUSION

The regenerative stenting technology offers a promising new endovascular treatment to regenerate diseased arteries through *in situ* tissue engineering, while preventing the need for invasive replacement surgery. The stent has the potential to treat both shorter and longer lesions, such as needed for below-the-knee indications. The resorbing character of the stent avoids a chronic foreign body response, as well as late-term thrombosis. Moreover, the rather unique capacity to restore the arterial endoluminal elastin is thought to be a key feature to prevent progressive restenosis. Future work is aimed at evaluating the regenerative capacity of the stent in clinically relevant large-animal models.

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ACKNOWLEDGMENTS

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Extent of Calcification in Heart Valve Tissue Engineering; a Systematic Review and Meta-Analysis of Large-Animal Studies of Pulmonary Valve Replacement.

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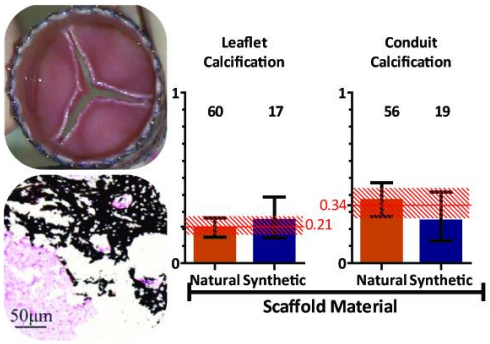
INTRODUCTION

Tissue-engineered heart valves are emerging alternatives to current valve prostheses, prospecting life-long replacement options that can adapt to a changing hemodynamic environment. Calcification, a pathological complication for biological valve protheses, has been reported in preclinical tissue-engineered valve studies.[1-3] However, a systematic analysis of its occurrence is missing. Here we aim to i) systematically review reported calcification of pulmonary tissue-engineered valves in large-animal studies, and ii) analyze the influence of tissue engineering methodology (*i.e.*, choice of scaffold material, cell pre-seeding) and animal model (*e.g.*, animal species and age) on calcification.

METHODS AND RESULTS

Overall, 80 studies were included in baseline analysis, and 41 studies containing 108 experimental groups were included in meta-analysis. The remaining studies could not be analyzed due to their non-existent or limited reporting on calcification. Overall calcification event rate was 35% (95% CI [28-43%]). Calcification was more prominent (p=0.023) in the arterial conduit region (34% [26-43%]) compared to the valve leaflets (21% [17-27%]) (Figure 1). Meta-analysis showed no significant differences in degree of calcification between tissue engineering strategy nor between animal models. Importantly, much variability between individual studies was observed in the degree of calcification as well as the quality of analysis and reporting thereof, hampering adequate comparisons between studies.



CONCLUSIONS AND DISCUSSION

These findings underline the need for improved analysis and reporting of calcification in tissue-engineered valves. It also necessitates control-based research to further enlighten the risk of calcification for tissue-engineered transplants in comparison to current options. Together, this can bring the field of tissue-engineered valves forward towards safe clinical use.

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>> 15:30 - 16:30 | Trans S1 - Translational session I
Chairpersons: Yves Bayon & Maria Pereira
Location: Room E

15:30 | KL Translational I - From Quantum Chemistry to Drug Eluting Stents: a Historical Narrative of How The Electro-Grafting of Vinylic Polymers Ended Up in Patients
Christophe BUREAU, AlchiMedics S.A.S., Paris, France

16:00 | O1 Translational I - Novel Fermentation based functional bio-material advances - GMP compliant non animal derived Collagen and bacterial Cellulose
Tim SMONIK, Evonik, Essen, Germany

16:15 | O2 Translational I - Development of new tissue-engineered vascular grafts: implantation, conservation and sterilization.
Diane POTART, BioTis INSERM U1026, Université de Bordeaux, Bordeaux, France

From Quantum Chemistry to Drug Eluting Stents: a Historical Narrative of How The Electro-Grafting of Vinylic Polymers Ended Up in Patients

Christophe Bureau, Ph.D[1], Jianhua Sun, Ph.D[2]

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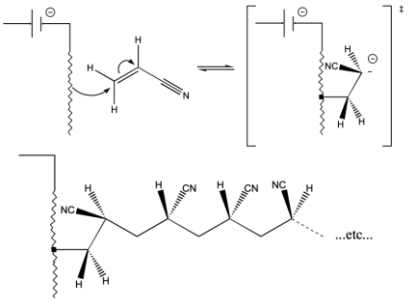
INTRODUCTION

The electro-grafting of vinylic polymers is an electrochemical coating technology thanks to which polymer brushes can be grown on conductive or semi-conductive surfaces. Initially invented at the CEA (Center for Atomic Energy) in the 80's, it remained a topic of fundamental research at the crossroads of quantum chemistry, theoretical electrochemistry and surface analysis until it was spun-off in 2001 to create the startup AlchiMedics. As of 2022, more than 1 million patients are implanted with cardiovascular and neurovascular devices embarking AlchiMedics' electro-grafted layers, showing proven clinical benefits, in particular regarding healing after implantation. This paper is a collection of some of the key scientific milestones that made these products reach their target, arranged along a historical narrative of the joint partnership between AlchiMedics and Sinomed. It is also a testimony, the story of the ups and down of creating a startup from fundamental research and leading it to a finish line in the real world.

EXPERIMENTAL METHODS

Electro-grafting is a coating technology in which a conductive surface is cathodically polarized in an organic bath containing vinylic monomers[1]:

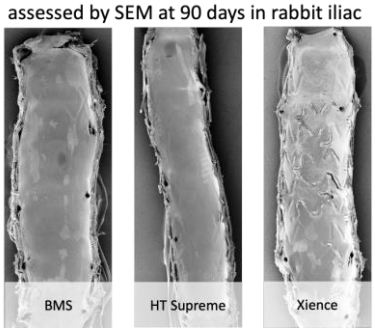


The reaction leads to ca. 200 nm polymer brushes covalently bonded to the electrode surface. These films are used as anchoring "Velcro" layers to strongly bind 5-7 um layers of PLGA containing Sirolimus. The PLGA structure is adjusted to secure a 30 day release in vivo, which is shorter than most leading Drug Eluting Stents (DES) on the market[2].

RESULTS AND DISCUSSION

Comparison with benchmark DES (Cypher™, Xience™ ...) shows that the *e*G™ /PLGA coated stent has superior healing capabilities, promoting full coverage by active endothelial cells at 2 months in a rabbit iliac artery model. Studies in transparent artificial arteries further demonstrate that the migration speed of endothelial cells is faster on electro-grafted layers compared to bare 316L surfaces, suggesting a possible booster role of the electro-grafted layer in the early healing observed[3].

Percent endothelial coverage,
assessed by SEM at 90 days in rabbit iliac



The BuMA™, HT Supreme™ and NOVA™ DES, developed and marketed by Sinomed on the basis of this technology, are now implanted in more than 1 million patients worldwide, with superior clinical outcomes and enhanced patient safety, including among complex lesions that have a higher rate of adverse events[4].

CONCLUSION

The BuMA™, HT Supreme™ and NOVA™ stents from Sinomed are the result of a strong collaboration between a French startup, created on the basis of fundamental research from the CEA, and a leading Chinese medical device company now listed on the STAR market at Shanghai stock exchange. It delivered a series of cardio- and neuro-vascular DES targeted at superior healing post-implantation.

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The authors wish to thank the CEA, and the private equity and investor community, without which none of this would have been possible.

Novel Fermentation based functional bio-material advances - GMP compliant non animal derived Collagen and bacterial Cellulose.

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Building on alliances and internal asset set-up Evonik addresses unmet needs in innovative materials ready to be used in next generation implants and in line with future regulatory requirements.

Cell culture assets using complex physiological and tissue models inhouse allow for convincing application PoC in many matrix polymer systems.

Evonik recently acquired JeNaCell, a specialist in the development and manufacture of biosynthetic cellulose with a well-defined design and a structure that can be controlled. This cellulose has already generated innovative and pioneering product solutions in the field of medicine, cosmetics, technology, and pharmaceutics. It can be formulated in a variety of forms and matrixes with applications as biomaterials, for example to strengthen wound healing.

The innovation of a non-denaturated triple helical endogeneous Collagenoid system with unprecedented immunological safety and solubility profiles, which can be modified via different chemical and biochemical methods, is now available at commercial scale to replace animal derived collagen in medical and pharma applications.

Different key biological parameters will be presented, animal models included.

First customer application results using our novel collagen system will be presented.

Development of new tissue-engineered vascular grafts: implantation, conservation and sterilization.

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INTRODUCTION

To meet the clinical need for small diameter vascular graft, we developed a new generation of human tissue-engineered vascular graft (TEVG) entirely made of biological material. Vessels were produced by weaving yarn of Cell-Assembled extracellular Matrix (CAM). Study objectives were: 1) to assess the graft behavior in the arterial circulation of immunosuppressed rats to evaluate its in vivo functionality, 2) to identify non-denaturing conservation and sterilization methods in order to simplify the manufacturing process.

EXPERIMENTAL METHODS

CAM sheets were produced in vitro by human skin fibroblasts after 8 weeks of culture in DMEM/F-12 with 20% FBS and 0.5 mM Na L-ascorbate and devitalized before use (frozen/airdried). Ribbons (35/TEVGs, 2-mm-wide) were assembled using a custom-made circular loom into 8-mm-long TEVGs with an internal diameter of 1.6 mm. Woven grafts were terminally sterilized with Ethylene Oxide (EtO) and implanted into the abdominal aorta of immunosuppressed rats. TEVGs were explanted after up to 12 months, and echo doppler was performed before sacrifice. Hematoxylin & Eosin and Masson Trichrome (MT) & Verhoeff stanning were performed, as well as smooth muscles and endothelial cells immunostaining.

CAM ribbons (5-mm-wide) were conserved in different conditions (-80°C, -20°C, 4°C dry or wet, Room Temperature (RT) dry or wet), and mechanically tested after rehydration after 1 year with a tensile test. CAM ribbons (5-mm-wide) were sterilized using different methods: gamma irradiation at high and low dose rate on dry and wet samples, EtO and supercritical CO2 (scCO2), and compared to control (sterile production). They were subcutaneously implanted in immunodeficient rat for up to 10 months and mechanically tested. HE, MT and Alcian Blue stanning were performed, as well as immunostaining (M1/M2).

RESULTS AND DISCUSSION

Twenty rats were successfully implanted, demonstrating graft implantability and the absence of transmural leakage. After 12 months, US images revealed flow through the graft, and weaving pattern conservation. Preliminary results showed formation of a complete neo-media at 3 months. No or very little cell infiltration in the graft was observed.

After 1 year, only samples conserved dry at 4°C and RT showed a decrease in rehydrated cross section area and an increase in maximum force, reminiscent of the behavior of dry CAM. We believe that those samples had diminished ability to rehydrate, which could be problematic for clinical use. For other groups, no mechanical parameters changed after 1 year. Before implantation, maximum force only decreased for the gamma wet group. In this condition, delamination of ribbons was observed with MT. After implantation, the control group showed a steady max force decrease with time, while the other groups displayed a sharp decrease after 2 months, and then no change. After 10 months, no significant difference in maximum force was observed between groups. Histological analysis is ongoing.

CONCLUSION

In this study, we showed that our woven TEVG can function for up to a year in the arterial circulation of rats. We also demonstrated the long-term conservation of our CAM in conditions compatible with hospital storage. Finally, multiple methods remain promising options for graft sterilization.

ACKNOWLEDGMENTS

The authors would like to thank the European Research Council (Grant no: 785908) for providing financial support to this project.

>> 17:15 - 18:15 | YSF & Young Researchers BIOMAT – Workshop II
Chairpersons: YSF and Young Researchers BIOMAT
Location: Room C

17:15 | How to keep young researchers in a good mental shape?
An BOGAERTS, HSE Department, KU Leuven, Leuven, Belgium

17:45 | Navigating The Early Academic Career, With a Smile
Vianney DELPLACE, Nantes Université, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, Nantes, France

How to keep young researchers in a good mental shape?

Dr. An Bogaerts
HSE Department, KU Leuven, Leuven, Belgium
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This workshop will focus on the project 'Mental Health Matters' of the KU Leuven University (Belgium). Via the project, young researchers are inspired to discover new habits in the context of staying healthy and in a good mental shape during their career. Recently, a light-hearted and invigorating podcast was produced for and by KU Leuven researchers. Two hosts talk with other PhD students and Postdocs about their mental well-being. Through the different episodes, different questions are tackled, for example: How do you stay confident and resilient in a highly competitive world? How do you deal with stress, isolation or anxiety? How do you create a good work/life balance? And how do you stay motivated during your research process? Colleagues and professors share their experiences and advice. In addition to the podcast series, new initiatives are planned in the next months.
In this workshop we will explain the different actions within the project of 'Mental Health Matters' and focus on how we tailored those initiatives to our target groups.

Navigating The Early Academic Career, With a Smile

Vianney Delplace

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"The most beautiful experience we can have" is the description that Einstein gave of the fundamental emotion of true science. Yet, in the competitive world of biomedical research, this experience is often counterbalanced by frustration, fear and uncertainty. This is especially true in the early years of an academic career, when one can easily lose track of the purpose and meaning of one's work, and forget about the joyful moments in the everyday life of a researcher.

Why doing research, then? Why persevering? How to make it through? In this semi-formal motivational talk addressed to the community of students and young investigators, we will seek to look on the bright side of our work, and rediscover what is so great about research. Sharing thoughts and reflections on the first ten years of a young career, we will try to identify the pitfalls to avoid on the way to obtaining a PhD or becoming a permanent researcher, but also highlight the many unique opportunities. The discussion will revolve around the notions of success, expectations, goals and paths, perspectives, passion, creativity, life/work balance, and more, through personal life experience, inspiring quotes, open questions, and direct interactions with the audience. Yes, research is awesome! Come and see why…

>> 17:15 - 18:15 | PSOP-11 - Cardiovascular tissue engineering
Chairpersons: Nicolas L'Heureux & Teresa Simon-Yarza
Location: Room F

17:15 | O1 Cardiovascular - 3D in situ ECM deposition: a new class of biohybrid material for cardiac repair
Albane CARRÉ, Université Paris Cité, INSERM U1148, Paris, France

17:30 | O2 Cardiovascular - Poly(alkylene terephthalate)s for Cardiovascular Applications: Effect of Molar Mass and Alkyl Chain Length on Surface Properties and Biocompatibility
Lenny VAN DAELE, Polymer Chemistry and Biomaterials Group (PBM), Centre of Macromolecular Chemistry (CMaC), Department of Organic and Macromolecular Chemistry, Ghent University, Ghent, Belgium

17:45 | O3 Cardiovascular - High-throughput Screening to Mimic Tunica Media SMCs Alignment in Porous Blood Vessel Scaffolds
Klaudia JURCZAK, University of Groningen, Groningen, The Netherlands

18:00 | O4 Cardiovascular - First Implantation of an Allogeneic Woven Tissue-Engineered Blood Vessel Made of Cell-Assembled Extracellular Matrix
Fabien KAWECKI, Univ. Bordeaux, Inserm, BioTis, UMR1026, Bordeaux, France

3D *in situ* ECM deposition: a new class of biohybrid material for cardiac repair

Albane Carré[1*], Emmanuelle Poque[2], Christelle Harscoat-Schiavo[2], Pascale Subra-Paternault[2], Teresa Simon-Yarza[1*]
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[2] Université de Bordeaux, Institut CBMN UMR 5248, Pessac, France
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INTRODUCTION

Ischemic heart diseases are one of the main global health issues that led to 9 million deaths in the last 30 years and are estimated to affect 1 845 individuals per 100 000 the global population by the next decade[1]. In the event of survival, the patient's heart remains weakened due to a thinning of the myocardial wall[2].

Our goal is to develop a biohybrid material closely mimicking the native cardiac tissue to reinforce the myocardial wall, providing a healthy environment to the cardiac cells to favor tissue repair. This material, obtained by *in situ* ECM deposition, combines the tuned physical-mechanical properties of a polysaccharides-based 3D hydrogel (HG) and the specific biological composition of a cell secreted cardiac extracellular matrix (ECM) coating.

EXPERIMENTAL METHODS

Hydrogel fabrication The 3D porous HG was obtained as previously described[3]. Briefly, Dextran and Pullulan were chemically cross-linked with sodium trimetaphosphate. The HG underwent a double freeze-drying process; the first one to tune the porosity, the second one after incubation in a gelatin solution (1 mg/mL) to create a thin physical coating of the pores.

Hydrogel characterization Porosity, swelling ratios, water content and enzymatic degradation rate were evaluated as per standard protocols[4]. Young Modulus has been evaluated both in bulk and through a nanoindentation mapping and verified to match the native cardiac tissue.

Hydrogel cellularization and in situ ECM deposition Two types of fibroblasts have been used: immortalized fibroblasts cell line (3T3 Balb/c A31), used to optimize cell culture parameters, and primary fibroblasts (Rat Primary Cardiac fibroblasts; RPCf). 3T3 fibroblasts were vacuum seeded at high concentration in the HG to maximize the cellularization homogeneity. Cell morphology and distribution have been observed using confocal microscopy and cellular metabolic activity has been evaluated up to 21 days. The ECM coating, naturally secreted by the fibroblasts, has been visualized with Masson's trichrome coloration at different time points. RPCf were seeded at high concentration and cultured following the same parameters as the 3T3 cells.

Biohybrid decellularization Once the cells secreted a sufficient quantity of ECM, after 10 to 21 days of culture, two decellularization methods were evaluated. The golden standard decellularization method, using sodium dodecyl sulfate was compared to an innovative supercritical-CO_2 optimized method to obtain a final cell-free biohybrid material with conserved macro architecture. Elimination of cells was observed with confocal microscopy and ECM coating architecture conservation was evaluated through Masson's trichrome coloration. DNA quantification was performed using a Nanodrop device, total protein quantification was evaluated with a BCA assay and specific proteins (collagen type I, laminin and fibronectin) were used for immunostaining and observation with confocal microscopy.

RESULTS AND DISCUSSION

Consistent with previous studies[3,4], our polysaccharides-based HG presented a porosity of approximately 40%, a swelling ratio of 11 (after 48h of hydration), a final water content of ca. 98% and an enzymatic degradation rate of 1.9 mg/min.

Preliminary bulk rheology evaluation resulted in a Shear Storage modulus of ca. 3kPa and surface rheology Young modulus ranging from 20 to 25kPa, in accordance with native cardiac tissue data found in literature[5]. Optimization of the 3T3 fibroblasts culture parameters pointed out a maximal cellularization homogeneity at a seeding density of 20 x 10^6 cells/mL. 21 days cell culture allowed a maximal coverage of the porous scaffold with a stable metabolic activity (see Figure below). Histological colorations testified of a protein layer covering the HG porosities. Current experiments are now being conducted with RPCf to obtain a native specific ECM coating of the HG. Sc-CO2 optimized decellularization method has been proven to disturb the cell distribution with only few nuclei remaining, that can easily be eliminated with a DNAse treatment.

To conclude, our results testify of the suitability of *in situ* cardiac ECM deposition on polysaccharide scaffolds to prepare a biohybrid materials.

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ACKNOWLEDGMENTS

The authors would like to thank the French Research Agency ANR (Grant no: ANR-20-CE18-001 EXCALYBUR) for providing financial support to this project and Université de Paris (Idex Emergences POLCA).

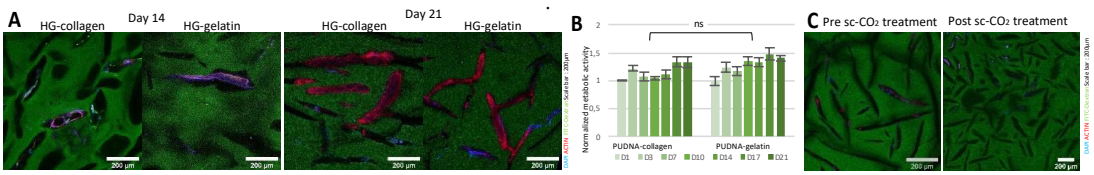

Figure: **A** 3T3 Balb/c A31 cell cluster and conformation after 14 and 21 days of culture within gelatin-coated hydrogel and collagen-coated hydrogel (used as control). **B** Analysis of 3T3 Balb/c A31 cells metabolic activity over 21 days of culture. No significant differences were observed (Kruskal-Wallis and Dunn's multiple comparisons test). **C** Confocal image of cells distribution and morphology after 7 days of culture prior to sc-CO2 decellularization protocol and after sc-CO2 decellularization. For all confocal images, HG are observable due to 1% Dextran FITC in the formulation. Cells are stained with DAPI and Phalloidin-TRITC. Scale bar 200µm.

Poly(alkylene terephthalate)s for Cardiovascular Applications: Effect of Molar Mass and Alkyl Chain Length on Surface Properties and Biocompatibility

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INTRODUCTION

Cardiovascular diseases are the leading cause of death worldwide, causing over 30% of all fatalities. Many cardiovascular diseases involve atherosclerosis and the subsequent occlusion of arteries. Treatments for this include balloon angioplasty (with or without stenting) and bypass grafting surgery.[1]

Poly(ethylene terephthalate) (PET) is a frequently used polymer in cardiovascular applications, especially as a vascular graft material. However, its high stiffness leads to compliance mismatch with the human blood vessels, resulting in altered haemodynamics, thrombus formation and eventually graft failure.[2] Poly(alkylene terephthalate)s (PATs) are very similar to PET, but they have a longer alkyl chain length, which decreases their stiffness. Nonetheless, other interesting properties of PET are still maintained (e.g. inert, stable and non-inflammatory), which renders them perfect candidates as new materials serving cardiovascular applications.[3]

In this work, we evaluated a homologous series of PATs with varying molar masses for their surface properties and biocompatibility. Furthermore, we investigated endothelial cell adhesion and proliferation, since endothelialisation is crucial in the context of cardiovascular applications such as vascular grafts and stents.

EXPERIMENTAL METHODS

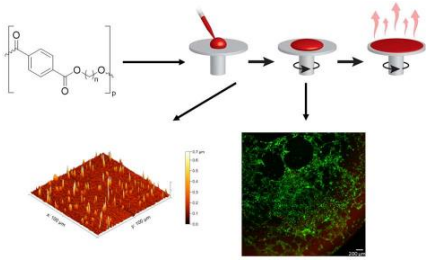
PATs with an alkyl chain length varying from 8 to 12 and $PAT_{(n=9)}$ with molar masses in the range of 28-206 kg/mol were synthesized via a catalyst-free single-step solution polycondensation reaction. Spincoated samples were subjected to atomic force microscopy (AFM) and static contact angle (SCA) determination to assess the surface roughness and wettability, respectively. Cytotoxicity was evaluated via both direct and indirect cell tests using human umbilical vein endothelial cells (HUVECs). Finally, endothelial cell adhesion and proliferation were evaluated via an MTS assay and live/dead staining. Cell tests were performed in six-fold and one-way ANOVA was used to determine statistically significant differences ($p < 0.05$).

RESULTS AND DISCUSSION

In general, an increase in molar mass led to an increase in root-mean-square surface roughness for spincoated samples of $PAT_{(n=9)}$ (from 14.8 to 63.3 nm). On the other hand, an increased number of methylene units resulted in a decreased surface roughness (from 191.5 to 41.5 nm), including an odd-even trend where the PATs with an odd number of methylene units gave rise to a systematically lower roughness ($p < 0.05$) compared to PATs with an even number of methylene units. Furthermore, the root-mean-square roughness never exceeded 200 nm. The SCA varied between 79° and 94° and although subtle differences could be observed in surface wettability, no clear trends could be established when varying the above-mentioned parameters.

Cytotoxicity tests performed on $PAT_{(n=9)}$ revealed that the relative viability exceeded 70%, indicating no major cytotoxic effects.

MTS and live/dead assays demonstrated that HUVECs adhered to the PAT substrates. After 7 and 14 days, cell proliferation on the developed materials was generally superior compared to PET and tissue culture plastic (TCP) and a confluent endothelial cell layer was established. Finally, microscopy imaging demonstrated that on some PAT samples, a certain amount of HUVECs showed an altered cellular morphology, indicating tubular organization (see Figure below), while this was not the case for PET nor TCP.



CONCLUSION

Based on this research, we can conclude that the synthesized PATs are non-cytotoxic and support endothelial cell adhesion and proliferation. Since they also exhibit a lower stiffness, they could be a very interesting alternative for PET serving cardiovascular applications.

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The authors would like to thank the Fund for Cardiac Surgery and the Research Foundation Flanders (FWO) (grant number 1SA2720N) for funding this research.

High-throughput Screening to Mimic Tunica Media Smooth Muscle Cells Alignment in Porous Blood Vessel Scaffolds

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Key words: *blood vessel engineering, high throughput screening platform, porous* poly(trimethylene carbonate)

Introduction:

Although the replacement of live animal models in biomedical research and reduction of animal distress led to the rapid development of blood vessel scaffolds, the ideal vascular model still remains a challenge and prevents the standardized studies in cardiovascular diseases (CVD) research. The engineered artery should resemble the 3D geometry of native blood vessel and reconstitute its three layers including tunica intima, tunica media, and tunica adventitia (1). The medial layer consists of circumferentially oriented smooth muscle cells (SMCs), which are responsible for the contractile function of a blood vessel. We hypothesized that the accurate representation of the cellular orientation of SMCs is paramount to the success of a functional blood vessel scaffold. In this study, we translate optimal topography as determined via high-throughput screening (HTS) to the fabrication of a vascular scaffolds from biodegradable and biocompatible polymer - poly(trimethylene carbonate) (PTMC) (3). Our research focuses on directing smooth muscle orientation hence, we are investigating the biophysical factors such as anisotropic topography using in house high throughput screening platform (HTS). The technology enables to identify optimum material parameters in a single cell experiment at the same time evading adverse effects of cell-material interactions (4).

Methods:

The PTMC films were prepared by the means of freeze extraction and exhibited interconnected porous structures and gel content around 80%. The film morphology was investigated using scanning electron microscopy (SEM). To promote continuously varying surface parameter, PTMC was directly imprinted from stretched and plasma oxidized PDMS molds as described previously (5). The SMCs derived from porcine origin were further used in alignment and proliferation screening on PTMC imprinted topographical gradients and porous PTMC films.

Results and discussion:

The SEM data showed that pore size diameter is in range of 0.5 m to 140 m on the air side of the PTMC film and from 0.7 m to 181 m on the bottom side. A mean pore diameter was 11.3 m and 22.3 m on the air and bottom side of the PTMC film, respectively. The PTMC anisotropic topography gradients were characterized via atomic force microscopy (AFM). Measurements of created wrinkles were taken along the surface gradient. AFM data revealed that the wavelength (W) and amplitude (A) of the wrinkles increased from the least exposed side of the prism mask – W: 1.2 m, A: 155nm; to the most exposed side of the prism mask – W: 5.6 m, A: 1264 nm.

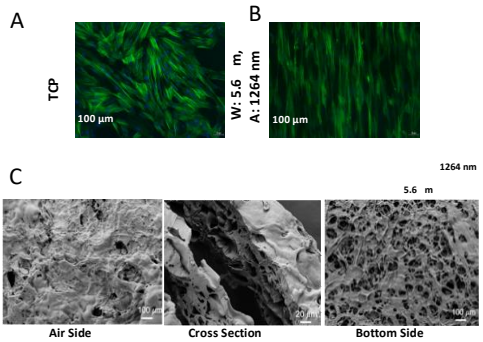

Figure 1 *SMCs expression of smooth muscle actin after 3 days of culture on a tissue culture plate (**A**) and a wrinkle gradient (**B**) (smooth muscle actin expression is shown in green; cell nuclei is visible in blue (DAPI). SEM images of porous PTMC prepared by freeze extraction method (C).*

Conclusion:

We performed a topography screening on SMCs to determine the optimum topography for alignment which were successfully transferred to PTMC via imprinting technology. In parallel we identified the optimum method for creating the porosity in PTMC films pivotal for the flow of nutrients in a blood vessel model. In the next steps, the combination of the surface topography and porous structures will be incorporated to the PTMC films. Development of such tissue engineered vascular scaffolds can provide a real benefit in pre-clinical testing of CVD.

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First Implantation of an Allogeneic Woven Tissue-Engineered Blood Vessel Made of Cell-Assembled Extracellular Matrix

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INTRODUCTION

Small diameter artery obstructions cause heart attacks, leg amputations, and strokes [1]. Current commercial vascular grafts are made from synthetic materials that are stiff, not remodeled by cells, recognized as foreign bodies, marked by chronic inflammation, and prone to infections [2].

Our research group has developed a biological material synthesized in the laboratory by human or ovine dermal fibroblasts [3,4] and so called Cell-Assembled extracellular Matrix (CAM). Recent data have shown that CAM produced by allogeneic fibroblasts (from a donor) can be devitalized (dehydrated) without damaging its structure [3] and does not elicit an innate immune response [5]. This opens the door to an allogeneic production strategy of CAM sheets and threads that have the advantage of being available on demand and more economically feasible. This study aims to demonstrate for the first time the short-term performance of an allogeneic woven tissue-engineered blood vessel (TEBV) made of CAM in a large animal model.

EXPERIMENTAL METHODS

Since we aim to graft our TEBV in an allogeneic context, ovine CAM sheets matured for eight weeks were produced *in vitro* using ovine fibroblasts. Five-mm-wide ribbons of CAM were obtained after cutting CAM sheets using a custom-made machine with circular blades. Circumferential CAM threads were produced by twisting two ribbons using a calibrated rotating motor. Woven TEBVs (n=10, 5-cm length, inner diameter = 4.1 ± 0.3 mm) were produced using a custom-made circular loom. For each TEBV, one circumferential CAM thread was inserted repeatedly between a mobile and a fixed set of 51 tensioned longitudinal ribbons of CAM, creating a plain weave pattern. Woven TEBVs (n=4) were tested for suture retention, burst pressure, and transmural permeability. Woven TEBVs (n=6) destinated for implantation were sterilized using ethylene oxide gas.

TEBVs were grafted in anesthetized sheep as a 5-cm-long interpositional carotid graft (**Fig. 1**) as part of a protocol approved by an ethics committee in accordance with national animal care guidelines. Doppler echography follow-up was realized immediately after implantation, as well as one- and four-weeks post-implantation to show graft patency.

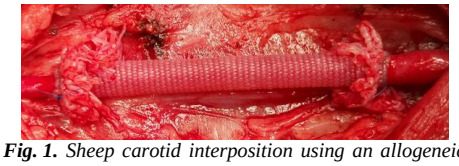

Fig. 1. *Sheep carotid interposition using an allogeneic woven TEBV made of CAM.*

RESULTS AND DISCUSSION

In vitro characterization of the woven TEBV revealed supraphysiological mechanical properties suitable for implantation with an average suture retention of 890 ± 140 gf and a burst pressure of 4260 ± 1160 mmHg (native ovine carotid = 2610 ± 860 mmHg). In addition, woven TEBV showed a low transmural water permeability of 27 ± 16 $mL.min^{-1}.cm^{-2}$, which is below the threshold of 50 $mL.min^{-1}.cm^{-2}$ often recommended for clinical use.

Surgical interpositional grafting of the woven TEBV was successfully realized without any complication during the procedures. No blood leakage was observed at the anastomosis or through the wall after restoring the flow, indicating that the TEBV can be easily sutured to the native vessel using standard techniques and instruments. In addition, excellent patency with no signs of thrombosis, stenosis, or chronic inflammatory reaction, was observed by echography for up to four weeks.

CONCLUSION

This study demonstrates the short-term performance of an allogeneic TEBV, made exclusively of CAM and produced using a textile-inspired approach, as an interpositional vessel graft.

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ACKNOWLEDGMENTS

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>> 17:15 - 18:15 | PSOP-12 - Hydrogel I
Chairpersons: Joao Mano & Sylvia Fare
Location: Room A

17:15 | O1 Hydrogels I - A Universal Nanogel-based Coating Approach For Medical Implants
Devlina GHOSH, University of Groningen, University Medical Center Groningen, Department of Biomedical Engineering, Groningen, The Netherlands

17:30 | O2 Hydrogels I - Cell-instructive 3D Printed Multilayered Biomaterials embedded in Photocrosslinkable Hydrogels for Vascular Tissue Engineering
João BORGES, Department of Chemistry, CICECO – Aveiro Institute of Materials, University of Aveiro, 3810-193 Aveiro, Portugal

17:45 | O3 Hydrogels I - Ex vivo and in vivo evaluation of injectable and porous hydrogels as support for muscle repair
Cloé PARET, PGNM-INMG, CNRS UMR 5310 - INSERM U1217, Claude Bernard university, Lyon, France

18:00 | O4 Hydrogels I - Thermosensitive shrinking behavior of biopolymer-based hydrogels for high resolution printing
Martina VIOLA, Department of Pharmaceutical Sciences (UIPS), Faculty of Science, Utrecht University, Utrecht, The Netherlands; Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands

A Universal Nanogel-based Coating Approach For Medical Implants

Damla Keskin[1], Devlina Ghosh[1*], Reinier Bron[1], Clio M. Siebenmorgen[1], Guangyue Zu[1], Theo van Kooten[1], Max J.H. Witjes[2], and Patrick van Rijn[1]
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INTRODUCTION

Materials for biomedical applications (like implants) have revolutionized healthcare system by providing enhanced drug delivery at targeted sites or by restoring tissue and organ functions.(1) However, secondary complications such as infection and inflammation due to microbial growth and biofilm formation, immune response in the host body can lead to the failure of the implant inside the human body.(2)
Coatings have been regarded as an excellent possibility to induce desired responses or prevent complications, as the bulk implant material does not need to be altered.(3) Owing to diverse physicochemical properties of implants **(Figure 1)**, the coating strategy required for polymeric materials is generally different than bioglass, ceramics, or metal-based implants. In order to enhance the coating applicability, a more relevant and better translatable universal method for coating all implant materials, regardless of their composition, will tremendously impact the field of biomedical coating developments **(Figure 2)**.

EXPERIMENTAL METHODS

Synthesis of nanogel particles. p(NIPAM-*co*-APMA) nanogel (nGel) particles were synthesised by precipitation polymerization reaction.
nGel coating. Upon activation, the surface rendered negative charge while the positively charged nGel particles attach onto it by electrostatic interactions.
Characterization techniques. Particles were characterized by Dynamic Light Scattering (DLS) and zeta potential, while the nGel coated implant surfaces were analyzed by atomic force microscopy (AFM).
Coating stability. The stability of the coating was determined in vitro by exposing nGel coated teflon surfaces to phosphate-buffered saline (PBS) and fetal bovine serum (FBS), while mimicking physiological conditions in a shaker incubator for 21 days.

RESULTS AND DISCUSSION

The DLS measurements showed that the average hydrodynamic diameter was 540.63 ± 11.2 nm and positive zeta potential, +15.83 ± 0.11 mV at 24°C was attributed to the presence of protonated primary amine groups. The nGel coating was applied on 11 materials belonging to different classes of implants. AFM images showed closely packed, homogenous nGel-layer on all the distinct surfaces, irrespective of their physicochemical properties. AFM images of nGel coated Teflon and fluorescence images captured by In vivo Imaging System (IVIS) indicated the retainability of the coating in both PBS and FBS media at 21 days (data not shown).

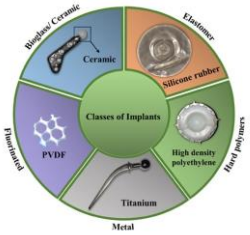

Figure 1. Schematic representation of the different classes of medically relevant implant materials.

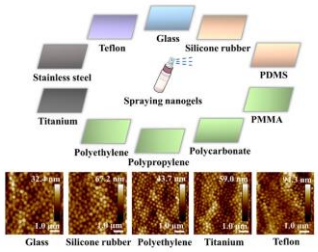

Figure 2. Application of spray coating technique (universal approach) that can be implemented on every class of material. AFM images show formation of homogeneous coating on the surface.

CONCLUSION

The nGel coating strategy was successfully translated to all the 11 unique medically relevant materials; hence we call it a universal coating approach. In future, different functions such as, antifouling, antibacterial properties, or imaging modalities can be incorporated into the coating, thereby creating a highly functional and potentially multimodal system depending on the desired application.

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Cell-instructive 3D Printed Multilayered Biomaterials embedded in Photocrosslinkable Hydrogels for Vascular Tissue Engineering

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INTRODUCTION

Bioengineering large 3D vascularized functional tissue constructs to restore, regenerate, or even substitute damaged tissues and organs remains as the major goal of tissue engineering and regenerative medicine (TERM).[1] Bottom-up approaches have opened exciting routes to develop 3D tissue-like constructs for pursuing advanced regenerative therapies.[2] Layer-by-Layer (LbL) assembly has proved to be a highly versatile technology to engineer hierarchical extracellular matrix (ECM)-mimetic biomaterials by resorting to a myriad of biomolecules exhibiting complementary interactions.[3] However, the lack of a carrier platform that could endow the developed biomaterial structures with superior mechanical stability to be administered in the human body extensively limits the use of the LbL technology stand-alone. Hydrogels are the most promising 3D platforms for cell encapsulation, controlled therapeutics delivery, and construction of 3D tissue-like constructs.[4] Herein, we report the bioengineering of hybrid 3D cell-biomaterial constructs encompassing (i) bioinstructive LbL-functionalized 3D printed capillary-like structures embedded in (ii) photopolymerizable hydrogels to promote the formation of tissue-mimetic vascularized networks *in vitro* and be potentially used as injectable systems in TE strategies.

EXPERIMENTAL METHODS

The build-up of bioactive multilayered thin films encompassing oppositely charged chitosan (CHT) and arginine (R)-glycine (G)-aspartic acid (D) grafted alginate (ALG-RGD) was monitored *in situ* by quartz crystal microbalance. Then, similar bioinstructive $(CHT/ALG\text{-}RGD)_n$ multilayers were templated on 3D printed ALG microfibers. Either uncoated or LbL coated ALG fibers were embedded in a methacrylated xanthan gum (XGMA) pre-hydrogel solution to develop robust 3D constructs after crosslinking with UV light, as confirmed by rheological measurements. The constructs were immersed in EDTA to obtain bioinstructive hollow tubular microstructures in which human umbilical vein endothelial cells (HUVECs) were cultured for 3 days in culture medium either with or without FBS and the cell adhesion, viability and spreading studied (Figure 1).

RESULTS AND DISCUSSION

ALG microfibers were 3D printed and LbL coated with $(CHT/ALG\text{-}RGD)_6$ bilayers prior to being encapsulated within photocrosslinkable XGMA hydrogels. The formation of liquified microtubular structures was accomplished by EDTA-induced chelation of the ALG fiber, as unveiled by the flow of a fluorescent dye aqueous solution injected through the microchannel. With the aim of forming prevascular networks, HUVECs were seeded in either the inner walls of $(CHT/ALG\text{-}RGD)_6$ LbL-functionalized or LbL-free microchannels (control) and the *in vitro* cell viability assessed after 3 days by confocal laser scanning microscopy (CLSM), revealing a higher cell adhesion in the LbL-functionalized microchannels.

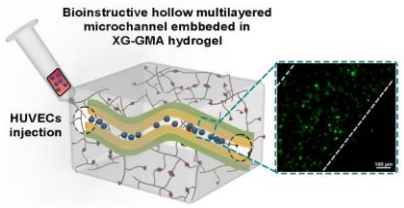

Figure 1. Hybrid 3D construct and CLSM image of HUVECs cultured for 3 days in FBS-free culture medium in the inner walls of the bioinstructive LbL microchannel.

CONCLUSION

Hybrid 3D constructs denoting a higher number of adherent and viable cells in the bioinstructive LbL-functionalized microchannels were bioengineered, holding great promise for the development of endothelial cell-lined tubular networks as blood vessel substitutes. The versatility imparted by the methodology herein proposed opens new avenues for engineering a wide array of 3D prevascularized tissue-like constructs to be used as injectable systems for modular TERM.

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Ex vivo and *in vivo* evaluation of injectable and porous hydrogels as support for muscle repair

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Keywords: Injectable-porous hydrogels; Skeletal muscle regeneration; animal experiment.

INTRODUCTION

Volumetric muscle loss (VML) resulting from traumatic incidents drastically decreases muscle regeneration capacity and lacks treatments[1]. Hydrogels are promising materials for the repair of damaged tissues, providing a hydrated and versatile support for cells. For VML, porosity and injectability of hydrogels are crucial parameters to perfectly fill muscle defects and to allow cell infiltration and efficient vascularization[2]. Recently, a biocompatible and biodegradable injectable effervescent porous hydrogels (EPH) of poly-lysine dendrimers (DGL) cross-linked by polyethylene glycol (PEG-NHS) has been developed[3]. These hydrogels present highly tailorable mechanical properties and inherent cell interactions[4] which allow for an efficient support of myoblast proliferation, differentiation and fusion into contractile myofibers, *in vitro*. These breakthrough results highlight the strong therapeutic potential of injectable and porous hydrogels to improve the repair of VML, which needs to be evaluated in *ex vivo* and *in vivo* experiments.

Therefore, the aims of this study were (i) to evaluate the ability of EPH formulations to be injected in muscle defects in an efficient fashion and to intimately bind to muscle defect margins, and (ii) to characterize muscle cells colonization of the injected porous matrices from the defect margins and subsequent muscle formation *ex vivo* and *in vivo*, in relevant mice and rat VML models.

EXPERIMENTAL METHODS

The ability of the hydrogels to intimately bind to the margins of a muscle defect was evaluated by crosslinking various hydrogels compositions between two pieces of beef muscle, followed by traction assays to determine force and elongation at break in comparison to muscle or hydrogel samples alone. The concentration and composition of EPH was modulated to determine optimal conditions allowing a swift crosslinking of porous structure formation after injection in 5 mm wide muscle defects created in commercial muscle pieces.

To evaluate the ability of muscle cells to migrate into injected EPH, an *ex vivo* VML model in mice and *in vivo* VML model in rats were developed. For *ex vivo* experimentation, VML were obtained using a 3 mm biopsy punch in the quadriceps of sacrificed mice. After hydrogel injection and crosslinking, the whole muscle was dissected and placed in culture medium (DMEM-15% FBS) for up to 10 days. Muscle were then fixed and cryo-sectioned. Cells colonization, proliferation and spreading were quantified using image analysis after immunostaining for DAPI, myoblast determination protein 1, myogenin and myosin heavy chain. For *in vivo* experiments, VML were created using a 5 mm biopsy punch in the tibialis anterior muscle of anesthetized rats, followed by injection and suturing of the covering skin.

RESULTS AND DISCUSSION

To allow cell infiltration from muscle defect into EPH, an intimate contact with the defect margins is mandatory. An efficient binding of the hydrogel onto muscle tissue was observed, possibly due to the presence of amine groups on the muscle surface, which can covalently react with the NHS function during hydrogel crosslinking. Tractions assays confirmed such a strong covalent binding as elongational breaks were obtained in the hydrogel rather than at the interface with muscle (Fig 1A).

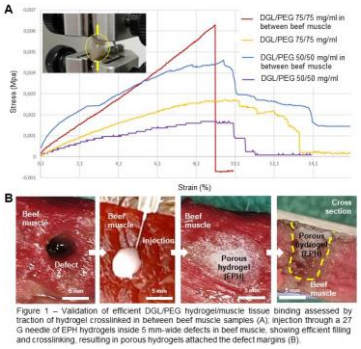

Figure 1 – Validation of efficient DGL/PEG hydrogel/muscle tissue binding assessed by traction of hydrogel crosslinked in between beef muscle samples (A); injection through a 27 G needle of EPH hydrogels inside 5 mm-wide defects in beef muscle, showing efficient filling and crosslinking, resulting in porous hydrogels attached the defect margins (B).

By careful selection of EPH formulation parameters (concentration, temperature, pH) it was possible to inject through a 27G needle into a muscle defect and achieve crosslinking of the porous matrices within 10 seconds, filling the defect with a porous hydrogel, in intimate contact with the remaining muscle margins (Fig 1B).

These successful parameters could be applied to inject EPH into mice VML defects. Explants, maintained *ex vivo* confirmed the ability for cells present in the remaining muscle to penetrate into EPH porosity after 3 and 4 days (Fig 2A,B). While identification of cells phenotype over 10 days is ongoing, this successful cellular invasion allowed to initiate the evaluation of EPH's muscle supportive capacity in rats VML models, *in vivo* (Fig 2C).

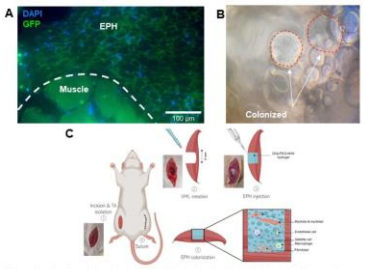

Figure 2 – Experimental protocol of VML defect in rats, filled with injected effervescent porous hydrogel (EPH) (A). EPH injected in mouse quadriceps and maintained in *ex-vivo* culture, showing efficient cellular infiltration in the hydrogel after 3 days (B, 10 µm section, DAPI = cell nucleus) and 4 days (C, phase contrast microscopy).

CONCLUSION

We validated the potential of EPH hydrogels to be readily and easily injected into muscle defects, in view of their evaluation as VML supportive therapy. The ongoing identification of cells that colonized the injected EPH *ex vivo*, as well as the analysis of the repair mechanism at play in EPH-filled VML models *in vivo* will further indicate if cells progenitors can migrate from the defect margin within EPH and differentiate into myotubes and myofibers.

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Thermosensitive Shrinking Behavior of Biopolymer-based Hydrogels for High Resolution Printing

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INTRODUCTION

Printing high resolution constructs with complex geometries and sizes below 100 μm of soft hydrogel materials is not only a challenge, but also of high interest in biofabrication for the regeneration of renal tubules and blood vessels. Hydrogel shrinking techniques (complexation or pH-dependent) have recently been proposed to print high resolution 3D scaffolds which are subsequently shrunken to go beyond the maximum resolution guaranteed by the printer. Since both complexation and pH-dependent techniques impose a non-cell-friendly environment, in this work we propose a thermosensitive hydrogel that reduces its dimension when exposed to a temperature increase to 37°C.
This thermosensitive shrinking method is based on the lower critical solution temperature (LCST) of poly N-isopropyl acrylamide (pNIPAM).

EXPERIMENTAL METHODS

Hydrogels based on biopolymers (gelatin and silk fibroin) were functionalized with methacryloyl moieties, crosslinked with NIPAM through UV exposure and the shrinking behavior was evaluated upon heating above the LCST (Fig.1). The hydrogels were applied to volumetric 3D printing, the printability, the ability to maintain geometry and proportions following shrinking were evaluated.

RESULTS AND DISCUSSION

Both GelMA/NIPAM and SilkMA/NIPAM hydrogels reduce their size in volume by a factor 2 when heated to 37 °C. This shrinking effect was found to be fully reversible when samples were cooled back below the LCST. After the shrinking both hydrogels showed an increase in the gel strength and an increase in the Young's modulus (Fig. 1).

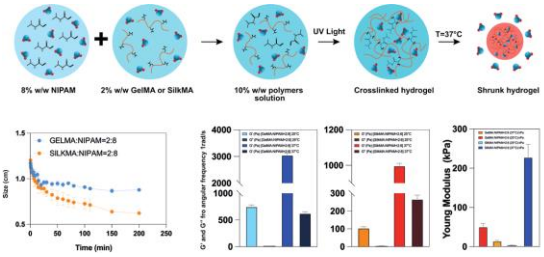

Fig. 1; Graphical representation of NIPAM – GelMA (or SilkMA) hydrogel formation and shrinking behavior when exposed to 37°C; kinetic of shrinking for both GelMA:NIPAM and SilkMA:NIPAM hydrogels and mechanical characterization of the same hydrogels.

Both volume reductions and cell viability of conditionally immortalized proximal tubule cells were observed to be correlated with increased ratios of NIPAM to methacroylated-polymer concentrations while keeping total polymer concentrations at optimal 10% (w/w).

Positive figures were printed with the volumetric printer with a resolution of 600 μm at room temperature that was enhanced to 30 μm upon shrinking (Fig. 2). For the negative figures a resolution enhancement from 200 to 50 μm was reached.

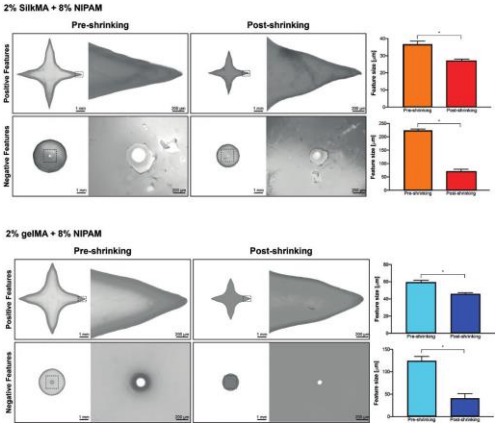

Fig. 2; GelMA:NIPAM and SilkMA:NIPAM positive and negative features printed with volumetric printing.

CONCLUSION

This work shows a versatility of materials applicable to volumetric printing with the ability to reach resolutions above the technical capacity of the machine, also demonstrating an inclination of cells to grow on the stiffened hydrogel following shrinking.

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ACKNOWLEDGMENTS

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>> 17:15 - 18:30 | CS2 - Clinical Session II: Osteoarticular repair
Chairpersons: Jean-Christophe Fricain & Michael Doser
Location: Room B

17:15 | KL Osteoarticular repair - Clinical Applications of Enamel Matrix Derivative, a Biomimetic and Inflammation-modulating Biomaterial
Nathalie WEBER, Institut Straumann AG, Basel, Switzerland

17:45 | O1 Osteoarticular repair - Influence of glenoid microdrilling on tissue remodeling at the pyrocarbon hemi-implant interface
Rémy GAUTHIER, Univ Lyon, CNRS, INSA Lyon, UCBL, Matéis, Lyon, France

18:00 | O2 Osteoarticular repair - Preclinical study design and validation of a model of intervertebral disc degeneration for the evaluation of glyco-functionalised biomaterials
Kieran JOYCE, CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland, Galway, Ireland

18:15 | O3 Osteoarticular repair - 3D-printed Biocompatible and Biodegradable PLA Scaffolds with Optimized Architecture and BMP-2 Dose to Repair a Sheep Metatarsal Critical-size Bone Defect
Charlotte GAROT, U1292 Biosanté, Equipe CNRS EMR 5000 BRM, Université Grenoble-Alpes, INSERM, CEA, Grenoble, France

Clinical Applications of Enamel Matrix Derivative, a Biomimetic and Inflammation-modulating Biomaterial

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INTRODUCTION

Enamel matrix proteins (EMPs) are a group of highly conserved proteins secreted during tooth development[1].

Original experiments conducted by Lars Hammarström demonstrated that EMPs could promote the regeneration of all three periodontal tissues, namely the cementum, the periodontal ligament and the alveolar bone in an animal model[2].

This pioneering work paved the way to an enormous body of research related to the characteristics, preclinical and clinical use of these proteins and led to the development of a medical device containing an enamel matrix derivative (EMD) commercialized under the brand name Emdogain®.

As of today much evidence supports the biomimetic mode of action of EMD for the regeneration of periodontal defects lost due to periodontitis[3], as well as its inflammation-modulating properties[4].

Clinical evidence on EMD gathered in over 200 clinical trials show that its use is beneficial in a wide range of applications in dentistry[5] and for dermal wound healing[6].

Institut Straumann AG's latest sponsored study[7] will be presented.

EXPERIMENTAL METHODS

A prospective, paired sample, multicenter, pilot study evaluated non-surgical periodontal treatment (NSPT) with (test) and without (control) Emdogain® in 51 patients presenting with moderate to severe periodontitis. The primary outcome variable was change in clinical attachment level (CAL) after 12 months. Secondary variables included bleeding on probing (BoP), and percent of pockets converted to sites no longer requiring surgical treatment.

RESULTS AND DISCUSSION

CAL changed significantly ($P < 0.001$) from baseline to 12 months for both treatment modalities (test = -2.2 ± 1.5 mm versus control = -2.1 ± 1.3 mm); the difference between groups was not significant. A significant difference, favoring test conditions, was observed in sites no longer requiring surgical treatment; 79.8% of test versus 65.9% of control sites. BoP decreased significantly more ($P < 0.05$) in test sites (BoP at 17.8% test versus 23.1% control).

CONCLUSION

In this study the adjunct use of Emdogain® with NSPT resulted in significantly greater improvements in overall periodontal health with less frequent BoP and a higher number of sites no longer requiring surgical treatment.

These findings contributed to the clinical evidence required for the indication widening of Emdogain® to NSPT.

With over 140 publications on EMPs in the past twelve months[8], these proteins seem to hold the interest of the scientific community. In-vitro and pre-clinical research on EMPs show that these proteins have an effect on many cell types and that some of them are more widely expressed in the body than just during tooth development as initially thought. A lot remains to be learned about them and they may find applications in a broader array of applications in regenerative medicine in the future.

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ACKNOWLEDGMENTS

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Influence of glenoid microdrilling on tissue remodeling at the pyrocarbon hemi-implant interface

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INTRODUCTION

The medical care of osteoarthritis (OA) in young patients glenohumeral joint remains challenging. While total shoulder arthroplasty (TSA) is the gold standard method to treat OA, hemiarthroplasty (HA) is preferred as a more conservative methods in young patients with high physical demands. Such surgical procedure allows for the glenoid conservation while replacing the humeral head by a hemi-prosthesis (Figure 1). Still, HA using metallic heads is associated with a rapid erosion of the glenoid cavity. In that context, new materials, such as pyrocarbon (PyC), have been recently used as humeral head and show interesting short-term outcomes[1]. PyC promotes chondrocytes expression of cartilage-like tissue markers, *in vitro*[2].

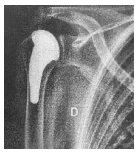

Figure 1: Shoulder radiograph after HA

As an additional method, to prevent from glenoid cartilage degeneration, micro holes can be drilled in the glenoid subchondral bone. Such microdrilling allows for bone blood and marrow to provide a large range of cell recruitment needed for the further tissue regeneration. While this technique reaches good clinical outcomes[3] there is no clinical data on the synergy between HA using PyC humeral head and glenoid bone microdrilling (MD).

CLINICAL CASES TISSUES ANALYSIS

In the present study, the tissue remodeled at the PyC-glenoid interface of 1 clinical case with and 1 clinical case without MD will be investigated. On these different clinical cases, tissues samples at the glenoid-PyC interface were harvested at the time of revision surgery. The tissues samples were then fixed, embedded in paraffin, and cut in sections. Sections were stained with Hematoxyline-Eosine-Safran (HES), Safranin O, or immunolabelled for collagen I and collagen II. Additionally, the amount of adsorbed phospholipids (PL) on PyC surface was measured. PL coming from the synovial fluids are known to play a major role in the joint lubrication. In that context, immediately after the surgery revision, the explanted PyC humeral head was subjected to ethanol-chloroform (1:2, v:v) washes. The solution was then used for a further lipidomic analysis through gas chromatography.

RESULTS AND DISCUSSION

The tissue between the PyC implant and the glenoid subchondral bone from the case without MD appeared as fibrocartilaginous. The main cell population is composed of fibroblast-like cells, while a small number of chondrocyte-like cells embedded in a fibrous matrix are observed. Conversely, the tissue from the case with MD did not showed a fibrillar structure. Additionally, Coll II was expressed intracellularly by chondrocyte-like cells trapped in an articular cartilage-like matrix (Figure 2 left). Interestingly, Coll II was also expressed around vascular canals, in the cartilage-like layer (Figure 2 left) and within the subchondral bone (Figure 2 right). Some fibrocellular matrix rich in Coll I was also observed.

Regarding the PL amount, a lower amount (0.0035 nmol/mL) was found on the implant without MD compared to the implant with MD (0.121 nmol/mL). The larger amount of PL on glenoid with MD may derived from bone marrow that can reach the implant surface.

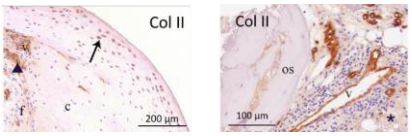

*Figure 2: Coll II immunostainings (arrows) in the cartilage-like tissue close to the PyC implant (left) and in the subchondral bone (right). v: vascular canals, f: fibrocellular matrix, c: homogeneous matrix, os: bone, *: bone marrow.*

Together, these results suggest that bone MD influences the course of tissue remodeling close to the implant interface. A hypothesis is based on the determinant role played by the PL as a lubricant. It is known that the cartilage integrity is controlled in part by bone mechanobiological nature[4]. The PL-aided lubrication of the implanted-joint could allow a suitable mechanical transmission from the implant toward the subchondral bone. Chondrogenesis may originate from this underlying bone as suggested by the presence of Coll II in vascular canals.

In that context, understanding the role of PL in the mechanotransduction of articular joint is a great challenge that needs to be raised in order to improve articular implant longevity and the patient quality of life. The amount of PL together with the shape of the PyC humeral head may be of major importance, in terms of mechanical load transmission. This might be determine the course of a neo-cartilage remodeling at the implant interface through the mechanical stimulation of the microdrilled subchondral bone.

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Preclinical study design and validation of a model of intervertebral disc degeneration for the evaluation of glyco-functionalised biomaterials

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INTRODUCTION

Glycosylation, a post-translational modification on peptides, plays a role in immune response, cell adhesion and migration, inflammation and cell survival[2]. Inflammatory and degenerative processes alter protein glycosylation in IVD injury and ageing[3–5], demonstrating increased sialylation (Neu5Ac). 3F-peracetyl Neu5Ac (Neu5Ac-inhib) is an ideal candidate molecule to inhibit sialyltransferase enzymes to assess the downstream effects of sialylation inhibition. This study proposes to validate a dog model of IVD degeneration and outlines a preclinical study design to study the effect of a Neu5Ac-inhib loaded hyaluronic acid (HA) hydrogel as a regenerative therapy.

EXPERIMENTAL METHODS

Dog IVDs were obtained from client-owned donated specimens from Utrecht University. Tissue analysis was performed using histology, lectin histochemistry and *N*-glycan analysis by MALDI-FTICR MS. Neu5Ac-inhib was loaded into the fabricated HA hydrogels and investigated in an *in vitro* model of IVD degeneration using dog NP cells. The HA hydrogel was synthesised by cross-linking sodium hyaluronate 1% with 4-arm PEG amine using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl-morpholinium chloride. The hydrogel was characterised by quantifying unreacted amine groups and rheological assessment of physicochemical properties. Animal experiments were carried out with appropriate ethical approval. Six beagle dogs (Nosan Beagle; Nosan Corporation, Kanagawa, Japan) underwent induced disc degeneration and following 4 weeks were treated with intradiscal injection of sham saline, hydrogel alone, hydrogel + low dose Neu5Ac-inhib, hydrogel + high dose Neu5Ac-inhib. Eight weeks post-degeneration, final radiological and MRI assessments were obtained, and routine histological analysis is ongoing[6]

RESULTS AND DISCUSSION

Lectin histochemical analysis of dog IVD revealed a hypersialylated tissue phenotype in increasing grades of degeneration. MALDI-FTICR further characterised *N*-glycan expression in these tissues. HA hydrogel was optimally cross-linked, exerted hydrolytic stability and resistance to enzymatic degradation with no cytotoxic effect. These findings indicate that optimally stabilised cross-linked HA hydrogel is a suitable delivery vehicle for glycosylation inhibitors *in vivo*. The efficacy of Neu5Ac-inhib release from a HA hydrogel was determined in dog NP cells (Fig. 1). Cytokine-induced inflammation

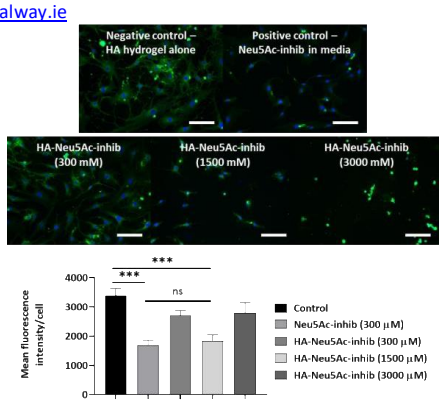

Fig 1. Neu5Ac-inhib loaded HA hydrogel effectively inhibits sialylation in canine NP cells. Scale bar = 100 µm

increased sialylation expression in dog NP cells, and sialylation inhibition effectively restored the glycosylation expression to physiological levels. The preclinical trial is complete, with results expected to validate *in vitro* findings and yield significant insight into the role of sialylation in IVD degeneration.

CONCLUSION

Compared to human degeneration, a similar glycomic response was observed in the dog IVD. This dog preclinical model was validated *ex vivo* as a suitable model for determining the efficacy of glyco-functionalised materials. The outlined preclinical study design captures the glycomic response to the delivery system and inhibitor dose. This study characterises the first glyco-functionalised system for IVD regeneration, paving the way for further trials targeting glycosylation-based disease targets, to regenerate the IVD in degeneration.

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3D-printed Biocompatible and Biodegradable PLA Scaffolds with Optimized Architecture and BMP-2 Dose to Repair a Sheep Metatarsal Critical-size Bone Defect

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INTRODUCTION

Critical-size bone defects are currently treated with autologous bone grafts. However, they are associated with limited availability, high postoperative donor-site morbidity, and inconsistency of repair in very large bone defects[1]. Scaffolds are being developed to replace the autologous bone graft[2]. For efficient bone repair, scaffolds should be biocompatible, biodegradable, osteoconductive, osteoinductive, and possess physico-chemical properties optimizing bone regeneration[3]. Among these physico-chemical properties, scaffolds should be porous[4], but the ideal pore size and shape have not been evidenced yet. The objective of this study was to design 3D-printed scaffolds made of a biocompatible and biodegradable polymer, polylactic acid (PLA) and having an optimized architecture. The surface of the 3D scaffolds was rendered bioactive via a thin film coating containing bone morphogenetic protein-2 (BMP-2). These bioactive medical devices were used to repair a sheep metatarsal critical-size bone defect.

EXPERIMENTAL METHODS

Different types of experiments were performed in vitro and in vivo. The aim in vitro was to assess the biocompatibility of the film-coated PLA scaffolds. In vivo, the aim was to repair a critical-size metatarsal bone defect in sheep and to optimize scaffold intern architecture.

Five biocompatibility assays were performed on 2D PLA discs to prove their biocompatibility: cytotoxicity in direct contact, direct cytotoxicity with extract, attachment, proliferation, and differentiation. For the 2 cytotoxicity assays, there were 6 experimental conditions (n=4): a negative control (thermanox), a positive control (latex), PLA alone, PLA + film, PLA + film + BMP30, and PLA + film + BMP60. For the other tests, there were 8 experimental conditions (n=3): the PLA conditions as for the cytotoxicity assays and the same conditions but on plastic.

In vivo, experiments were conducted in a 25-mm long segmental metatarsal bone defect in sheep using 3D-printed medical-grade PLA. Scaffolds had a cylindrical shape (14 mm in diameter and 25 mm in height). The influence of scaffold geometry on bone regeneration and on BMP-2 incorporation was studied in order to optimize scaffold architecture. To this end, 4 scaffold geometries were selected for implantation: Lines, Gyroid 1, Double Lines, and Gyroid 2 (**Figure 1**). The scaffolds were film-coated and post-loaded with BMP-2 at ~500 µg/scaffold, the effective loaded amount loaded being quantified using UV spectrophotometry. They were implanted and compared to negative controls (film-coated scaffolds without BMP-2). Bone regeneration was followed over 4 months. X-ray radiographs were acquired each month until euthanasia. Micro-computed tomography (µCT) scans were acquired after explantation, and histology was finally performed.

RESULTS AND DISCUSSION

The *in vitro* experiments validated the biocompatibility of the PLA implants, independently of their coating or BMP-2 loading. These same PLA implants were implanted subcutaneously in rats to assess the biocompatibility of implants *in vivo*. This experiment is still ongoing.

The repair of a sheep metatarsal critical-size bone defect showed that scaffold geometry had an influence on bone regeneration. Indeed, X-ray radiographs and µCT scans showed that the geometry Lines presented the fastest and strongest bone formation among the different geometries. The results for Gyroid 2 geometry are not complete yet but look promising. The geometry Gyroid 1 led only to a partial bridging of the bone defect and the geometry Double Lines led to a partial bridging of the defect in one case and to no bone repair at all in the other case (**Figure 1**). As the BMP-2 dose loaded in the different geometries was comparable, this means that scaffold geometry influenced bone regeneration kinetics and the amount of new bone formed.

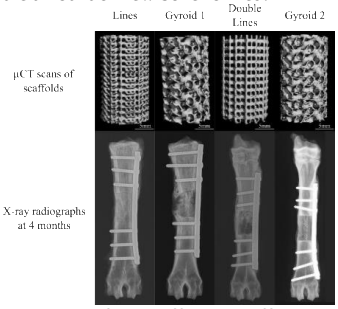

Figure 1. µCT scans of the different scaffold geometries and representative X-ray radiographs of implanted scaffolds at 4 months.

CONCLUSION

We showed that 3D-printed PLA scaffolds were biocompatible and biodegradable. We further showed that scaffold geometry influenced bone regeneration. The geometry Lines, coupled with an optimized BMP-2 dose (~500 µg/scaffold), led to the best bone regeneration without any adverse effects. This study investigated the influence of pore size and shape on bone regeneration but other geometrical parameters such as scaffold permeability could be important as well. Further studies should be conducted to pursue the optimization of scaffold geometry in view of optimal bone repair.

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ACKNOWLEDGMENTS

This work was supported by the French Agency for Research (ANR-18-CE17-0016, OBOE). CP is a senior member of the Institut Universitaire de France whose support is acknowledged.

>> 17:15 - 18:30 | Trans S2 - Translational session II
Chairpersons: Yves Bayon & Christophe Bureau
Location: Room E

17:15 | KL Translational II - Shinning a light on tissue repair: from the bench to the bedside
Maria PEREIRA, Tissium, Paris, France

17:45 | O1 Translational II - Inguinal Hernia Mesh with a Drug Delivery System
François AUBERT, Cousin Surgery, Wervicq, France

18:00 | O2 Translational II - Industrialization of mesenchymal stem cell derived extracellular vesicle manufacturing
Adam HARDING, Process Development Department, Lonza Cell and Gene, Geleen, The Netherlands

18:15 | O3 Translational II - A Novel Resorbable Polyester, Tailor-Made to Support as a Knitted Temporary Support for Primary Ventral & Incisional Hernia Repair
Robert VESTBERG, Medtronic – Sofradim Production, Trévoux, France

Shinning a light on tissue repair: from the bench to the bedside

Maria Pereira*, Rachel Motte, Elise DeVries

Tissium, Paris, France
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INTRODUCTION

Next generation biomaterials promise unprecedented impact in simplifying surgical procedures and promoting tissue repair. In particular, tissue adhesives have the potential to promote atraumatic repair of tissues. However, existing tissue adhesives are associated with a strong inflammatory response, weak adhesion, and/or poor control over the bonding process.[1]

To meet this need, a viscous, light activated adhesive that can be exposed to blood flow without compromising its adhesive strength, was developed.[2] The core technology, poly(glycerol sebacate) acrylate (PGSA), is based on the combination of naturally occurring compounds (glycerol and sebacic acid) to form a viscous pre-polymer that can be precisely applied to internal tissues during surgical procedures, both open and minimally invasive. Once applied to the target location, it is polymerized using an external blue light. The resulting bond is elastic, allowing the polymer to comply with the underlying tissue while remaining strongly adhered. Furthermore, this biocompatible polymer is biodegradable.

This versatile polymer platform can be used in several ways: In addition to being applied as a sealant or adhesive and polymerized on-demand, the pre-polymer can be used as a 3D printing resin to build high resolution scaffolds to support tissue repair. It can also be loaded with drugs and deployed potentially anywhere in the body creating a drug depot for local drug delivery.

This presentation will cover the innovation pathway related to the PGSA technology development as well as the process TISSIUM has used to translate it from the bench to the bedside to address various clinical needs.

EXPERIMENTAL METHODS

To translate PGSA technology from the bench to the bedside, TISSIUM has scaled up the manufacturing process for producing PGSA. Bench and animal studies as well as a first clinical study, have been conducted to evaluate the safety and performance of the material. The initial indications selected were identified through a rigorous assessment of unmet clinical needs to ensure maximum impact for both open and minimally invasive surgical approaches.

RESULTS AND DISCUSSION

Leveraging the PGSA polymer platform, TISSIUM has developed integrated solutions to address diverse clinical needs. For each specific indication, we designed a set of dedicated surgical instrumentation, in collaboration with surgeons, to maximize the usability and performance of the polymer. Furthermore, dedicated light sources, for open or minimally invasive procedures, were developed to promote the controlled polymerization in each surgical scenerio.

Our first product, a Vascular Sealant, has been evaluated in different large animal studies of open vascular carotid and aortic surgery. The pre-clinical results were then translated into a clinical setting in a prospective, single-arm multicenter study in patients requiring carotid endarterectomy using an ePTFE patch.[3] This product received CE mark in 2017.

The range of developed applications for the core polymer technology include two concepts that have shown promise in preclinical studies. We have designed a sutureless nerve coaptation system that demonstrated to be a promising alternative to microsutures and fibrin glue for strong and effective sensory and motor nerve repair in a rodent animal model. A solution for atraumatic hernia mesh fixation is also currently in development and its performance and safety has been assessed in a porcine model of laparoscopic intraperitoneal onlay mesh placement.

CONCLUSION

The novel PGSA polymer platform offers promising solutions for atraumatic tissue repair. Its unique ability to be leveraged as diverse solutions is due to the modular platform design: each use case leverages a polymer formulation and distinct surgical instrumentation. TISSIUM continues to innovate on these elements to design integrated solutions that can address a broad range of clinical needs.

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ACKNOWLEDGMENTS

Authors would like to thank the different collaborators that have been involved in developing PGSA as a new class of surgical materials and building TISSIUM's pipeline of products.

Inguinal Hernia Mesh with a Drug Delivery System

François AUBERT[1*], Stéphanie DEGOUTIN[2], Feng CHAI[3], Mickael MATON[3], Guillaume VERMET[1], Safa OUERGHEMMI[1] Bernard MARTEL[2], Nicolas BLANCHEMAIN[3]
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[2]Univ. Lille, CNRS, INRA, ENSCL UMR8207, UMET – Unité Matériaux et Transformations, F-59000 Lille, France
[3]Univ. Lille, Inserm, CHU Lille, U1008 – Controlled Drug Delivery Systems and Biomaterials, F-59000 Lille, France
[*] f.aubert@cousin-surgery.com

INTRODUCTION

The avoidance of post-herniorrhaphy pain can be challenging for hernia repair and has the greatest impact on patient's quality of life, health care utilization and cost to society. Visceral meshes, functionalized with an efficient drug carrier system ie hydroxypropyl beta-cyclodextrin polymer (polyHPβCD) coating, were developed to give a locally prolonged analgesic drug release. This technology has given rise to several patents and publications in various fields of biomedical applications: vascular [1], parodontal [2], visceral [3-4]. The in vivo pain-relief efficacy of ropivacaine loaded polyHPβCD functionalised polyester meshes in a rat model of visceral pain induced by colorectal distension (CRD) and the safety of functionalized meshes have been evaluated. After validation of the manufacturing processes at Cousin Surgery, premarket clinical study begans.

EXPERIMENTAL METHODS

Mesh functionalization and Ropivacaine (RVP) loading: Used meshes are knitted in multifilament polyethylene terephthalate (PET) "A1L" (38 g/m^2), manufactured by Cousin Surgery. The functionalization of A1L with polyHPβCD was based on a pad/dry/cure textile finishing process as previously reported [1-4].

A1L meshes were impregnated in CTR/NaH_2PO_2/HPβCD reactant solution ratio of 16/2/20/ named A1L-CD360. Functionalized meshes (1.5 x 1.5 cm) were loaded with 1 mg ropivacaine A1L-CD360+RVP.

Rat model for visceral pain assessment: The visceral pain was monitored in Male Sprague Dawley rats weighing around 200 g, who received the controlled isobaric colorectal distension (CRD) with a balloon introduced and inflated in the colon. The animal nociception was estimated by measuring the intra-colonic pressure required to induce a certain behavioral response.

In vivo safety: In vivo safety, pharmacokinetic profile and biodegradation were measured via histological analysis and high-performance liquid chromatography.

Statistics: GraphPad Prism 5.03.0001 was used for statistical analysis. Results are reported as mean ± SD for each experimental group. For CRD assays, nonparametric analysis using Kruskale Wallis ANOVA for pairwise comparisons was undertaken followed by Bonferroni correction. Pharmacokinetic parameters and homogeneity evaluation were compared with a two-side non-parametric Mann-Whitney U test. The $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results confirmed that the polyHPβCD on the functionalised meshes has a high adsorption capacity of ropivacaine and resulted in a sustained drug release in rats after mesh implantation

This was further reaffirmed by an elevated pain threshold (30%) up to 4 days after implantation in the rat CRD model, compared to 1-2 days for non-adapted meshes (Fig 1).

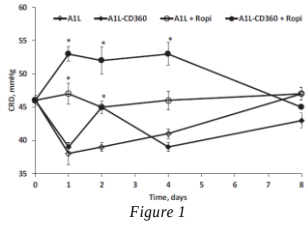

Figure 1

Neither polyHPβCD nor the loaded ropivacaine had a major impact on the inflammatory response (Fig 2) This evidence strongly suggests that polyHPβCD functionalised visceral mesh could be a promising approach for post-operative pain control by improving the intraperitoneal drug delivery and bioavailability.

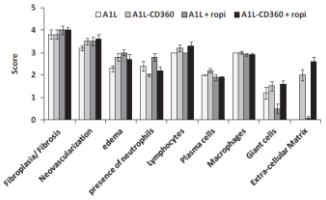

Figure 2

Safety evaluations and process validations on sterile finished products reported compliance according MDR 2017/745/EU.

CONCLUSION

The reported results in this abstract have been already published [5]. They conducted to the development of a medical devices that is today under clinical investigation on patients (NCT04033055).

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5.Chai *et al*, Biomaterials, 192, 2018

ACKNOWLEDGMENTS

Authors acknowledge Pr. Desreumaux and Mrs. Dubuquoy from Intestinal Biotech Development (INSERM U995) for their technical support in evaluating the pain reaction in vivo. We also would like to thank the "Plate-forme Ressources Experimentales (D.H.U.R.E)", University of Lille, for their support on animal studies and Oncovet Clinical Research (OCR) for histological analysis.

Industrialization of mesenchymal stem cell derived extracellular vesicle manufacturing

A. Harding[1], B.G.M van Dijk[1*], Paul Cahill[2], Verena Börger[3], R. Vyzasatya[1]

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[2] School of Biotechnology, Dublin City University, Dublin, Ireland
[3] Institut für Transfusionsmedizin, Universitätsklinikum Essen, Essen, Germany
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INTRODUCTION

With the increase in life expectancy of the population, the amount of implanted prostheses increases every year. Additionally, with the aging of the population, not all prostheses are a lifetime solution for patients. In many cases a revision prosthesis is needed, which can be often attributed due to aseptic loosening. This is a growing concern, as in such an inflamed environment the chance of successful bone engraftment is lower. A new generation of revision implant is needed, that has the capacity to also treat the local inflammation to ensure successful bone engraftment. Mesenchymal stem cells (MSCs) have shown promise in regenerative medicine and to treat autoimmune and inflammatory diseases. There is growing evidence that the immunosuppressing function of MSCs can be attributed to MSC-derived extracellular vesicles (MSC-EVs). Lonza is a partner in the EVPRO consortium, which aims to use MSC-EVs to counteract inflammation of hip revision prostheses. Before MSC-EVs can be used to be used to treat patients in clinical trials, they must be produced according to good manufacturing practice (GMP), and comply with appropriate regulatory guidelines. Within the EVPRO project, a GMP process is being developed, for both upstream (MSC expansion) and downstream (MSC-EV isolation and purification) manufacturing. The current academic partners use two different upstream approaches. Either multilayer culture flasks (static culture) or hollow fiber bioreactor (dynamic culture).

EXPERIMENTAL METHODS

First, a detailed gap assessment was performed of both approaches, investigating GMP compliance and scalability of these manufacturing processes (i.e., procedures, materials, equipment, preliminary data, process scale, analytical testing).

Next, the process is translated into a GMP ready process in the following way:

1) Feasibility studies. Establishing the manufacturing processes in the process development laboratories of Lonza using R&D materials and at R&D scale:

2) Process development studies to de-risk the process:

- Removing aseptic risk (e.g by closing handlings)
- Removing / replacing non-compliant materials
- Scaling up or scaling out the process
- Standardizing the process
- Establishing in-process control strategy
- Lock the process

3) Confirmatory studies. Run locked process at full scale.

RESULTS AND DISCUSSION

The main gaps identified are:

- Removing or substituting starting and raw materials with GMP equivalents
- Closing, de-risking and upscaling the upstream and downstream manufacturing process
- Establishing an in-process and release testing strategy.
- The 2D process has a scalability gap, and will only be scalable up to phase 2/3 clinical studies.

To address these gaps, Lonza focusses on the following development aspects:

- Material replacement → Removal of antibiotics, replacement of research grade materials.
- Upstream process → Closed seed-train and GMP compatible hollow fiber bioreactor.
- Downstream process → Closed and scalable downstream purification method.

Lonza has performed the feasibility studies and successfully transferred the upstream R&D process into the process development labs. The process development studies are ongoing and the following changes are already successfully implemented: 1) Replacement of RUO grade materials with GMP grade materials, 2) Removal of antibiotics of the culture, 3) Standardizing the static expansion, 4) Establish an in-process testing strategy. Furthermore, Lonza has identified the equipment and materials needed for the upstream and downstream GMP-ready process and is currently establishing this in the process development labs.

CONCLUSION AND FUTURE WORK

Although there are a considerable amount of gaps in the research processes, if addressed by process development, there are no gaps which could prevent transfer to a GMP facility. The GMP-ready process has been identified, and the development strategy is defined. The feasibility runs are successfully completed and first gaps have been closed.

Next, Lonza will further develop the process into a GMP-ready manufacturing process that can be tech transferred successfully into a GMP manufacturing facility. This will enable MSC-EVs to be used for clinical trials, both for follow-up programs of EVPRO, but also for other potential applications of MSC-EVs.

ACKNOWLEDGEMENTS

This project has received funding from the European Union's Horizon 2020 research and innovation action under grant agreement No. 814495-EVPRO (www.evpro-implant.eu)

A Novel Resorbable Polyester, Tailor-Made to Support as a Knitted Temporary Support for Primary Ventral & Incisional Hernia Repair

Robert Vestberg*, Julie Lecuivre, Emilie Payet, Amandine Radlovic, Yves Bayon

Medtronic – Sofradim Production, Trévoux, France
*robert.vestberg@medtronic.com

INTRODUCTION

Medtronic developed Transorb™ Self-Gripping Resorbable Mesh (Transorb™), a new fully resorbable synthetic mesh to reinforce soft tissue where weakness exists in procedures involving ventral hernia repair. The mesh is a macroporous mesh knitted from absorbable monofilament – composed of poly-L-lactide, poly-trimethylene carbonate copolymers (PLLA/TMC, 80/20 mol/mol). This polymer is designed as a triblock copolymer with the A-B-A' structure wherein the A and A' blocks each include polylactide, the B block includes polytrimethylene carbonate and polylactide. It was selected among a series of custom-made copolymers of lactide and trimethylenecarbonate and copolymers of lactide and caprolactone. It displayed the highest mechanical fatigue resistance profile, under cyclic loading conditions (Patent EP3628698A1, Walter et al., 2018).

EXPERIMENTAL SECTION (Fig.1)

PLLA/TMC was extensively characterized. Notably, its degradation profile as a mesh was investigated in real-time (37°C) and accelerated aging conditions, in Sorensen's phosphate buffer, at pH 7.4. Size exclusion chromatography (SEC) was used to monitor the molar changes over time in Mn and Mw. This was superimposed on the loss of mechanical properties, assessed by tensile tests, at same time points.

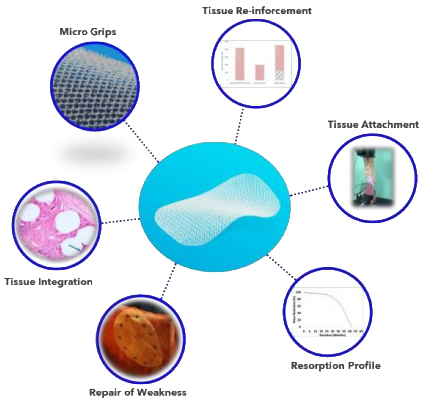
Using the minipig model of abdominal wall weakness (published by Martin et al, 2013 & Deeken et al, 2013), all abdominal wall defects were healed at 20 weeks after reinforcement with the new mesh. This was checked by a series of histology staining with semi-quantitative scoring of inflammation and new extracellular matrix formation. Additionally, the new mesh delivered statistically higher maximum force in the ball burst test when compared to the native abdominal wall and the in vivo performance of the new mesh was aligned with its behavior observed in an in vitro model of cyclic mechanical loading testing, mimicking the in vivo mechanical loading of the abdominal wall.

Transorb™ mesh include the ProGrip™ technology, i.e. microgrips reducing the need for traditional tack or suture fixation in the abdominal wall. The Transorb™ mesh attachment strength to tissues was also found to be statistically equivalent to the ProGrip™ Self-Gripping Polyester Mesh, the legacy non-resorbable ProGrip™ mesh (base of the knit in polyethyleneterephthalate and microgrips in polylactide), in a swine model. This should support the ease-of-use feature of the mesh, allowing its positioning on the hernia defect without additional fixation means. It should then save time in the operating room.

CONCLUSION

The preclinical tests of Transorb™ Self-Gripping Resorbable Mesh are compelling enough to clearly underline its clinical potential/benefit. Clinical evaluations of the mesh are planned.

Fig.1 Design & Evaluation of Transorb™ Self-Gripping Resorbable Mesh



>> 09:45 - 11:15 | EUR S - EUROPEAN PROJECTS SYMPOSIA
Chairpersons: Nicolas Blanchemain & Cristina Barrias
Location: Room C

9:45 | Eur S 01 - Open innovation test beds, a tool for the effective validation of new technologies on medical devices
Iraida LOINAZ, CIDETEC, Basque Research and Technology Alliance (BRTA), Donostia - San Sebastián, Spain, Donostia - San Sebastián, Spain

10:00 | Eur S 02 - cmRNAbone project: 3D Printed-Matrix Assisted Chemically Modified RNAs Bone Regenerative Therapy for Trauma and Osteoporotic Patients
Matteo D'ESTE, AO Research Institute Davos, Davos, Switzerland

10:15 | Eur S 03 - From the Teaming Phase 2 project Baltic Biomaterials Centre of Excellence consortium
Dagnija LOCA, Rudolfs Cimdins Riga Biomaterials Innovations and Development Centre of Riga Technical University, Riga, Latvia

10:30 | Eur S 04 - From pathobiology to synovia on chip: driving rheumatoid arthritis to the precision medicine goal (FLAMIN-GO)
Annalisa CHIOCCHETTI, Department of Health Sciences, Interdisciplinary Research Center of Autoimmune Diseases-IRCAD and Center for Translational Research on Autoimmune and Allergic Disease-CAAD, Università del Piemonte Orientale, 28100 Novara, Italy; Trusteck s.r.l. (TRUSTECK, Italy); Max Planck Institute (MPG, Germany); Science on the Street (SoS, Slovenia); REGENHU (reH, Switzerland) and EU CORE Consulting Srl (EUCORE, Italy) , Italy, Italy

10:45 | Eur S 05 - EVPRO - Development of Extracellular Vesicles loaded hydrogel coatings with immunomodulatory activity for Promoted Regenerative Osseointegration of revision endoprosthesis
Claudia SKAZIK-VOOGT, Precision engineering and Automation, Fraunhofer Institute for Production Technology, Aachen, Germany

11:00 | Eur S 06 - Background, Success and Failures to get an ERC
Riccardo LEVATO, Utrecht University, Utrech, The Netherlands

Open innovation test beds, a tool for the effective validation of new technologies on medical devices

Iraida Loinaz[1*]
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ABSTRACT

Europe's healthcare system faces two major problems: the wide gap in outcomes between patients and the ever-increasing costs of treatments, which result in an urgent need to create and incorporate value in healthcare. However, the new regulation with its strong focus on demonstrating safety and performance of novel medical devices (MD) together with the long and complex reimbursement processes, impose great challenges specially on highly innovative Small and Medium-sized Enterprises (SMEs) to maintain their competitiveness and innovation capacity in global competition.
The EU research project TBMED has been conceived within this framework and aims to create an Open Innovation Test Bed (OITB) for the development of high-risk medical devices. The main goal is to establish an acceleration platform to increase the access of high-risk MD to patients by facilitating their development and market introduction, while maintaining high quality standards. For that purpose, expert support will be provided from an early development stage to the optimised transformation of prototypes into valuable and innovative products. In that way, the platform will cover aspects related to technological services as prototyping, manufacturing and characterization, testing and regulation, alongside other aspects related to business development and market advise services. Therefore, it will provide services to cover the entire value chain of transforming a prototype into an innovative product and the access to these services will be facilitated by providing a single entry point.

This entire process will be performed by adapting the Quality-by-Design (QbD) approach currently used in the pharma industry. The QbD concept enhances product and process understanding together with process control, based on robust scientific knowledge and quality risk management. The use of statistically designed experiments for process validation will reduce the costs and variability of the manufacturing process. Counselling and advisory sessions with experts on clinical investigations and an advisory health technology assessment (HTA) team will make sure that important evidence on the safety and efficacy of the new devices and adequate comparators is generated during preclinical development.

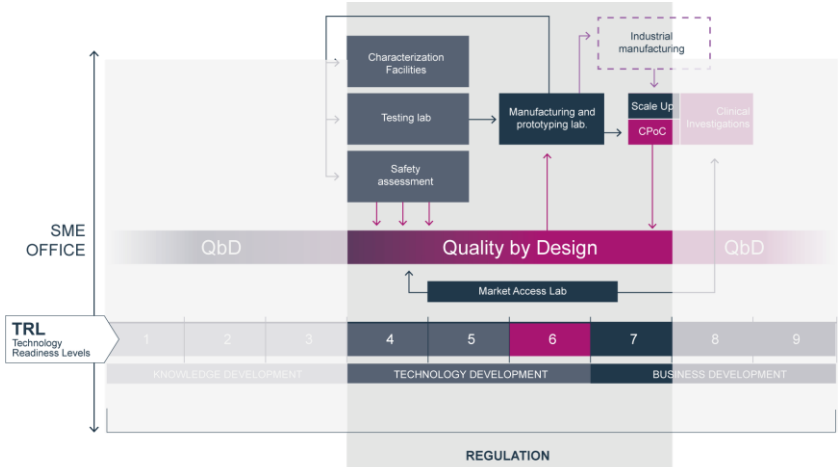

Figure 1: Structure of the Open Innovation Testing Bed

The established OITB will support highly innovative SMEs, companies that are interested in screening new MedTech developments or that want to expand into the Medical Devices sector, companies that need multicentered clinical trials or that need a re-assessment of their marketed products and companies with a Medical Device marketed outside EU, that want to obtain a CE mark.

TBMED project is currently being validated by providing services to 8 case studies, 4 of them internal to the project, and another 4 brought into the project through several Open Calls. Each of these case studies is at a different stage of the development process and the deliberate choice of these 8 cases has been made to cover a variety of devices and the whole development process, from TRL 3 to clinical trials (TRL 6/7). This will facilitate the development of an OITB suitable for a broad range of applications thus turning TBMED into a one-stop shop providing med-tech companies with open access at fair conditions.

cmRNAbone project: 3D Printed-Matrix Assisted Chemically Modified RNAs Bone Regenerative Therapy for Trauma and Osteoporotic Patients

Matteo D'Este[1], Daphne van der Heide[1, 2], Joëlle Amédée[3], Martin James Stoddart[1]

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[3] Inserm U1026, université de Bordeaux, Bioingénierie tissulaire, 33076 Bordeaux Cedex, France

INTRODUCTION

Due global ageing and changes in lifestyle, with people being increasingly physically active at later stages of life, bone traumatic injuries and fragility fractures caused by osteoporosis present a vast medical and socio-economic challenge. Available therapies are insufficient to provide reliable bone regenerative solutions.
The cmRNAbone project aims to create a novel bone regenerative therapeutic approach based on combination of chemically modified RNAs (cmRNAs)-vectors embedded in a 3D-printed guiding biomaterial ink tailored to patients need.

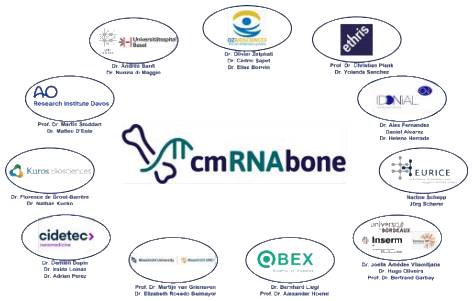
EXPERIMENTAL METHODS

To achieve our goal, sema3a, vegf, pdgf-bb and bmp7 cmRNAs targeting neurogenesis, vasculogenesis and osteogenesis are being synthesized and tested. For cmRNAs preservation and delivery, we are developing vectors based on lipids and polysaccharide nanocapsules. A functional Hyaluronan-Calcium Phosphate biomaterial ink has been developed according to the following design inputs: 1) can be loaded with cmRNAs-vectors to release them, and 2) having intrinsic osteoinductivity and presenting laminin-derived peptides for guiding sensory neurons and endothelial cells ingrowth, and 3) satisfying the rheological and shape-retention properties for extrusion-based 3D-bioprinting. As part of the project, a customized extrusion-based 3D printer has also been developed for fabrication of patient specific constructs.
A large effort is being carried out on deciphering regenerative mechanisms and optimizing dosage and ratio of cmRNAs, loading of cmRNAs-vectors in the ink, 3D-printing, etc, to demonstrate regenerative capabilities *in vitro* and *in vivo.* Lead formulations identified will be tested on rat models of long bone defect in nude rat as well as in rats with induced osteoporosis, using autograft as a control. To increase the robustness of the results, *in vivo* testing will take place at different project partners. The custom-made 3D printer being developed in the project will be installed at the preclinical facilities and used to produce the constructs to be implanted.
Each task of the project is being implemented with an overreaching attention on using methods, procedures, materials which are compatible with production under a regulated environment with the vision of a 1st in human trial. Towards this goal, partners facilities undergo audits and input is being received from clinical experts support group to ensure that Good Manufacturing Practice-like production for all regenerative tools, and regulatory and commercial strategies are realized.

RESULTS AND DISCUSSION

To achieve this ambitious plan, a consortium of 11 partners was gathered bringing in expertise spanning from biomaterials science, bone biology, chemical modification or RNA, delivery of genetic material, regulatory expertise, technology of 3D printing devices, management (Fig 1). The partners are: the AO Research Institute Davos, University of Basel, ETHRIS GmbH, EURICE - European Research and Project Office GmbH, CIDETEC, Kuros Biosciences BV, OZ Biosciences SAS, IDONIAL Technological Center, Qbex GmbH, Maastricht University, University of Bordeaux.

Fig. 1: *Partners involved in the cmRNAbone project*



CONCLUSION

The cmRNAbone project proposes a regenerative therapeutic approach providing robust spatial control of therapeutic bioactivity based on combination of novel cmRNA-vector embedded in an advanced biomaterial ink tailored to patients need and 3D-printed in the clinic. We aim to open new avenues in regenerative medicine for patients suffering from bone disorders and beyond, by establishing technological platforms to be applied to other clinical needs.

ACKNOWLEDGMENTS

This project has been made possible thanks to funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 874790. We express deep thanks to Prof. David Eglin for gathering this team and bring this idea into reality.

From the Teaming Phase 2 project Baltic Biomaterials Centre of Excellence consortium

Dagnija Loca

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INTRODUCTION

BBCE – Baltic Biomaterials Centre of Excellence

BBCE overall objective is to establish a joint research centre for advanced biomaterials development by three internationally recognized research institutions RTU, LIOS, RSU and clinical partner RSU IS operating in Latvia with a support from the international leading research institutes ARI, Switzerland and FAU, Germany, which main tasks in the project is support for research, research commercialization, knowledge transfer and management.

Strategic research direction of BBCE is development of patient specific personalized solutions for bone regeneration in 3 levels: biomaterial composition, geometry and bioactive compound delivery.

BBCE ensures full cycle of biomaterials for bone regeneration development, starting from the material design and characterization to preclinical investigations and clinical trials.

Within the project 6 scientific focus groups are established and all staff members are involved in wide range of training activities, including new methodologies on material development and analysis, IP protection, technology transfer and dissemination of open science.

Webpage: https://bbcentre.eu/

PROJECT COORDINATOR

Riga Technical University (RTU), Latvia

PROJECT PARTNERS

- Latvian Institute of Organic Synthesis (LIOS), Latvia Rīga Stradiņš University (RSU), Latvia
- LTD "Rīga Stradiņš University Institute of Stomatology (RSU IS), Latvia
- AO Research Institute Davos (ARI), Switzerland
- Friedrich- Alexander University of Erlangen-Nuremberg (FAU), Germany

ACKNOWLEDGMENTS

The authors would like to thank the EU Horizon 2020 Programme WIDESPREAD-01-2018-2019 (Grant agreement No. 857287).

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From pathobiology to synovia on chip: driving rheumatoid arthritis to the precision medicine goal (FLAMIN-GO).

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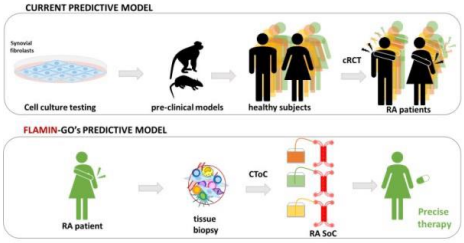
INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune inflammatory disease affecting more than 3 million people in the EU[1]. RA is characterized by joint inflammation leading to impaired mobility and chronic pain. No definitive cure exists for RA and there are no biomarkers of individual treatment response. Though several RA treatment options are available, 40% of RA patients fail to achieve an improvement in disease activity[2]. Thus, innovative trials and new drugs for RA represent an urgent clinical unmet need. FLAMIN-GO's project aims at developing a personalized next-generation joint-on-chip (JoC), that by effectively mimicking the complexity of a rheumatoid arthritic joint, created from each patient's tissues, will permit patient-specific clinical trials-on-chip (CToC). FLAMIN-GO's Consortium comprises 14 European partners.

RESULTS AND DISCUSSION

We will design and fabricate a multi-compartment microfluidic platform, for 3D culturing and perfusion of all the disease-relevant joint tissues, focusing on synovia which is the pathogenetic targeted tissue in RA. Our platform will also include the immune system (leukocytes) who sustains the disease, as well as cartilage and bone which are the end damaged tissues, leading to permanent disability. The integration with a lab-on-chip and several sensors will allow to monitor continuously culturing conditions and response to drugs. The figure below depicts the FLAMIN-GO's concept.



CONCLUSION

This personalized *ex vivo* model will be used for the screening of multiple commercial drugs, allowing the selection of the most adequate treatment for each RA patient.

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EVPRO - Development of Extracellular Vesicles loaded hydrogel coatings with immunomodulatory activity for Promoted Regenerative Osseointegration of revision endoprosthesis

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INTRODUCTION

Primary joint replacements can lead to aseptic loosening at the implant-bone interface as a result of chronic inflammatory processes caused by abrasion and corrosion[1]. Due to the chronic inflammatory changes in the bone or former implant site, the normal service life of a second, so-called revision prosthesis is considerably shortened. Medically managing the inflammation process is challenging as it is difficult to introduce drugs to the site of inflammation after implantation of the revision endoprosthesis. EVPRO overcomes this problem by developing a novel bio-instructive and adaptive coating for hip revision endoprosthesis able to control inflammation at the original anatomical location of the removed endoprosthesis and promote bone regeneration.

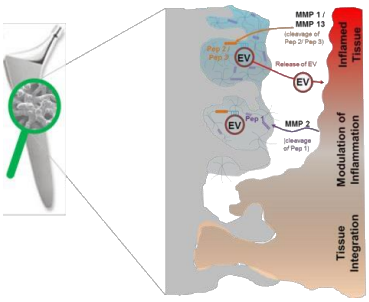
EXPERIMENTAL METHODS

EVPRO will use mesenchymal-stem/stromal-cell-derived extracellular vesicles (MSC-EVs), which have been found to promote comparable therapeutic activities as MSCs including anti-inflammatory effects[2,3]. In addition pro-regenerative effects of MSC-EVs in osteogenic settings have been reported[4]. The MSC-EVs will be encased in a biodegradable hydrogel coating grafted onto a microporous titanium dioxide (TiO_2) surface of the implant to control inflammation and enhance osseointegration.

RESULTS AND DISCUSSION

To realize such a major breakthrough we will introduce a new paradigm for anti-inflammatory coatings with the following functions: (i) mechanical interlocking of implant surface to bone, (ii) modulation of local inflammation in a self-adaptive manner and (iii) promotion of osseointegration. The figure below depicts EVPRO´s concept. To achieve these aims the following points will be addressed

1. Development of a GMP conformed large scale production of MSC-EVs based on the use of hollow fiber bioreactors (HFBR)
2. Establishing of an online quality check to select the most potential MSC-EVs using a microfluidic Lab-on-a-Disc (LoaD). The LoaD platform will integrate immuno-affinity methods with optical detection of EV profiling markers.
3. Production of implant protypes using additive manufacturing 3D printing technology Tritanium, mimicking the cancellous bone. In a second process step an electrolytic, plasma-induced process (PEO) is executed to generate the microstructure and nano-roughness.
4. The hydrogel is designed to prevent collapse, provide mechanical stability and control the release of MSC-EVs. This will be achieved using macromolecular building blocks cross-linked by peptides degradable by metalloproteinases, which are overexpressed during inflammation. Hence, the response of the coating is self-regulated.
5. Assessment of the functionality of the newly developed implant prototypes in vitro. The proof-of-concept will be obtained in vivo in a rat model



CONCLUSION

The EVPRO project is an integration of multidisciplinary emerging technology concepts which enables the successful resolution of the enhanced osseointegration of endoprotheses for osteo-articular tissue regeneration.
With impact beyond EVPRO, the actions will enables establishing feasible solutions for novel MSC-EV-biomaterial technologies that instruct and control tissue (re)generation.

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Background, Success and Failures to get an ERC

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PRESENTATION

The field of biomaterials is continuously evolving, and an increasing number of avenues for early career scientists are emerging, also thanks to the key role of biomaterial science in novel branches of regenerative medicine, biomedical research, biofabrication, 3D tissue culture and organoid technology.

In this session, we will discuss challenges, opportunities and some first hand experiences in the preparation of project proposals for competitive research funds, with a particular attention to the ERC funding scheme, which is uniquely characterized by its focus on scientific excellence and high-risk/high-gain projects.

ACKNOWLEDGMENTS

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>> 09:45 - 11:15 | SYMP-09 - ELECTRICAL STIMULATION AND CONDUCTIVE BIOMATERIALS IN TISSUE ENGINEERING: ADVANCES AND CHALLENGES

Chairpersons: Sahba Mobini & María Ujué González
Location: Room H

09:45 | KL Conductive biomaterials - Conducting biomaterials for regenerative bioelectronics – a story of electrochemistry
Maria ASPLUND, Department of Microsystems Engineering (IMTEK) , University of Freiburg; BrainLinks-BrainTools Center, University of Freiburg; Division of Nursing and Medical Technology, Luleå University of Technology; Department of Microtechnology and Nanoscience, Chalmers University of Technology; Freiburg Institute for Advanced Studies (FRIAS), University of Freiburg , Freiburg, Germany

10:15 | O1 Conductive biomaterials - Elongation-induced Crystallization by One-step Melt-spinning of Poly (L-lactic Acid) Fibers for Future Piezoelectric Bioapplications
Richard SCHÖNLEIN, 1) POLYMAT and Polymers and Advanced Materials: Physics, Chemistry and Technology, Faculty of Chemistry, University of Basque Country UPV/EHU, Donostia-San Sebastian, Spain; 2) POLYMAT and Department of Mining-Metallurgy Engineering and Materials Science, Faculty of Engineering in Bilbao, University of Basque Country UPV/EHU, Bilbao, Spain

10:30 | O2 Conductive biomaterials - Electroconductive Scaffolds Based on Gelatin and PEDOT:PSS for Cardiac Regenerative Medicine
Franco FURLANI, National Research Council of Italy - Institute of Science and Technology for Ceramics (ISTEC-CNR), Faenza (RA), Italy

10:45 | O3 Conductive biomaterials - Nanocellulose Composite Wound Dressings with Integrated pH Sensing Capabilities for Detection of Wound Infections
Elisa ZATTARIN, Laboratory of Molecular Materials, Division of Biophysics and Bioengineering, Department of Physics, Chemistry and Biology, Linköping University, Linköping, Sweden

11:00 | FP01 Conductive biomaterials - Novel Pulsed Electrodeposition Method for Hybrid Conductive Soft Hydrogel based on PEDOT/Alginate for Versatile Drug Delivery
Arua DA SILVA, Implantable Bioelectronics Laboratory. Department of Automatic Control and Systems Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

11:05 | FP02 Conductive biomaterials - The interplay of collagen/bioactive glass nanoparticle coatings and electrical stimulation regimes distinctly enhanced osteogenic differentiation of human mesenchymal stem cells
Poh Soo LEE, Institute of General Electrical Engineering, University of Rostock, Rostock, Germany; Max Bergmann Centre of Biomaterials, Technische Universität Dresden, Dresden, Germany

11:10 | FP03 Conductive biomaterials - Electrohydrodynamics based functional nanofibers electrically stimulate neuron regeneration
Menglin CHEN, Department of Biological and Chemical Engineering, Aarhus University, Aarhus, Denmark

Conducting biomaterials for regenerative bioelectronics – a story of electrochemistry

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INTRODUCTION

Bioelectronic medicine refer to technology which use electrical signals, either recorded from or injected into tissue, for therapeutic effect. In the central nervous system, applications range from non-invasive modulation of brain rhythms to implantable systems, even with the capability to selectively interface specific brain cells. In the peripheral nervous system, electrical stimulation has for long been used to treat chronic pain. More recently prosthetic limbs with artificial sensory feedback have been demonstrated or neurostimulation for motor restoration after spinal cord injury[1]. To date, research almost exclusively focus on the nervous system, to large extent overlooking the potential that bioelectronic medicine has to "medicate" also other tissues. For instance, the possibility to use electrical fields to guide regenerative processes deserves more attention.

Most mammalian cells exhibit some reactivity towards electrical fields, for instance aligning their growth (electrotrophy) or migration (electrotaxis) with the field applied. Different cells respond at different thresholds and some cells electromigrate towards the anode, while others towards the cathode. Directionality may even change depending on the field strength. To make things even more complex the same cells may behave differently depending on the type of culture, e.g. migration of solitary cells follow different rules that migration within a confluent cell layer.

Electromigratory and electrotrophic effects are most commonly studied *in vitro* and only a limited number of studies have fully taken the step to follow up promising *in vitro* data with actual *in vivo* electrical field stimulation. A reason for this is the lack of electrode materials that can cope with direct current stimulation directly in tissue. While neurostimulation typically involves impulses of a few hundred microseconds, electromigratory effect of cells may need hours of continuous electrical fields to fully develop. Thus, the signals needed to control growth and migration of cells, are more demanding for the electrode materials or, at least, require different characteristics. Based on this, I will talk about the necessity to move beyond metals, in order to support the development of regenerative bioelectronic therapies.

EXPERIMENTAL METHODS

Over the last ten years we have developed a range of techniques and materials suitable for stimulation in tissue. We have explored the extraordinary properties many of these materials have *in vitro* but also with implantable systems. Our materials portfolio is to large extent based on conducting polymers which we tailor to match specific applications. An interesting alternative is IrOx, which in its sputtered form have a variety of reactions that can support even DC current directly in tissue[2]. Last but not least, conducting carbon in the form of laser induced graphene (LIG) open up for completely new fabrication methodologies and corrosion free electrodes.

RESULTS AND DISCUSSION

I will talk about how electrical stimulation may be a possibility to accelerate repair of skin wounds, and why the choice of electrode materials is crucial for the success of such concepts. I will furthermore show how it is possible to translate such concepts to implantable regenerative bioelectronics, which potentially could guide regeneration of complex tissues such as the spinal cord.

CONCLUSION

Development of conducting biomaterials are key ingredients to unleash the full potential of future bioelectronic medicine.

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Elongation-induced Crystallization by One-step Melt-spinning of Poly (L-lactic Acid) Fibers for Future Piezoelectric Bioapplications

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INTRODUCTION

Piezoelectric poly (L-lactic acid) (PLLA) fibers with high molecular chain orientation are already applied in biomedical fields like biosensors or scaffolds for tissue regeneration.[1] Since fast relaxation reduces chain orientation while spinning fibers directly out of melt[2], two-step processes such as hot drawing of solid state fibers[3] is usually employed to induce piezoelectric properties. A one-step fabrication process of piezoelectric fibers directly out of melt is not documented in literature. In this work, supercooling of melt is applied to increase molecular chain relaxation time, preserving the orientation obtained by melt elongation. The aim is to achieve high molecular chain orientation to produce melt-spun piezoelectric PLLA fibers in a one-step process.

EXPERIMENTAL METHODS

PLLA with a D-isomer content of about 1.6 % (NatureWorks) was used to fabricate fibers out of melt via capillary rheometer (GÖTTFERT 25) with a rheotens unit. A supercooling, ΔT_{sup}, of 30 Kelvin below process temperature of 180 °C and elongation rates, $\dot{\varepsilon}$, from 0.2 to 66 s^{-1} was applied. The crystallinity was analyzed by differential scanning calorimetry (DSC) and wide-angle X-ray diffraction (WAXD). The molecular chain orientation has been examined by polarized optical light microscopy (PLOM).

RESULTS AND DISCUSSION

The fiber diameter ranges in between 111 and 553 µm depending on the elongation rate. DSC and WAXD measurements indicate that without supercooling ($\Delta T_{sup} = 0$ K) the fibers at low ($\dot{\varepsilon} = 0.2\ s^{-1}$) and high ($\dot{\varepsilon} = 66\ s^{-1}$; Figure 1a, top) elongation rate are amorphous. However, at a supercooling of 30 K at elongation rates of 29 s^{-1} and higher, elongation-induced crystal domains are generated (Figure 1a, center). According to WAXD spectrum, with increasing elongation rate the fraction of low dense-packed α' form decreases and the dense-packed α form increases. PLOM images in Figure 1b show that melt-spun fibers without supercooling (amorphous) exhibit no birefringence, whereas supercooling (elongation-induced crystal domains) leads to birefringence. Thus, the molecular chain orientation is increased and preserved due to crystallization. We suppose that the elongation-induced crystallization is favored due to the reduction of relaxation behavior by supercooling preserving already oriented molecular chains that act as crystal nuclei.

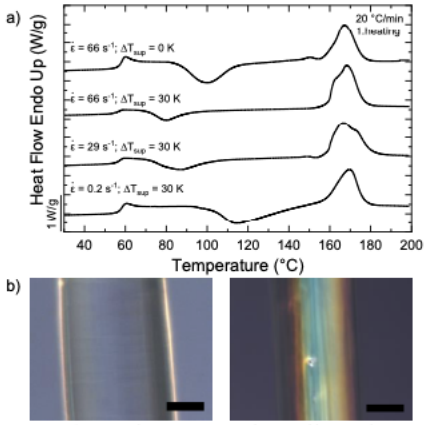

Figure 1. a) DSC 1.heating curves of PLLA fibers melt-spun at elongation rates, $\dot{\varepsilon}$, of 0.2 s^{-1}, 29 s^{-1} and 66 s^{-1} and at supercooling, ΔT_{sup}, of 0 and 30 K. b) PLOM image of melt-spun PLLA fiber with elongation rate, $\dot{\varepsilon}$, of 66 s^{-1} without (left) and with (right) supercooling (scale bar: 50 µm).

CONCLUSION

Elongation-induced oriented crystal structure in melt-spun PLLA fibers was achieved by the combination of high elongation rate ($\dot{\varepsilon} \geq 29\ s^{-1}$) and supercooled melt ($\Delta T_{sup} = 30$ K). The latter was able to preserve the orientation and to induce crystal form. The obtained orientation and crystal form within the fiber may enhance piezoelectricity, what will be examined in future works. One-step melt-spinning of piezoelectric PLLA fibers directly out of melt is a promising approach for biosensor and tissue regeneration applications.

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Electroconductive Scaffolds Based on Gelatin and PEDOT:PSS for Cardiac Regenerative Medicine

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INTRODUCTION

Cardiac tissue is an excitable system which is frequently damaged by diseases and display poor self-healing ability[1]. Recently, electroconductive materials emerged to support recovery of the degenerated electroconductive tissues by enhancing cell growth, adhesion and formation of functional networks[2]. Specifically, regenerative medicine approaches require suitable biomaterials able to mimic natural tissues properties, like biochemical milieu, spatial composition, electroconductivity and mechanical performance. Scaffolds are three-dimensional networks able to mimic the natural Extra Cellular Matrix (ECM) and gelatin is a biopolymer, derived by the denaturation of collagen, currently exploited to fabricate ECM mimics, since display most – excluding electroconductivity – of the above-mentioned properties. Simultaneously, attention was devoted to the development and the possible use of different conductive polymers, *i.e.* polymers able to conduct electrons, including PEDOT and poly-pyrrole, for regenerative medicine. Nevertheless, these conductive polymers can be exploited to fabricate mainly films[3]. On the other hand, conductive polymers can be combined with other polymers to develop hybrid three-dimensional conductive biomaterials[4].

In the present study we report an original method to form electroconductive scaffolds based on gelatin and PEDOT:PSS that can be implanted into the lesion of electroconductive tissues and to support regeneration.

EXPERIMENTAL METHODS

A liquid mixture containing gelatin from porcine skin and different PEDOT:PSS concentrations was transferred in Teflon molds and freeze-dried promoting scaffolds formation. Resulting scaffolds were stabilized by dehydrothermal (DHT) treatment at 160°C in vacuum conditions. Electroconductive properties of resulting materials were assessed by Electrochemical Impedance Spectroscopy (EIS), whereas the chemical structure was investigated by FTIR-ATR analyses. Mechanical properties of resulting scaffolds were investigated by compression tests by Dynamic Mechanical Analyses (DMA). The scaffolds stability and swelling ability were assessed by weighting samples after the incubation in physiological-like conditions up to 21 days. Scaffolds were then seeded with rat cardiomyoblasts cells (H9C2 cells). Cell viability and cell proliferation were assessed by Presto Blue assay at different timeframes up to 14 days. The cell viability was also confirmed by Live/Dead assay. On the other hand, the cell morphology and spreading ability were investigated by fluorescent microscopy analyses (after nuclei and cytoskeleton labelling) and by Scanning Electron Microscopy (ESEM) assay at different timeframes up to 14 days. Gene expression profile of cardiomyoblasts seeded on different scaffolds was finally investigated by real time PCR analysis.

RESULTS AND DISCUSSION

Dehydrothermal (DHT) treatment promoted the formation of bounds between gelatin and PEDOT:PSS and the formation of scaffolds endowed with a good interconnected porosity. Resulting scaffolds displayed an excellent stability and a good swelling ability in physiological-like conditions. Additionally, the presence of PEDOT:PSS enhanced the electroconductivity of resulting materials. On the other hand, the presence of PEDOT:PSS did not affect the mechanical performance of resulting scaffolds. Specifically, all scaffolds displayed a similar Young modulus comparable to mechanical performance of native electroconductive tissues[5]. All scaffolds did not show any cytotoxic effect towards cardiomyoblasts. The presence of PEDOT:PSS within the scaffolds promoted the cell adhesion, spreading and proliferation at early timeframes. At later timeframes (more than 7 days) the presence of PEDOT:PSS partially limited the cell proliferation, however the cells, grown on the scaffold, colonized the inner part of the 3D biomaterial showing their typical morphology.

CONCLUSION

This study showed an original method to fabricate electroconductive and biomimetic scaffolds based on gelatin and PEDOT:PSS. The presence of electroconductive polymers did not affect the mechanical performance of resulting scaffolds, but enhanced the conductivity of resulting materials. All the scaffolds displayed an excellent stability and resulted to be non-cytotoxic towards cardiomyoblasts. Additionally, the presence of PEDOT:PSS enhance cell proliferation.

These scaffolds can be proposed as implantable and biomimetic biomaterials for regeneration of electroconductive tissues, especially cardiac tissue, and as a promising 3D tissue model for *in vitro* biomolecules screening.

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Nanocellulose Composite Wound Dressings with Integrated pH Sensing Capabilities for Detection of Wound Infections

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INTRODUCTION

Wound infections are generally diagnosed by visual inspection, which requires the infection to have progressed to a level where clinical symptoms such as redness, swelling, pus and pain are clearly present[1]. At this stage, the microbial load is high, and the infection can be very difficult to treat. Early-stage detection and diagnosis of wound infections can enable a more efficient treatment and reduce the risk of developing non-healing wounds[2]. Infections typically trigger an increase in wound pH from slightly acidic pH (pH 4-6) to elevated alkaline values (pH 7-9), which tends to be manifested before other symptoms[3,4]. The integration of a pH sensor within the wound dressing would therefore facilitate monitoring of the wound status and improve treatment outcome.

Herein we present nanocellulose-based composite wound dressing materials with integrated colorimetric pH sensors for rapid and cost-effective real-time monitoring of wound pH, which allow for a direct readout by the patient or the healthcare provider.

EXPERIMENTAL METHODS

The pH responsive materials were fabricated by incorporating mesoporous silica nanoparticles (MSN) in the nanofibrillar network of bacterial cellulose-based (BC) wound dressings by self-assembly. A pH-responsive dye, bromothymol blue (BTB) was loaded and immobilized in the MSNs.

RESULTS AND DISCUSSION

The self-assembly process allowed for controlling sensor material characteristics, including a precise tuning of the amount of MSN in the BC. The incorporation of MSNs led to a major increase in specific surface area compared to pure BC, from 88 m^2/g to 469 m^2/g. The BTB loading could further be adjusted, producing materials with optimized color intensity to facilitate sensor readout. The composite material retained the favorable wound dressing properties of BC, such as excellent water retention and high conformability, allowing for moist wound healing and an intimate contact between the dressing and the wound tissue, respectively. Moreover, the composite materials show an increase in exudate holdup capacity (+35%) and moisture retention compared to BC. The fabrication technique is scalable and poses no restrictions on the size of the dressing, which make them suitable for pH sensing of both small and large wound areas. Furthermore, the addition of the MSNs reinforces the bacterial cellulose dressing, conferring them capacity to withstand high compressive forces without significant volume loss.

The composite materials allow for rapid sub-mm spatial pH discrimination, with a color change occurring within seconds of application. The color change is reversible, enabling monitoring of dynamic changes in wound pH. A quantitative pH map can be acquired using UV-Vis spectroscopy. The pH sensor performance was demonstrated in a porcine burn wound model infected with S. aureus (Figure 1), showing its effectiveness to rapidly discriminate infected from non-infected wounds.

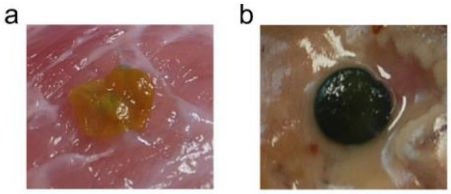

Figure 1. In vivo porcine wound model. pH responsive wound dressings applied on (a) non-infected wound, and (b) a wound infected with Staphylococcus aureus. Photographs were recorded 1 minute after application of the dressings.

CONCLUSION

We present a flexible and robust pH sensor with spatial resolution and clear readout both in vitro and in vivo.

The possibilities to create well-defined composites of two high-surface-area biomaterials can further enable the development of a wide range of devices for therapeutic and diagnostic applications for advanced wound care applications.

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This work was supported by the Swedish Foundation for Strategic Research (SFF) grant no. FFL15-0026 and framework grant RMX18-0039 (HEALiX), the Swedish Government Strategic Research Area in Materials Science on Functional Materials at Linköping University (Faculty Grant SFO-Mat-LiU no. 2009-00971), the competence center FunMat-II that is financially supported by Vinnova (grant no. 2016-05156), and the Knut and Alice Wallenberg Foundation (grant no. KAW 2016.0231).

Novel Pulsed Electrodeposition Method for Hybrid Conductive Soft Hydrogel based on PEDOT/Alginate for Versatile Drug Delivery

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INTRODUCTION

Conductive soft hydrogels offer a promising approach to either delivery specific molecules *in locu* or probe electrical signals from living tissue, being greatly important in bioelectronics applications[1].
In a previous work, our research group proposed a hybrid PEDOT/Alginate conductive soft hydrogel. However, using step potential to obtain the hybrid was very limited due to diffusional limitation at the electrode interface[2].
Herein, we are showing a novel electrodeposition pulsed method to 1) enhance the conductivity of the hydrogel and 2) homogeneously obtain the hydrogel layer. The hybrid conductive soft hydrogel was loaded with model drug for passive and electroactive release studies.

EXPERIMENTAL METHODS

Herein we developed a pulsed electrodeposition method to obtain an enhanced conductivity in the hybrid hydrogel. For this, we prepared the deposition solution containing 1% alginate (w/w), 0.5% calcium carbonate (w/w), 70 mM sodium dodecylsulfate (SDS) and 50 mM 3,4-ethylenedioxythiophene (EDOT). Using a gold wire as electrode, we applied +1.4 V (vs. Ag/AgCl/KCl 3M) for 500 miliseconds, followed by either 10 seconds resting at open circuit potential (OCP) or at 0.0V (vs. Ag/AgCl/KCl 3M). We used the traditional electrodeposition method of applying step potential for a time and just EDOT without alginate for comparison. When the electric potential is applied, it promotes the electrodeposition reaction of EDOT, which triggers other two chemical steps to obtain the alginate hydrogel. This mechanism is known as electrochemical-chemical-chemical (ECC) (**Figure 1**). We characterized its electrical properties by using cyclic voltammetry and electrochemical impedance spectroscopy. For the electroactive release investigation, we added 1 mg/mL of Fluorescein (model molecule for drug delivery) to the solution and encapsulated together with the hydrogel formation.

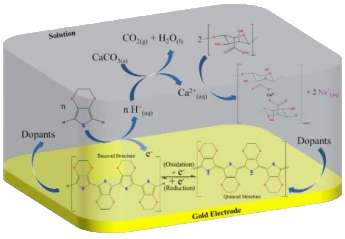

Figure 1. Schematic representation of the ECC mechanism for electrodeposition of PEDOT/Alginate hydrogel.

RESULTS AND DISCUSSION

The pulsed method enabled fine control of hydrogel growth kinetics. From 1 to 120 polymerization pulses it follows order zero ($t_{1/2}$ = 22 pulses), while after 120 pulses it follows first order ($t_{1/2}$ = 252 pulses) growth kinetics. The hydrogel produced using OCP pulsed protocol generated the most electroactive hydrogel. It presented lower total impedance and higher charge storage capacitance. Additionally, we were able to control the crosslinking of the alginate layer to promote either a dense (1.5-3% calcium carbonate) or a loose (0.5-1% calcium carbonate) hydrogel. Based on this step, the alginate layer can be disassembled within 2-3 hours (loose) or resist decomposition for more than 36 hours (dense) in PBS solution. The conductive soft hydrogel can be loaded with Fluorescein for releasing study (**Fig. 2**). In the passive release, we used the conductive property of the hydrogel as a sensor, monitoring the capacitance and correlating it to the passive release of desired molecule, with excellent correlation between the capacitance and the amount of molecule quantified by fluorescence. Furthermore, we demonstrate the electroactive release is fully controlled by electric potential. Full release (100%) when -1.2V and full holding (0%) when +0.8V was applied for 40 minutes. Additionally, intermediate gradient release was achieved by applying pulses at -1.2V was applied for 3, 8 and 30 seconds.

Figure 2. Picture of the PEDOT/Alginate hydrogel loaded with Fluorescein.

CONCLUSION

In the current work we described a novel pulsed method of obtaining a hybrid conductive and soft hydrogel of PEDOT/Alginate.

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ACKNOWLEDGMENTS

The authors would like to thank funding from ERC Starting Grant: IntegraBrain (804005).

The interplay of collagen/bioactive glass nanoparticle coatings and electrical stimulation regimes distinctly enhanced osteogenic differentiation of human mesenchymal stem cells

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INTRODUCTION

The unique ion release mechanisms and physical properties of bioactive glass nanoparticles (BGN) to promote cell proliferation, osteogenic differentiation and angiogenesis are attracting great interest in the field of bone tissue engineering [(1)] and also demonstrated in our recent publication on collagen coatings [(2)]. Moreover, the application of electric field (EF) stimulation to enhance osteogenic differentiation in place of conventional biochemical supplements has also gained more attention [(3)]. Interestingly, the interplay of BGN and EF was less investigated and their proficiency to induce / support osteogenic differentiation is unclear. In this study, we aimed to elucidate the synergistic effects of BGN and EF for osteogenic differentiation of human mesenchymal stem cells (hMSC). Further, the influences of continuous versus intermittent EF regimes were also our key interest.

EXPERIMENTAL METHODS

Collagen (Col) coatings with BGN were prepared at a 1:1 (w/w) ratio and air-dried on glass coverslips (Ø 13 mm). hMSC from two donors were investigated and seeded at 10,000 hMSC/cm^2. Coverslip with only Col was used as controls. Next, the coverslips were cultured in cell culture chambers and exposed to either continuous or intermittent EF stimulation regimes (*Fig. 1*) for 28 days on a transformer-like coupling (TLC) system designed to exert pure EF [(3)]. Importantly, the osteogenic differentiation medium was supplemented only with 50 µM ascorbic acid 2-phosphate and 10 mM β-glycerophosphate. Dexamethasone was omitted to show the proficiency of BGN and EF to initiate osteogenic differentiation. Samples were collected every 7 days. ALP activity was used as an indicator for osteogenic differentiation and calcium accumulation for mineralization (n=4). Real-time qPCR (n=3) was performed to determine the gene expression profiles at each condition.

RESULTS AND DISCUSSION

The results from this study showed the potential of BGN to initiate osteogenic differentiation at early time points. Further, the stability of collagen coatings was enhanced when both BGN and EF were applied. By coupling BGN with a continuous (12/12) EF regime, an obvious increase in ALP activity was observed as early as day 7. When coupled with an intermittent (4/4) EF regime, a higher calcium accumulation was documented on day 28 (*Fig. 1*). Further, each EF regime had shown preferences on osteogenic differentiation pathway.

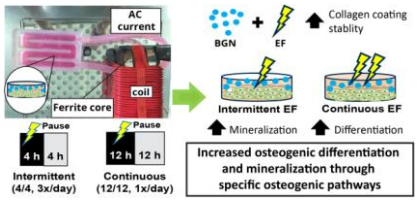

Fig.1: The experimental approach (left) and summarized results (right) of this study.

CONCLUSION

In summary, our results had illustrated the synergistic effects of BGN and EFs in different regimes on osteogenic differentiation that can be further exploited to enhance current bone tissue engineering and regeneration approaches. Further enhancement could be accomplished by doping metal ions to the existing BGN to achieve coatings / scaffolds with tunable electrical conductivity. It would be done with an objective further to enhance osteogenic differentiation through BGN and EFs interplay.

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Electrohydrodynamics based functional nanofibers electrically stimulate neuron regeneration

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INTRODUCTION

Alongside the widely studied pathways of biochemical regulation by chemokines, cytokines and growth factors, one often-overlooked but significant influence over the behavior of biological systems is electrical signaling. Voltage gradients among all somatic cells (not just excitable nerve and muscle) control cell behavior, and the ionic coupling of cells into networks via electrochemical synapses allows them to implement tissue-level patterning decisions, which is called developmental bioelectricity. Electrical modulation is therefore a potential target for many new therapies for a range of diseases and biological functions.
We have pioneered various new techniques to enable artificial, biomimetic, nanofibrous scaffold substrates to mediate cell behavior using electrical stimulation. Anisotropy, photocatalytic stimulation and assembly process as three design parameters for the neuron guide conduits were addressed here for peripheral nerve regeneration.[1]

EXPERIMENTAL METHODS

After optimizing the topographical patter, the MEW patterned PCL scaffolds were firstly treated by 1,6-hexamethylenediamine /isopropyl alcohol solutions for 8 h at 37 °C to achieve surface amino functionalization. Then, the modified PCL scaffolds were immersed in a GO colloidal dispersion for 1h and subsequently protonated g-C_3N_4 dispersion for 1h. Scanning electron microscopy (SEM), atomic force microscopy (AFM), Raman spectroscopy, X-ray photoelectron spectroscopy (XPS) and fluorescence microscopy were used to characterize the modifications. The neurite outgrowth on the PCL-GO-C3N4 scaffolds under mono- chromatic light irradiation (450 nm) was studied. Finally, based on the anisotropic micropatterned structures, NGCs assembly for implantation were explored.

RESULTS AND DISCUSSION

MEW PCL scaffolds with rectangular anisotropic patterns (1–2, 1–3) promoted significant longer average neurite extension length than those with isotropic 1-1 pattern. After the surface functionalization of g-C_3N_4 nanoparticles (Fig. 1a,b) by electrostatic interaction (Fig. 1c), The blue fluorescence originated from g-C_3N_4 was observed (Fig. 1d, e). The PCL-GO-C_3N_4 scaffolds were then used for stimulating PC12 cells differentiation under a blue LED light irradiation (450 nm). As shown in Fig. 1g-i, after differentiation for 7 days, cells cultured under light irradiation exhibited greater neurite outgrowth than those without stimulation. Lastly, combining a thermo-responsive PNIPAM membrane with geometry defined MEW micropatterns in a bi-layer system provided a spontaneously-forming Neural guidance conduit.

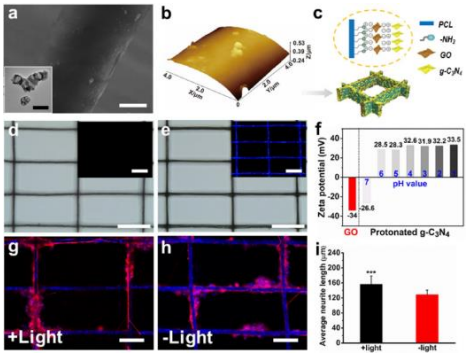

Fig. 1 Effect of photocatalytic stimulation initiated by g-C_3N_4 decorated polymeric scaffolds on neurite outgrowth. (a) SEM and (b) AFM images of a single PCL-GO- C_3N_4 fiber. The inset shows a typical TEM image of g-C_3N_4 nanoparticles. Scale bars: 5 μm in SEM image and 200 nm in TEM image. (c) Scheme diagram of PCL-GO-C_3N_4 scaffold. Bright field and fluorescence images (the insets) of (d) PCL-GO and (e) PCL-GO-C_3N_4 fibers, using 360 nm light excitation. Scale bars: 200 μm. (f) Zeta potential of the GO colloids, and protonated g-C_3N_4 dispersed in DI water at various pH values. Immunofluorescence images of PC12 cells differentiated on PCL-GO- C_3N_4 scaffold for 7 days (g) with and (h) without light stimulation. Cells were stained with TUJ1 (red) and Hoechst (blue). (i) Average neurite length analysis of cells cultured on PCL-GO-C_3N_4 scaffold with/without light treatment.

CONCLUSION

In this work, anisotropy, photocatalytic stimulation and assembly process as three design parameters for the NGCs were addressed for peripheral nerve regeneration. Unitedly addressing the three aspects, MEW based NGCs may possess great potential for repairing peripheral nerve injuries.

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ACKNOWLEDGMENTS

The authors would like to thank the Carlsberg Foundation (Grant no: CF19-0300) for providing financial support to this project.

>> 09:45 - 11:15 | SYMP-10 - NANOFIBROUS MEMBRANES FOR BIOMEDICAL APPLICATIONS

Chairpersons: Cathy Ye & Nazely Diban Gómez
Location: Room E

09:45 | KL Nanofibrous materials - Design and Fabrication of Multifunctional Micro-/Nanofiber Meshes for Healthcare
Andreas LENDLEIN , Institute of Chemistry & Institute of Biochemistry and Biology, University of Potsdam , Potsdam, Germany

10:15 | O1 Nanofibrous materials - Core-shell electrospun nanofibers incorporated with Silver Nanoparticles for inhibition of microorganisms
EDVANI CURTI MUNIZ, State University of Maringá, Maringa -Brazil and Federal University of Piaui, Teresina-Brazil

10:30 | O2 Nanofibrous materials - Engineering of dual-stimuli responsive nanofibrous magnetic membranes for localized cancer treatment
Paula SOARES, i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, Caparica, Portugal

10:45 | O3 Nanofibrous materials - An Improved Wet-electrospun Technology for Fabrication of High Porous PCL Matrix for Cartilage Tissue Engineering
Haoyu WANG, Institute of Orthopaedic & Musculoskeletal Science, UCL, London, UK

11:00 | FP01 Nanofibrous materials - Electrospun PNIPAAm-based fibers for pH- and thermo-responsive localized drug release
Adriana GONÇALVES, CENIMAT | i3N, Department of Materials Science, NOVA SST, Caparica, Portugal

11:05 | FP02 Nanofibrous materials - Topography-induced modulation of cell behaviour by alumina ceramic textiles
Deepanjalee DUTTA, Institute for Biophysics, University of Bremen, Bremen, Germany

11:10 | FP03 Nanofibrous materials - 3D membrane of electrospun fibers for cell culture
Bénédicte FROMAGER, Institut Européen des Membranes, Univ Montpellier, ENSCM, CNRS, Montpellier, FRANCE

Design and Fabrication of Multifunctional Micro-/Nanofiber Meshes for Healthcare

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INTRODUCTION

Modern applications in healthcare require devices, which need to fulfil complex demands for multifunctionality.[1] Structural functions, degradation behavior, bioinstructivity and controlled drug release capabilities are typical examples for functions, which might be combined in a device. The integration of several functions in one device is challenging, especially if such features need to be adjustable almost independently from each other. The extracellular matrix is an example for a highly multifunctional biological material and might as such serve as a source of inspiration for synthetic biomaterial systems. The collagen fiber network of soft tissues is the starting point for conceptualizing multifunctional fiber meshes from copolymers and multiblock copolymers.

RESULTS AND DISCUSSION

Functions can be implemented in polymeric materials by different methods[2]. On the molecular level chemical moieties can provide hydrolytically cleavable bonds or reversible chemical crosslinks.

The hierarchical architecture of materials and devices can be used to integrate functions at different length scales. Whereby such functions can be designed orthogonally or sequentially linked, which might enable a translation of processes from the molecular to the macroscopic level. Finally, novel functions can result from a smart combination of different materials in a multimaterial system. In this presentation fiber meshes from synthetic copolymers are considered as a design concept for multifunctional materials.

Examples are given for a covalently crosslinked material and a thermoplastic elastomer. The fiber diameter[3] and the pore size[4] in meshes of chemically crosslinked fibers are explored with regard to shape-memory and actuation capabilities. The multifunctionality of a fiber mesh from degradable, physically crosslinked multiblock copolymers enables its use as epicardial patch system, which can guide postinfarct myocardial remodeling processes[5].

CONCLUSION

Fiber meshes provide options for implementing functions on different hierarchical levely. On the molecular level the degradation behavior can be adjusted by the number and distribution of hydrolysable bonds. The phase morphology and orientation macromolecules in single fibers influence the elastic properties relevant for the cell-material interaction. The geometrical arrangement of the fibers in the mesh determines pore sizes and the number of potential contact points between the fibrous scaffolds and the cells growing on this structure. The combination of these effects can result in a targeted bioinstructivity, which is required for inducing and modulating regeneration in tissues.

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Core-shell electrospun nanofibers incorporated with Silver Nanoparticles for the inhibition of microorganisms

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INTRODUCTION

Electrospun nanofibers have been extensively investigated in the biomedical field. This is due to their unique tissue structures, with small pores, ultrafine diameter, extensive surface area, and being relatively light[1]. In this scenario, nanofibrous matrices (NM) obtained by electrospinning are potential candidates for obtaining new medicines and in the manufacture of personal protective equipment, such as face masks[2]. Therefore, in the present study, we evaluated the NM obtained from uniaxial and coaxial electrospinning. The fiber's core was composed by a polymeric blend of poly(vinyl alcohol) and chitosan with incorporated silver nanoparticles (AgNPs) as antimicrobial agent (PVA/CHT-AgNPs). The fibers were coated by polycaprolactone (PCL), as shell, targeting the inactivation of microorganisms.

EXPERIMENTAL METHODS

Firstly, fibers of PVA/CHT (70:30 %-w, aqueous acetic acid at 2%-v) with or without $AgNO_3$ (0.1 µmol L^{-1}) and of (PCL 10 %-w, chloroform and DMF 50:50 %-v) solutions were prepared through uniaxial electrospinning using 10 mL syringes with ∅ of 0.7 mm needle. The mixture was kept in the syringe under constant pressure with a flow of 0.65 ml h^{-1}, 23 kV and at distance of 10 cm between the needle tip and the surface of the metallic collector. After, at the same conditions used for uniaxial, coaxial fibers were obtained (core PVA/CHT with and without AgNPs; and shell PCL). It is worth noting that the coaxial needle (∅ of 0.7 mm for both channels) was homemade. All NM obtained were evaluated by scanning electron microscopy (SEM), infrared spectroscopy (ATR), X-ray energy dispersion spectroscopy (EDS), thermogravimetric analysis (TGA) and X-ray diffraction (XRD). The mechanical properties were evaluated with a texturometer (Stable Micro Systems, model TA-TX2). The evaluated properties were maximum tensile strength (MPa), elongation at break (%), and modulus of elasticity or Young's (MPa). The *in vitro* release studies were performed in simulated physiological fluid (pH 7.4) and bactericidal activity was evaluated in *Escherichia coli* and *Staphylococcus aureus* through inhibition halo method.

RESULTS AND DISCUSSION

The uniaxial PCL electrospun fibers with high degree of uniformity and average diameter of 500 nm were obtained. The uniaxial PVA/CHT electrospun fibers with or without AgNPs showed high degree of uniformity, absence of beads and average thickness of 200 nm. Furthermore, the EDS spectrum clearly demonstrates the presence of AgNPs in the fibers (signal at 2.98 eV), Figure 1. In addition, the yellow color of the as-obtained NM refers to the success in the *in situ* AgNPs preparation. Furthermore, coaxial NM of [PVA/CHT: core; PCL: shell] with or without AgNPs presented certain degree of uniformity in terms of thickness (average diameter ca. 300 nm), despite the presence of some beads. In addition, it is noted that the as-obtained core-shell fibers possess intermediate diameters referring uniaxial fibers, as expected[3]. Overall, essays show that AgNPs are rapidly released from PVA/CHT fibers, as monitored by electronic absorption at l =430 nm. The released AgNPs had average hydrodynamic diameter of 20 nm and polydispersity index of 0.5, ideal characteristics to obtain antimicrobial effects[4]. Furthermore, the surface potential of released AgNPs was close to zero (-4.5 mV). Additionally, compositional characterizations by ATR, TGA and XRD confirmed the presence of all constituents of the polymer blend in the core with some interactions as compared to uniaxial fibers. On the other hand, the PCL shell showed the absence of interactions with core polymers, a crucial factor for efficient formation of core-shell electrospun fibers. The analysis of the mechanical properties revealed that the core constituted by PVA/CHT reduces mechanical performance of fibers in terms of maximum tensile strength, elongation at break (%), and Young modulus. However, the *in situ* incorporation of AgNPs recovers to a large extent these mechanical properties. Moreover, all fibers incorporated with AgNPs presented considerable inhibition halo both in gram-positive and gram-negative bacteria. In addition, the fibers were tested against Sars-cov-2 and showed 99.99% of viral inactivation after only 30 minutes of contact (BR patent in process) and absence of cytotoxicity in healthy cells.

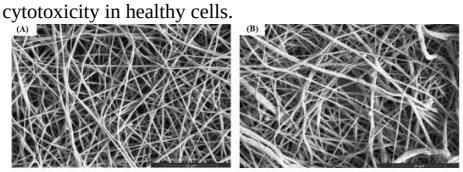

Figure1. (A) SEM images of uniaxial fibers; and (B) coaxial fibers, both with incorporated with AgNPs.

CONCLUSION

The set of obtained results indicated the efficiency and potential of coaxial fibers incorporated with AgNPs in the inactivation of microorganisms. Electrospinning is potential effective ally in obtaining MN for drugs and individual protection equipment developing.

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Engineering of dual-stimuli responsive nanofibrous magnetic membranes for localized cancer treatment

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INTRODUCTION

Cancer is a major worldwide problem, with an estimated 19.3 million new cases and 10 million cancer deaths in 2020. Cancer diagnostic and treatment have significantly improved in recent years, which increased life expectancy. Nevertheless, current treatment limitations and the elevated incidence, mortality, and cancer disease heterogeneity.[1] In recent years, magnetic nanoparticles (MNPs) demonstrate a considerable potential to substantially improve cancer treatment. Their unique properties, particularly the ability to respond to an external magnetic field, enable their use as imaging probes and as magnetic hyperthermia agents. Additionally, magnetic responsiveness can be used as a trigger for controlled drug release.[1,2] Magneto-responsive devices can provide a real-time response upon application of an external magnetic field. An external magnetic field as the trigger is optimal since it has good tissue penetration without almost any physical interaction with the body.[3,4]

The present work focuses on the development of dual-stimuli responsive nanofibrous membranes, embedded with thermoresponsive PNIPAAm microgels and MNPs for a synergic effect between magnetic hyperthermia and controlled drug release as an alternative localized cancer treatment for solid tumors.

EXPERIMENTAL METHODS

Superparamagnetic iron oxide nanoparticles were successfully synthesized by chemical co-precipitation technique and stabilized with oleic acid (OA) and dimercaptosuccinic acid (DMSA). Thermoresponsive PNIPAAm microgels with a lower critical solution temperature of 32 ºC were synthesized thought surfactant free emulsion polymerization. Poly(vinyl alcohol) (PVA) was used as fiber template and processed by electrospinning techniques using a homemade equipment.

RESULTS AND DISCUSSION

PNIPAAm microgels and MNPs were incorporated into the nanofibers (average diameter of 180 nm) through colloidal electrospinning and dual-stimuli responsive nanofibrous membranes were produced. To avoid their dissolution in aqueous medium, the membranes were submitted to physical crosslinking. Mechanical studies demonstrated that physical crosslinking using temperature of the nanofibrous membranes increased their mechanical parameters and that the presence of microgels and MNPs act as a reinforcement in the fibers. The swelling ability of the membranes is shown to decrease in the composite membranes when compared with plain PVA membranes due to the presence of MNPs. Magnetic hyperthermia assays were performed during 10 min of an AC magnetic field application with magnetic flux density of 300 G and 418.5 kHz of frequency. The results showed promising results regarding the heating ability of the magnetic membranes. PVA fibers incorporated with DMSA coated MNPs were capable of reaching therapeutic temperature (around 42 ºC) for hyperthermia treatment, indicating that the device is a viable option in cancer treatment. In drug release assays, PVA fibers with a model drug, doxorubicin (DOX), were produced and controlled release was studied in different pH solutions to simulate in vivo conditions (4.5 – endosomes/lysosomes, 6.5 – tumor microenvironment, and 7.4 – physiological pH) at 37 ºC. The results showed a more significant release of DOX at pH =7.4. Finally, to evaluate the cytotoxic effect of the device, cytotoxicity assays of PNIPAAm microgels and PVA fibers were performed in Vero and SaOs-2 cells. All assays reveal the absence of cytotoxicity indicating the possibility to use device in biomedical applications.

CONCLUSION

A dual-stimuli responsive nanofibrous membrane was successfully developed and the present work demonstrates its potential for magnetic hyperthermia and controlled drug release that shows potential as an alternative localized cancer treatment.

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An Improved Wet-electrospun Technology for Fabrication of High Porous PCL Matrix for Cartilage Tissue Engineering

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INTRODUCTION

Cartilage tissue regeneration requires the scaffolds to have interconnecting networks for cell migration and waste transport, tissue mimicked structure to support cartilage regeneration [1]. In this area, the electrospinning technique was widely used owing to its potential to generate ECM-mimic fibrous structure. However, scaffolds produced by conventional electrospinning have a density fibrous structure, limited cell ingrowth. Wet electrospinning, as a promising method that can generate high porosity [2] and 3D structure [3], has received attention.

In this study, we developed a modified wet-electrospinning set-up, fabricating high porosity PCL 3D scaffold, which overcome the disadvantages of conventional electrospinning and could be applied in cartilage tissue engineering.

EXPERIMENTAL METHODS

The improvised set-up includes a high voltage applied needle, as shown in Fig 1. A pump mounted with syringe connected with needle. And a liquid bath filled with ethanol as collector. A stir bar was placed in the liquid bath, driven by magnetic stirring, creating a vortex. A polystyrene (PS) sphere (D = 8 ± 1 mm) was added and floated on the liquid surface and rotated via the vortex. The electrospun fibre then entangled with the floated rotated PS spheres, forming a high porosity scaffold.
PCL solution (13 wt.% in DMF/Chloroform (2/8 v/v)) were electrospun in with the following parameters: 15cm, 2.5ml/h, 13kv.

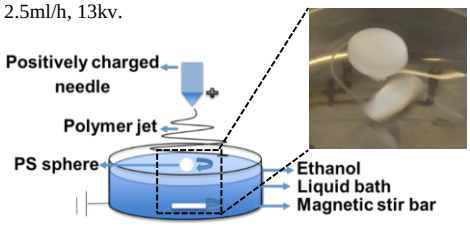

Figure 1 high porosity wet-electrospinning fabrication set-up and a photo on-site.

The resultant matrix was examined by SEM and gravimetric method to determine their structure and morphology.

RESULTS AND DISCUSSION

The examination revealed that the matrix obtained has a high porous structure with a porosity of 99.5 ± 0.1%. Two processing parameters, the concentration of ethanol and stir bar rotation speed were researched and found that the rotation speed significantly affects the porosity in that lower rotation speed results higher porosity (Fig 2). This could be attributed to the dynamic liquid flow tightens the structure. The SEM examination demonstrated that the fibre diameter of this wet-electrospun scaffold was 1.60 ± 0.4 µm. The mechanical testing revealed the PCL matrix has a compressive module at 1.34 Pa. The loosen structure and the high porosity scaffold imply the potential for cell infiltration improvement, which could overcome the disadvantages of conventional electrospinning (2D structure and high density fibre).

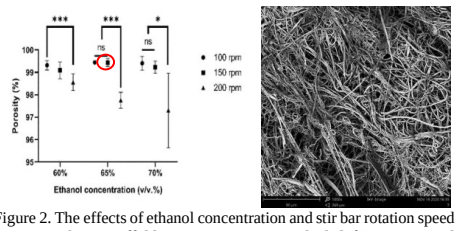

Figure 2. The effects of ethanol concentration and stir bar rotation speed on cotton shape scaffold porosity were researched (left).'ns', '*' and '***' mean P > 0.05, P < 0.05, and P < 0.005 respectively. The red circle indicates the sample processing parameters for SEM imaging (right).

CONCLUSION

An improvised wet-electrospinning machine has been developed, and high porous PCL matrix has been fabricated and examined. The study demonstrated the resultant PCL Matrix has a porosity of 99.5%, with nanofiber of 1.6 micrometer (average). The results indicated high potential to fabricate biomimetic matrix for cartilage tissue engineering.

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ACKNOWLEDGMENTS

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Electrospun PNIPAAm-based fibers for pH- and thermo-responsive localized drug release

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INTRODUCTION

Poly(N-isopropylacrylamide) (PNIPAAm) is a biocompatible polymer that exhibits a negative temperature response with a lower critical solution temperature (LCST) of around 32 ºC. The transition temperature of PNIPAAm, along with the conformational change that comes with it makes this polymer particularly interesting for biomedical applications such as controlled drug release, biosensing and tissue engineering [1]. An important and requirable characteristic of thermosensitive polymers for applications in biomedical applications is the possibility of tuning the LCST to values near or above physiological temperature. In the present work we hypothesize that PNIPAAm copolymerization with hydrophilic monomers, like acrylic acid and acrylamide, can be used to increase its transition temperature. To test this hypothesis we explored the use of RAFT polymerization to synthetize different PNIPAAm based copolymers. Reversible addition fragmentation transfer (RAFT) polymerization (Fig. 1) was used, since it provides well-defined polymers with targeted molecular weights and narrow molecular weight distributions [2].
Finally, the produced PNIPAAm based copolymers with a LCST near physiological temperature were used as fiber template in electrospinning technique to produce thermoresponsive fibrous scaffolds for drug delivery applications.

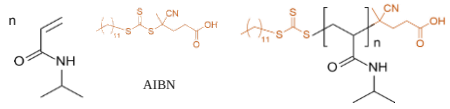

Figure 1 - Scheme of RAFT polymerization of PNIPAAm.

EXPERIMENTAL METHODS

PNIPAAm based copolymers were synthesized by RAFT polymerization. NIPAAm was used as a monomer and acrylic acid (AAc) and acrylamide (AAm) were used as co-monomers. 2,2'-Azobis(isobutyronitrile) (AIBN) and 4-cyano-4- [(dodecylsulfanylthiocarbonyl) sulfanyl] pentanoic acid (CDTP) were used as an initiator and chain transfer agent (CTA), respectively. Different monomer ratios were studied. Electrospinning technique was used to produce pH- and thermosensitive fibers using the previously obtain copolymers and the membranes were later crosslinked to increase their water stability.

RESULTS AND DISCUSSION

pH- and temperature-responsive copolymers were designed and prepared by reversible addition fragmentation transfer polymerization. Successful copolymerization with AAc and AAm was confirmed through FTIR and ^{1}H NMR analysis. The LCST of PNIPAAm based copolymers in different pH mediums was determined. PNIPAAm-AAm copolymers showed an increase in the LCST with the increase in AAm content, independent of the pH value of the medium. In contrast, PNIPAAm-AAc copolymers showed a pH-dependent thermosensitive behavior where the transition temperatures rises with AAc content at pH 6.5 and 7.4 but it decreases at pH 4.5. pKa of acrylic acid is 4.7, which means that at lower pH values (e.g 4.5) most of the carboxylic groups of AAc are protonated. This leads to a higher hydrophobicity of AAc segments in a more acidic medium, therefore, lowering the LCST [3]. Stress test were performed to evaluate the mechanical parameters of the membranes. The swelling ability of the membranes was also evaluated in different pH medium.
Contact angle assays were performed with different pH solutions and showed a decrease in the hydrophilicity of the surface of the membrane when heated to temperatures above their LCST, confirming the pH- and thermoresponsive behavior of the fibers.

CONCLUSION

PNIPAAm based thermoresponsive copolymers were successfully synthesized via RAFT polymerization. The LCST of PNIPAAm was tuned to temperatures near human body temperature through the copolymerization with hydrophilic monomers such as acrylic acid and acrylamide. The obtained copolymers were successfully electrospun and gave rise to pH- and thermoresponsive fibers which opens great opportunities for controlled and localized drug release.

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Topography-induced modulation of cell behaviour by alumina ceramic textiles

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INTRODUCTION

Substrate topography as well as changes in the physical microenvironment of cells play a major role in cell adhesion and cytoskeletal changes, thereby influencing intracellular signaling and other cellular properties.[1] In particular, scaffold porosity is known to influence cellular integration into a host tissue, which is especially important for wound healing. Therefore, we studied how 3T3 fibroblasts and HaCaT keratinocytes interact with microporous alumina textiles as a potential scaffold material for wound repair.

EXPERIMENTAL METHODS

Alumina textiles (Zircar Ceramics Inc., USA) were cleaned by 5 min immersion into piranha solution and stored dry after washing with deionized water.

To study the metabolic cell activity on the textiles, adherent NIH 3T3 mouse fibroblasts and HaCaT keratinocytes were used. After 24h, 72h and 120h of cultivation, presto blue assay was performed in triplicates for each substrate type.

For subsequent cell morphology analysis, actin filaments and cell nuclei were stained with iFluor phalloidin 647 (AbCam) and Hoechst (NucBlue Live ReadyProbes Reagent, Thermo Fisher Scientific) for 30 min, respectively, mounted and imaged using an inverted fluorescence microscope (Ti-E– V5.30, Nikon, Tokyo, Japan). The morphology of the textiles and the fixated cells was further studied with SEM using previously described method.[2]

For immunostaining of fibronectin and E-cadherin, cells were grown for 72h. Then the cells were fixed by 30 min fixation in 4% PFA, permeabilized with 0.1% TritonX-100, and blocked by non-specific binding blocking buffer (0.3M glycine and 1% BSA in 1X PBS), for 1hr. Then, the respective primary antibody was applied and incubated overnight at 4°C. Thereafter, the respective second antibody was used for 1hr at 4˚C in the dark. The samples were then stained with Hoechst for 15 mins and imaged with our inverted fluorescence microscope.

RESULTS AND DISCUSSION

The ceramic textiles supported the adhesion of keratinocytes and fibroblasts up to 120h in culture and growth on textiles was comparable to the growth on standard tissue culture (TC) plates. For HaCaTs, on microporous alumina textiles a distinct difference in morphology was observed compared to standard TC plates and alumina-coated glasses. On alumina textiles, HaCaTs exhibited an elongated cell shape while they grew in clusters on both reference substrates. Moreover, the unique woven topography of the alumina textiles induced visible changes in the actin cytoskeleton, as observed by phalloidin staining. SEM analysis further revealed that HaCaTs had formed multiple contact points along the filaments of the alumina textiles hence inducing changes in the cell morphology and adhesion pattern. Fibroblasts also adhered well to microporous alumina textiles and grew along individual filaments yet with minimal changes in their morphology compared to TC plates and alumina coated glass. Inspired by the differences in HaCaT morphology, further analysis of protein expression in keratinocytes on alumina textiles revealed upregulation of fibronectin expression followed by downregulation of E-cadherin. 3T3 fibroblasts on the other hand showed negligible changes in protein expression. The observed differences in the expression of cell-specific maker proteins in HaCaTs indicate that topography-induced changes in cell adhesion might be related with the epithelial-mesenchymal transition (EMT) of epithelial cells, which could be beneficial in respect to tissue regeneration during wound healing. In the future, it will be highly interesting to explore if inorganic ceramic textiles can trigger beneficial cell signaling pathways in addition to supporting cell growth.

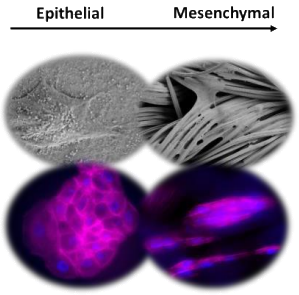

Figure 1: Topography-induced changes in cell behavior by alumina textiles.

CONCLUSION

In summary, our results suggest that microporous alumina textiles, although being an inorganic biomaterial, are very attractive scaffolds for skin tissue engineering. In the future, the observed topography-driven induction of possible EMT-like behavior of keratinocytes on alumina textiles could potentially promote native wound repair mechanisms.

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3D membrane of electrospun fibers for cell culture

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INTRODUCTION

Cell therapy, which is organ or tissue regeneration from injection and growth of stem cells, raises a lot of hope for treatment of diseases. However, the direct injection does not work out so well. Thus, biocompatible membranes, allowing stems cells to growth and differentiate, are needed. 3D membranes of electrospun fibers have been developed by both IEM and INM. Their application extends from drug screening to cell therapies. In fact, this scaffold is purely synthetic, reproductible, low cost to produce and fibers show fluorescence properties. Moreover, it is inert, biocompatible, it can be sterilized and inserted into well plates. Studies[1-2] have shown that migration and growth of stem cells can be studied realistically since fibers imitate extracellular medium.

EXPERIMENTAL METHODS

3D nanofiber matrix

Nanofibers were produced by electrospinning a solution of 10% w/w Polyacrylonitrile (Sigma Aldrich) in DMF (Sigma Aldrich). A voltage of 20kV and a flow rate of 2.4 ml/h were applied. Multi-walled carbon nanotubes (MWCNT, Nanocyl) were added in the electrospun solution. After overnight drying at room temperature, the nanofibers were put in a chamber furnace for heat treatment. Before biological use, the matrixes were cut and sterilized in a classical autoclave.

FTIR

ATR-FTIR Spectrum was recorded on Nexus Spectrum.

Scanning electron microscopy (SEM)

Scanning electron micrographs were captured of sample mounted onto SEM stubs with carbon tape (HITACHI S-4800, 2keV, 5mm working distance).

Tomography Electron Microscopy

Samples were embedded in LR White resin and cut with an ultramicrotome Leica UC7. TEM analyses were performed on a JEOL 2200FS microscope, equipped with a field emission gun (FEG) and an in-column Omega-type energy filter, operated at 200 kV. Images were acquired on an CCD Gatan UltraScan 4000 camera.

DiameterJ[3]

DiameterJ algorithm was used to analyze SEM images of 500 resolution.

Cell culture and migration assay

GBM cells were cultured in U-bottomed 96-well plates (25 000 cells/well) in DMEM/F12 medium supplemented with glucose, glutamine, insulin, N_2, Epidermal Growth Factor and Fibroblast Growth Factor. After 24h, they were dissociated and put on nanofiber matrixes. After 15 minutes, 500 µl of DMEM/F12 medium supplemented with fetal bovine serum (0.5%), fungizone and B27 were added. They were put in incubator at 37°C, 5% CO_2. After 5 days, medium was removed and washed with PBS twice. Then, cells were fixed with formaldehyde solution (1%) in PBS and permeabilized with triton (0.5%)/serum (5%) solution in PBS. After 30 min in incubator, nuclei were colored in Hoechst (1/1000). Fibers were then placed on slides, dried, recovered by mounting liquid and another slide above, left overnight in fridge and analyzed by epifluorescence microscopy.

RESULTS AND DISCUSSION

The study of spectrum before and after heat treatment shows that PAN has undergone aromatization and dehydrogenation. The functional groups on the surface enable the formation of hydrogen bond between nanofibers and cell surface, which make the matrix biocompatible, and will enable us to coat the nanofibers with biomolecules such as MEC proteins.

With the parameters chosen, we obtain a membrane composed of 0.716±0.046 µm diameter nanofibers and pores of 27.11±3.302 µm². As showed in previous study[2], the morphology of nanofibers, the dimension of pores and the constitution of the mesh enable cells to infiltrate, migrate and proliferate.

To better understand the impact of MWCNT on mechanical properties, we analyzed membranes with TEM. The comparison between TEM images of fibers without MWCNT and those with 0.05% MWCNT shows that MWCNT are all inside the fibers and towards their lengthwise. Over the 195 sections of fibers, 19 contained MWCNT. They are well incorporated within PAN solution and kept through electrospinning process.

The analysis of fibers with DiameterJ showed that neither the diameter and mesh hole area nor the orientation are significantly impacted by MWCNT.

Results show that GBM migration is maximal when they are cultured on nanofibers matrixes containing 0.0015% MWCNT. Moreover, we can see that migration is directional, following fibers orientation. These results show that GBM can migrate in the nanofibers.

CONCLUSION

We produced a 3D nanofiber matrix whose physical and chemical property can be modified and studied independently to produce an environment mimicking *in vivo* ECM. Moreover, it is suitable for cell culture. Thus, it represents a relevant tool to study ECM role in cell migration, proliferation, and differentiation.

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>> 09:45 - 11:15 | SYMP-11 - TISSUE ENGINEERING INSPIRED TISSUE AND ORGAN MODELS: RECENT TECHNOLOGICAL ADVANCES AND ROAD TO THE BEDSIDE AND MARKET

Chairpersons: Gianluca Ciardelli & Alessandra Roncaglioni
Location: Room A

09:45 | KL Tissue & Organ engineering - The role of physiologically based kinetic modeling in interpreting the results of in vitro cell-based toxicity assays
Ronette GEHRING, Institute of Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

10:15 | O1 Tissue & Organ engineering - Porous Polysaccharide-Based Scaffolds Promote Hepatocytes Autoassembly into Functional Spheroids and Improve Survival After Acute Liver Injury in Mice
Teresa SIMON-YARZA, Université Paris Cité, INSERM U1148, Paris, France

10:30 | O2 Tissue & Organ engineering - Fibrin microgels for dermal papilla cell encapsulation as an alternative to spheroid cultures
Cristina QUÍLEZ LÓPEZ, Department of Bioengineering and Aerospace engineering, Universidad Carlos III de Madrid, Leganés, Spain

10:45 | O3 Tissue & Organ engineering - Hybrid scaffolds to create long-term in vitro co-culture model for targeted treatment of ovarian cancer
Elly DE VLIEGHERE, Polymer Chemistry and Biomaterials Group, Centre of Macromolecular Chemistry, Ghent University, Belgium and Cancer Research Institute Ghent (CRIG), Ghent University, Belgium

11:00 | FP01 Tissue & Organ engineering - Innovative luminescent porous 3D-printed scaffolds based on 13-93B20 bioactive glass and persistent luminescent particles for bone bioengineering.
Amel HOUAOUI, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland

11:05 | FP02 Tissue & Organ engineering - Organo-mineral 3D-printed scaffolds for bone regeneration
Baptiste CHARBONNIER, RMeS Lab - INSERM U 1229 - Nantes University, Nantes, France

The role of physiologically based kinetic modeling in interpreting the results of *in vitro* cell-based toxicity assays

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Keywords: Physiologically-based kinetic models, risk assessment, toxicokinetics

INTRODUCTION

The risk a chemical poses to human health is a function of its potential to damage cells (toxicocynamics), coupled with the concentration to which cells are exposed *in vivo* (toxicokinetics) (Figure 1).

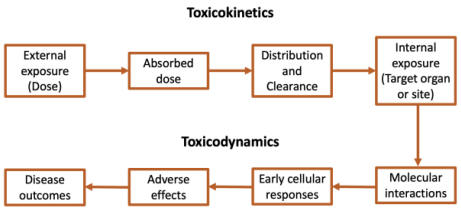

Figure 1: The toxicokinetic and toxicodynamic processes that link between external exposure and adverse health outcomes

Cell-based *in vitro* assays measure the former. The latter is dependent on the biokinetic properties of the chemical and often needs to be predicted using *in silico* mathematical models. Physiologically-based kinetic (PBK) models are important tools for simulating the toxicokinetics of a chemical compounds and predicting internal chemical concentrations at the organ, cellular and even subcellular levels for a given human exposure scenarios[1].

EXPERIMENTAL METHODS

PBK models are *in silico* mathematical models that conceptualize the body as a system of interconnected compartments coupled by differential equations that describe the processes that govern the fate and transport of compounds between these compartments. Parameter values for these models are set *a priori* based on a combination of experimental data and theoretical knowledge of the compound, human anatomy and human physiology.

RESULTS AND DISCUSSION

PBK models for predicting chemical concentrations in cardiac tissue have been developed[2]. These can be adapted to account for the characteristics of different *in vitro* models, as well as the putative molecular targets and key events associated with the toxicodynamics of the chemical compound being studied.

CONCLUSION

PBK models are useful tools for predicting of time-concentrations profiles of chemicals in heart tissues to allow a meaningful interpretation of *in vitro* concentration-response data and predict *in vivo* outcomes.

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Porous Polysaccharide-Based Scaffolds Promote Hepatocytes Autoassembly into Functional Spheroids and Improve Survival After Acute Liver Injury in Mice

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INTRODUCTION

Acute liver failure is associated with high short-term mortality rates of about 70%. Despite liver transplantation is the second most common solid organ grafting, less than 10% of the global transplantation needs are currently met.[1] Numerous liver tissue engineering approaches based on cellularized biomaterials have been proposed,[2] however none of them fulfils all the requirements for clinical application.[3]

In the present work, we optimized the autoassembly of human hepatic cell line HepaRG into polarized functional hepatic spheroids within a GMP compatible porous polysaccharide-based scaffold (pSC). For the first time, this liver engineered construct has been transplanted and challenged in a mice model of acetaminophen (APAP)- induced acute liver failure.

EXPERIMENTAL METHODS

Scaffold fabrication and HepaRG spheroids obtention. Briefly, porous pSCs were obtained by cross-linking and freeze-drying of a solution of the two polysaccharides pullulan and dextran, as previously described.[4]

HepaRG were amplified in 2D following Biopredic International standard for two weeks and subsequently seeded into the scaffold's pores. After self-assembly into spheroids in the first week, HepaRG were differentiated for 2 weeks with DMSO addition in order to obtain hepatocytes and biliary-like cells.

In vitro functional tests. Hepatic functions such as albumin, total bile acid and urea secretion, glycogen content and cytochrome activities were evaluated in HepaRG spheroids obtained after differentiation and their values compared to constructs cultivated in basal medium. Furthermore, expression of mature hepatic markers was assessed after two weeks of differentiation culture protocol.

Transplantation studies. pSCs containing differentiated HepaRG spheroids were implanted for one month on the left lateral and right superior lobes in healthy NOD/SCID male mice (APAFIS #20962-2019051615276341). Blood was collected at different time points to assess transaminases dosage and human albumin secretion in plasma. After animals were euthanized, livers and liver-linked pSCs were analyzed by immunohistochemistry.

In a second study, 12 hours fasting period and lethal i.p. administration of APAP were applied 3 hours prior to scaffold implantation. Mice survival rate with and without HepaRG spheroids implantation was followed over one week.

Mann-Whitney test analysis, two-way Anova for kinetics and log-rank (mantel-cox) test analysis for survival curves were used.

RESULTS AND DISCUSSION

Pullulan-dextran pSCs promoted HepaRG autoassembly into spheroids, characterized by a diameter of less than 150 µm and absence of necrotic core. After differentiation, mature and polarized hepatocytes and functional biliary-like cells were obtained (**Fig.1**).

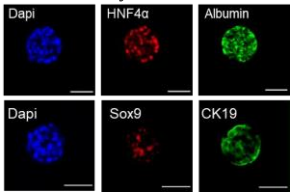

Figure 1. Representative images of HepaRG in pSCs after 2 weeks in differentiation media, expressing hepatocyte (HNF4α, Albumin) and biliary (Sox9, CK19) markers (scale bar 50 µm).

One month after transplantation, no signs of liver toxicity, nor inflammatory and fibrotic processes were found. Immunohistology analysis demonstrated the presence of the spheroids and pSC integration to the host liver. Human albumin was detected in mice sera up to one month. Remarkably, pSCs containing HepaRG spheroids improved survival rate after acute liver failure by 35% compared with empty pSC. Thus, supporting HepaRG spheroids functionality *in vivo* and therapeutic potential of our cellularized constructs (**Fig. 2**).

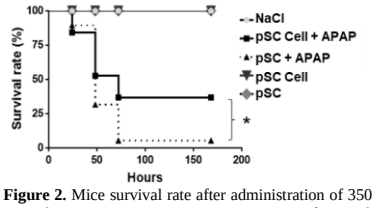

Figure 2. Mice survival rate after administration of 350 mg/kg APAP toxic dose. N = 20 per group + APAP, N = 6 for NaCl and pSC Cell (pSC loaded with HepaRG) and 3 for pSC (empty pSC) *p < 0.05.

CONCLUSION

A polysaccharide-based scaffold, cost-efficient, GMP compatible and easy to produce, has been developed to support hepatic spheroids assembly and differentiation with promising outcomes for the treatment of acute liver failure upon implantation.

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ACKNOWLEDGMENTS

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Fibrin microgels for dermal papilla cell encapsulation as an alternative to spheroid cultures

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INTRODUCTION

Cellular spheroids have been described as an appropriate culture system to re-program dermal papilla fibroblast (DPc) stem fate and successfully induce hair follicle formation[1]. Nonetheless, low viability values are associated to this culture system, reason why new alternatives need to be explored. Human blood plasma as a source of natural fibrinogen with encapsulated fibroblast has been successfully used for skin regeneration[2]. Using the same principle, in this work we propose the use of human blood plasma for the generation of fibrin microgels (FM) with encapsulated DPs for cell reprogramming. With that purpose, microgel morphology, cell viability and protein expression were analyzed and compared to the spheroid culture.

EXPERIMENTAL METHODS

FM were prepared by mixing platelet poor/rich plasma (PPP/PRP) at a final fibrin concentration of 2.4mg/mL, $CaCl_2$ (0.016%(w/v)), Amchafibrin (0.0016% (w/v)) and NaCl 0.9% to dilute fibrin concentration. To analyze the effect of cell density, 375, 750, 1500 or 3000 cells/µL. DPc were encapsulated diluted in cell culture media. FM were generated by the deposition of 2µL of the working solution on the lid of a cell culture plate and 15 minutes at 37ºC for gelification. For cell mediated contraction measurement, images of FM were taken and processed at different timepoints to determine changes in area. Cell viability was analyzed using Live/Dead assay by adding 2µM Calcein-AM and EthD-1 solution to the FM an incubated for 30 minutes at 37ºC. Additionally, proliferation was characterized by the incorporation of BrDU into the cell nucleolus. Cell reprogramming and extracellular matrix remodeling was characterized by the positive expression of Alkaline phosphatase (ALP), Versican (Ver), α-Smooth Muscle Actin (αSMA) and Collagen IV proteins in the confocal microscope.

RESULTS AND DISCUSSION

Morphology analysis showed a decrease in size of a 75% in the first 48 hours for FM with 3000, 1500 and 750 cells, showing a mean diameter of ~550µm, close to a human DP (Figure 1). Conversely, this reduction in size was only of a 25% if 6000 DPc were encapsulated. This could be explained by an unbalance of the cell number and the available RGB motifs for cells attachment, being crucial the cell/volume ratio. Additionally, cell viability within the FM with 750-3000DPc exhibited values that range from 45- to 85% (Figure 2, right). These values improved cell viability more than a 50% when compared to the spheroid system for all cell densities. On the other hand, DPc positively expressed all the protein markers after 48h in culture, being DPc successfully reprogrammed to their stem state. Moreover, after 15 days in culture fibrin matrix was replaced to a proteoglycan rich extracellular matrix similar to that found in native human DP (Figure 2, left).

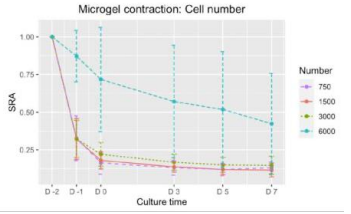

Figure 1: DPc mediated contraction of FM for different cell numbers.

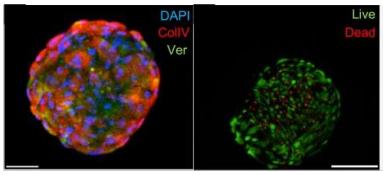

Figure 2: Matrix remodelling in FM after 15 days in culture (left) and DPc viability within FM after 48h in culture (right). Sacle bar: 100 µm.

CONCLUSION

Here we propose and alternative culture system based in FM that showed a similar morphology, cell protein expression and extracellular matrix composition than native human DP. Furthermore, this system showed improved cell viability compared to that of spheroid culture systems, being a reliable culture system to mimic DP structure that can be used to induce hair follicle morphogenesis.

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ACKNOWLEDGMENTS

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Hybrid scaffolds to create long-term *in vitro* co-culture model for targeted treatment of ovarian cancer

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INTRODUCTION

A subtype of ovarian cancers exhibits a KRAS mutation, and is susceptive to targeted therapy exploiting a MEK-inhibitor (e.g. trametinib). These therapies usually give a good initial response. However, patients often become resistant to treatment and exhibit recurrent disease[1]. To study resistance mechanisms, long-term clinically relevant models, recapitulating the tumor environment, are needed.

EXPERIMENTAL METHODS

Hybrid scaffolds were produced by extrusion-based 3D-printing of acrylate-endcapped urethane-based poly(ethylene glycol) (AUPPEG8k)[2] exploiting the 3D Bioplotter (SysEng Bioscaffolder, Hünxe, Germany) to ensure similar dimensions in swollen state as a peritoneal metastasis nodule. The scaffolds are cylinder-shaped with the following dimensions: 5mm diameter, 4mm height, 500µm pore size and 300µm strut diameter. Plasma treatment was exploited to improve the compatibility with a gelatin-methacryloyl (2w/v% GelMA solution containing 2 mol% Irgacure 2959 relative to the amount of methacrylamide moieties) coating, enabling cell interaction. The scaffolds were seeded with a mixture of ovarian cancer cells and cancer associated fibroblasts (CAFs) encapsulated in a type I collagen gel (2mg/ml)[2]. *In vitro* cultures were monitored by fluorescence microscopy and bio-luminescent imaging (BLI)[3]. Scaffolds are randomized into four treatments group based on bioluminescent signal; control (0.1% DMSO), Trametinib (1nM Trametinib, C988930 Bioconnect, Huissen, The Netherlands)), Luminispib (10nM Luminispib, ORB154741, Bioconnect) and a combination thereof (1nM Trametinib and 10nM Luminispib). Culture medium is refreshed 2 times a week with medium containing the active compounds.

RESULTS AND DISCUSSION

The encapsulated cells self-organize into spheroids within the scaffold pores within 48h. The red labeled CAFs initiate spheroid formation and form collagen fibers. These fibers are used by the green labeled cancer cells to migrate and form spheroids (fig 1A-B). These spheroids are stable during long time culture (>1 month) and are firmly connected to the struts through the collagen fibers (fig 1C-D).

Scaffolds containing the spheroid co-cultures are used to evaluate long-term treatment effects on the cancer cells within the 3D co-culture environment. All treated scaffolds show an initial response. In mono-treated scaffolds, the cancer cell viability drops to 10-20% which remains stable for over 40 days. Conversely, the cancer cell viability for the combination treatment decreases below 1% (fig 1E).

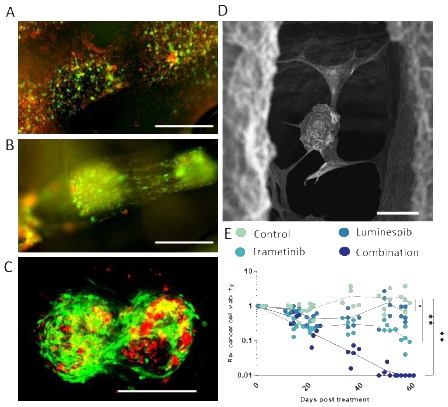

Fig 1. Fluorescent images of hybrid scaffolds with ovarian cancer cells (green) and CAFs (red) immediately (A) and 10h post-seeding (B), confocal fluorescent image 1 week post-seeding (C) and SEM image 1 month post-seeding (D). The scale bars represent 50µm. E) Relative cancer cell viability of treated scaffolds determined by bio-luminescence.

CONCLUSION

Hybrid scaffolds mimic the tumor and its environment on a cellular and biophysical level. The 3D co-cultures allow long-term therapy evaluation on cancer cells and CAFs. In addition, this long-term evaluation allows to investigate the role of the tumor environment on therapy resistance. 2D monocultured treated ovarian cancer cells show a complete response within 5 days[4], while in 3D co-cultures, a significant part of the cancer cells remains viable even after 60 days of treatment. These cells are at risk of becoming resistant and inducing recurrent disease. Combining trametinib with a HSP90 inhibitor (luminespib) could possibly circumvent this.

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Innovative luminescent porous 3D-printed scaffolds based on 13-93B20 bioactive glass and persistent luminescent particles for bone bioengineering.

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INTRODUCTION

Complex and critical-size bone defects require innovative devices presenting osteoproperties: from osteocompatibility to osteogenesis. Due to its ability to release ions in solution and trigger signaling pathways leading to "osteocompetent responses", bioactive glass (BAG) appears as a pertinent solution to face the needs in bone engineering[1]. However, imaging BAG *in-vivo* remains a difficulty as they are typically radio-transparent and therefore cannot be detected easily and unambiguously radiographically[2]. But it is crucial to track glass behavior in bone implants in order to allow an easy follow-up of the dissolution, mineralization and resorption of the scaffold after implantation[3]. The proposed alternative is to use persistent luminescence (PeL) microparticles (MPs) which would allow one to track *in-situ* glass behavior while improving cells proliferation and osteogenic differentiation by using low light therapy[4].

In this study, porous 3D scaffolds based on 13-93B20 BAG and different PeL MPs were developed. The effect of these PeL MPs on the scaffolds' bioactivity and their cytocompatibility using human fat stem cells (HFSCs) were investigated.

EXPERIMENTAL METHODS

The porous scaffolds combining 13-93B20 BAG and MPs with blue, green, turquoise, and red PeL were obtained by robocasting with a ratio of 90/10 (weight %) respectively.

The bioactivity *in-vitro* of the scaffolds was studied in Simulated Body Fluid (SBF) for up to two weeks. The ion release was quantified using ICP-OES, and the materials were observed and analyzed by SEM-EDX.

The cytocompatibility of the scaffolds with the different PeL MPs was investigated using HFSCs. The scaffolds were excited under white light for 15min every hour for the duration of the test.

RESULTS AND DISCUSSION

The scaffolds were obtained by 3D-printing and exhibit PeL after being charged with white light indicating that the fabrication method used to prepare the scaffolds does not affect the spectroscopic properties of the PeL MPs (Figure 1).

The *in-vitro* bioactivity test of all the investigated scaffolds, in SBF, showed a decrease of [P] and [Ca] concentrations overtime, suggesting a precipitation of an apatite like-layer which was confirmed by SEM (Figure 2A) and EDX.

The HFSCs viability experiments, in direct contact with the PeL scaffolds and under subsequent excitation and

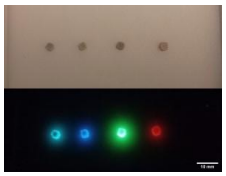

Figure 1: 3D-printed scaffolds before (top) and after (bottom) excitation under white light (Scale bar 10mm).

emission, showed that the cells are alive and numerous after 24h of incubation except for the scaffolds with red PeL MPs where a much lower density of cells is seen (Figure 2B). It must be investigated if this is due to a toxicity of red PeL MPs or due to the red-light emission which the cells cannot bear. After selecting the most appropriate PeL MPs for the targeted applications, the osteoinduction capacity of these materials and their mineralization will be studied *in-vitro* and *in-vivo*.

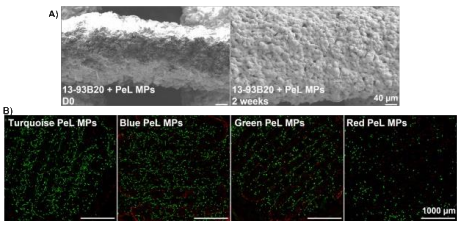

Figure 2: A) SEM images of the scaffolds with PeL MPs before and after 14 days of immersion in SBF. B) Live-Dead images of the scaffolds with PeL MPs after 24h with HFSCs.

CONCLUSION

Our results demonstrate that the PeL MPs do not inhibit the bioactivity of our scaffolds. Cellular experiments reveal promising behavior toward the potential use of these scaffolds in hard tissue regeneration and mineralization. This work not only opens the path to bioimaging but also to the photo-release of molecules of therapeutic interest.

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ACKNOWLEDGMENTS

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New and Easy 3D Cell Culture Method Investigation by Using Peptide and Pectin

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INTRODUCTION

In pharmaceutical and biomaterial studies, new applications developed are first tried on cell culture- *in vitro* models. In a conventional cell culture study, biomaterial activity tests are performed using cells culture on plates in two dimensions, however, lack the simulation of the extracellular matrix (ECM)[1]. In 3D cell culture systems, cellular interaction is supported in many dimensions, and cells grow within physical limits, as in living tissue. Thus, cell behavior and morphology show similar characteristics to cells in natural tissues[2].

Hydrogels based on polymer chain structures can encapsulate solvent and various content and these structures are suitable for cell life due to their high-water content and has the potential to mimic the natural ECM[3]. An ECM consists of interlocking protein fibers and sugar chains[1] that represent an important potential in the development of ECM-like 3D cell culture. In our project, an easily accessible, feasible, optimized and modifiable biomolecular combination of **pectin** as the plant-derived sugar component of the ECM, and **peptide amphiphilic nanofibers** as protein-like components[5] was tested for 3D cell culturing.

EXPERIMENTAL METHODS

1) Pectin isolation was isolated from sugar beet pulp powder state by extraction methods and final product was freeze-dryed.

2) Determination of the Percentage of Esterification of Pectin is measured by titration method.

3) Determination of Protein Contaminants on Pectin was determined using the Qubit™ Protein Assay Kit and the Qubit fluorometer.

4) Fourier Transform Infrared Spectrometer (FTIR) was performed in order to determine the purity of pectin, pectin-specific regions.

5) Cell Culture was maintained by Saos-2 cell line by growing in DMEM containing 10% Fetal Bovine Serum (FBS), 1% penicillin/streptomycin, 2 mM L-glutamine cell culture media.

6) Hydrogel Construction and 3D Cell Culture

100 µL of 10mM KHH oligopeptide solution with distilled water and 100 µL of 2wt% pectin-cell solution with DMEM was mixed to construct hydrogel. Then, cells were incubated for various days for viability tests by Alamar Blue Assay and microscopic analysis by crystal violet dye and fluorescent microscope.

7) For Characterization of Hydrogels, the swelling ratio, the viscoelastic properties and stability of hydrogels were tested. The morphology and porosity of the hydrogel was determined by SEM.

8) Testing the Success of Bone-Like Mineralization of the SaOS-2 Cell Line in 3-D Cell Culture was determined by Alizarin Red staining and cetylpyridinium for quantitative analysis.

9) Statistical Analyses were performed using GraphPad Prism software. Differences between groups was determined by One-Way ANOVA tests.

RESULTS AND DISCUSSION

We were able to synthesize a one step, easily forming 3D cell culture method with sugar beet pectin and KHH oligopeptide. We have proved the stability of hydrogels by the stability tests and successfully incubated various hard and soft tissue lines. Experiments of cell incubation gave closer results to reality because it supports the spread, growth, and differentiation of cells as in living tissue. The success of the encapsulation has been proven with microscope imaging, cell viability, biomineralization tests and gene expression experiments are still ongoing. Moreover similar to native tissue, 3D gels embedded into parafilm and tissue-like morphology were obtained from microtome sectioning. In addition, this method supported the ECM environment for other cell types. Eventually, we have succeeded the development of a new facile 3D culturing method.

The statistical analysis was performed with a p value of ≤ 0.05 was considered statistically significant.

CONCLUSION

Our goal was to produce a single step, easily forming and, low cost and effective method for cell culturing. To test this new 3D method for *in vitro* applicability, we designed osteoregeneration model by SaOS-2 cell line to support cell viability and to induce biomineralization and osteoblast differentiation. With this recently reported system, different cell lines also could be cultured indicating practical future applications of the developed 3D model.

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Organo-mineral 3D-printed scaffolds for bone regeneration

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INTRODUCTION

Developments in the field of computer-aided design and additive manufacturing have allowed significant improvements in the design and production of ephemeral scaffolds with biologically relevant features to treat bone defects [1]. Their benefits versus standard scaffolds have already been acknowledged. Unfortunately, the clinical and manufacturing workflow to generate personalized scaffolds is still source of inaccuracies which may lead to a poor fit between the implant and patients' bone defects [1]. This may result in drastic consequences on the regenerative outcomes: e.g., poor osteointegration of the scaffold, reduced bone formation, delayed or non-union. Furthermore, most scaffolds display a non-adapted mechanical behavior (e.g., fragility, brittleness, low stiffness), an inappropriate biodegradability rate and a mediocre potential to promote the formation of new vascularized bone tissues. Tackling these issues, organo-mineral scaffolds with evolutive mechanical properties were 3D-printed: from deformable scaffolds after production to stiff scaffolds after implantation

EXPERIMENTAL METHODS

Alpha tricalcium phosphate (α-TCP) and anhydrous trimagnesium phosphate (a-TMP) were used as reactive inorganic powders. They were obtained by heat treatment of apatitic TCP (1360°C-15h, air quenching) and hydrated TMP (1050°C-5h) rods, respectively. Rods were crushed using an agate mortar and pestle and the powder sieved between 20 and 40 µm.As organic phase, 6% w/v hyaluronic acid (2.6 MDa, HTL Biotechnology) was dissolved in a 50% w/w D-glucose solution. Organo-mineral cementitious pastes were prepared by mixing 60 and 50% w/w of reactive α-TCP and a-TMP, respectively. Disks (Ø = 5 mm, h = 1 mm) were printed by robocasting (R-Gen 200, RegenHu) using 25G cones following a rectilinear and gyroid pattern. These macroporous scaffolds were implanted in non-critical calvarial defects (rat model) with or without total bone marrow (N = 6 scaffolds per condition). Animals were euthanized after 7 weeks, and scaffold degradation and bone formation was assessed by micro-computed tomography X (µCT), scanning electron microscopy (SEM, back-scattering) and histology (hematoxylin and eosin & toluidine blue/von Kossa stain). Deep-learning routine were developed for µCT and SEM quantitative analyses. Finally, a real size 3D-printed polymeric model of a cleft lip and palate deformity was used as a proof of concept: a scaffold, 15% larger than the intended defect, was robocasted then inserted within the defect; this simulating a surgical intervention (Fig. 1).

RESULTS AND DISCUSSION

The simulated procedure was a success, with a deformable scaffold that could be inserted into the defect without breaking and adjusted to the edges for an optimized bone-implant contact (Fig. 1).
Bone formation in calvarial defect could be observed for both calcium and magnesium phosphates (CaP & MgP, respectively) – based scaffolds up to their core (Fig. 2); the addition of bone marrow playing a significant role. Scaffold architecture has little influence on bone formation. Significant differences in scaffold biodegradation were observed: while CaP-based scaffold largely remained, MgP-based scaffold could hardly been observed.

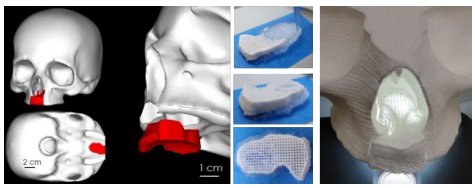

Fig. 1. Real size simulation of a surgical reparation of cleft lip and palate using 3D-printed scaffold 15% larger than the defect

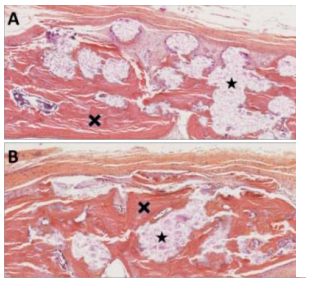

Fig. 2. Histological sections stained by hematoxylin and eosin of A. CaP-based scaffold and B. MgP-based scaffold; both supplemented with bone marrow

⋆ Material x Bone

CONCLUSION

The potential of 3D-printed organo-mineral scaffolds with evolving mechanical properties was demonstrated in this study. Biological response was driven by the inorganic phase composition. Further improvements of material formulations are currently ongoing, taking advantage of this proof of concept.

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ACKNOWLEDGMENTS

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>> 9:45 - 11:15 | SYMP-12 - POLYMER-BASED NANOPARTICLES FOR THERANOSTICS ? FROM FORMULATION TO SCALE-UP

Chairpersons: Alberto Rainer & Fillipo Rossi
Location: Room B

9:45 | KL Nanoparticles & Theranostic - Engineering Conformable Polymeric microMESH for the Delivery of Combination Therapies against Gliomas
Paolo DECUZZI, Laboratory of Nanotechnology for Precision Medicine, Genova, Italy

10:15 | O1 Nanoparticles & Theranostic - Extracellular Hyperthermia for the treatment of advanced cutaneous melanoma
Beatriz SIMÕES, i3N/CENIMAT, Department of Materials Science, NOVA School of Science and Technology, NOVA University Lisbon, Campus de Caparica, Caparica, Portugal

10:30 | O2 Nanoparticles & Theranostic - Multifunctional polydopamine nanoparticles as a platform for treating colorectal cancer
Matteo BATTAGLINI, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera, Italy

10:45 | O3 Nanoparticles & Theranostic - Ultrasound responsive polymer microbubbles for a targeted treatment of thrombotic diseases
Louise FOURNIER, INSERM U1148, LVTS, Université Paris Cité, Bichat Hospital, Paris, France

11:00 | FP01 Nanoparticles & Theranostic - Cytotoxicity of nitric oxide releasing Pluronic F-127 hydrogel containing silica nanoparticles loaded with cisplatin towards breast cancer cell
Amedea BAROZZI SEABRA, Center for Natural and Human Sciences, Federal University of ABC, Santo André, Brazil

11:05 | FP02 Nanoparticles & Theranostic - Peptide Mediation of Nanoparticles To Cross The Blood-Brain Barrier – A Platform For Brain Drug Delivery
Catarina I. P. CHAPARRO, CENIMAT/i3N (Centro de Investigação em Materiais), NOVA School of Science and Technology (FCT-NOVA), Almada, Portugal; Instituto de Medicina Molecular, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Engineering Conformable Polymeric microMESH for the Delivery of Combination Therapies against Gliomas

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INTRODUCTION

Despite tremendous advancements in early cancer diagnosis, targeted therapies, and the advent of cancer immunotherapies, glioblastoma and pediatric high-grade gliomas continue to be the most aggressive and less curable forms of any cancer. The current standard of care has not changed over the past 20 years (Stupp protocol, 2005), still relying on maximal safe resection of the malignant tissue followed by adjuvant radiotherapy and chemotherapy with temozolomide (TMZ). This treatment plan provides only a modest improvement in life expectancy (a few months) and various degrees of therapy-induced complications. Here, a novel compart-mentalized, flexible micro-implant – microMESH – for the sustained and localized delivery of a variety of agents (small molecules, inhibitors, antibodies, nanomedicines), and combinations thereof, is described.[1]

EXPERIMENTAL METHODS

microMESH is obtained via a top-down fabrication approach relying on two replica molding steps (**Figure 1A**). In its final configuration, microMESH appears as a dual-compartment system comprising a poly(vinyl alcohol) (PVA) microlayer, carrying docetaxel nanoparticles (L-SPN) and a network of poly(lactic-co-glycolic acid) (PLGA) strings, carrying the hydrophobic molecule diclofenac (DICL) (**Figure 1B**).[1] After fabrication, the in vitro therapeutic efficacy of microMESH is tested on U87-MG GFP+ spheroids, which represent a standard 3D cell model of glioblastoma, in addition to conventional experiments on U87-MG monolayers. Finally, three orthotopic murine models of glioblastoma are used to test in vivo the therapeutic potential of microMESH, namely U87-MG without tumor removal; U87-MG with tumor removal; and patient-derived cancer stem cells (CSC) with tumor removal. The microMESH therapeutic efficacy is assessed via bioluminescence analyses and animal survival (**Figure 1C**) and compared to multiple control groups, including TMZ, DTXL and L-SPN.[1]

RESULTS AND DISCUSSION

The multi-step procedure for the microMESH fabrication is depicted in **Figure 1A**. Scanning electron and confocal fluorescent microscopy images of the PLGA network, loaded with curcumin (green), and PVA microlayer, loaded with RhB-nanoparticles (red), are shown in **Figure 1B**. These demonstrate the precision of the fabrication process and flexibility of the PLGA micronetwork. The bioluminescence data for the U87-MG tumor removal model are presented in **Figure 1C** together with the survival data up to 270 days.

CONCLUSION

The hierarchically engineered drug depot microMESH has been designed, realized, and validated to deliver combination molecular/nano-therapies to malignant gliomas. The modular structure of microMESH allows the loading of a broad variety of therapeutic molecules and nanomedicines. The superior performance of microMESH, as compared to more conventional systemic and local treatments, is explained with its ability to deform and adsorb over complex biological surfaces and deliver with a precise schedule anti-cancer and anti-inflammatory drugs. microMESH could improve the prognosis of the most aggressive brain tumors and possibly other malignancies with high local recurrence.

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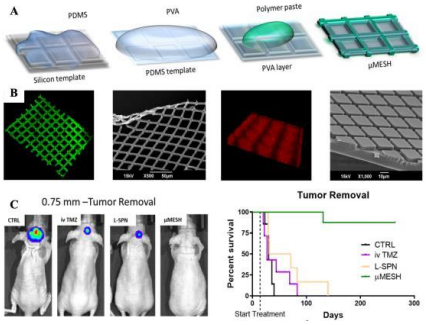

Figure 1. A. Fabrication steps. **B.** Dual compart-mentalized system. **C.** Preclinical results in mice. [1]

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Extracellular Hyperthermia for the treatment of advanced cutaneous melanoma

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INTRODUCTION

Cancer remains a leading cause of death worldwide. Melanoma is an aggressive type of skin cancer which originates from genetic mutations in melanocytes. Targeted therapy and immune checkpoints inhibitors have been the current therapeutical approach to treat metastatic melanoma. However, the low response rate, melanoma acquired therapy resistance and toxicity effects have limited the clinical outcomes of these therapies. Magnetic fluid hyperthermia is an emerging heat-based cancer therapy aiming to induce apoptosis of malignant cells by locally increasing the temperature at the tumor site. This therapy uses superparamagnetic iron oxide nanoparticles (SPIONs) as agents and have already been employed in clinical practice for brain tumors. SPIONs have biocompatibility properties and, due to their size, only become magnetic when an external magnetic field is applied, being extensively study for bio-medical applications. Yet, the internalization of SPIONs by cancer cells negatively affects their magnetic-responsiveness leading to lower levels of cell death by magnetic hyperthermia. [1,2] We hypothesize that by blocking the internalization we can introduce a new mode of extracellular magnetic hyperthermia.

EXPERIMENTAL METHODS

In this work, we studied the SPIONs internalization dynamics in WM983b metastatic melanoma cell line and used small-molecular inhibitors of endocytosis to block the nanoparticles internalization with the aim to improve magnetic fluid hyperthermia by performing it extracellularly. The SPIONs used were synthesized by chemical co-precipitation and further stabilized with (3-aminopropyl)triethoxysilane (APTES). The interaction between the SPIONs and melanoma cells was assessed in 2D and 3D tumour models by Prussian blue staining (optical microscopy) for several time points of cell exposure to the stabilized SPIONs. The nanoparticles' intracellular location was assessed by confocal microscopy, functionalizing the APTES stabilized SPIONs with Rhodamine B fluorophore. The inhibition capacity of five small-molecular inhibitors (chlorpromazine, methyl-β-cyclodextrin, nocodazole, latrunculin B and genistein) was qualitatively and quantitatively evaluated by confocal microscopy. *In vitro* intra and extracellular magnetic hyperthermia was performed to evaluate the effect of internalization on the SPIONs heating capacity.

RESULTS AND DISCUSSION

A qualitative analysis of microscopy images revealed that the internalization process of SPIONs in melanoma cells starts with a rapid interaction between the cell membrane and the nanoparticles surface, followed by an endocytic mechanism of uptake to allow internalization. It was determined that methyl-β-cyclodextrin inhibitor efficiently blocked the SPIONs internalization with cellular uptake of 14%. Our specific absorption rate (SAR) results, representative of SPIONs heating capacity, confirmed the expected lower values for intracellular magnetic hyperthermia (63 ± 7 W/g), which increase when SPIONs are in the extracellular environment due to cellular uptake blockade by small-molecule inhibitor (91 ± 16 W/g).

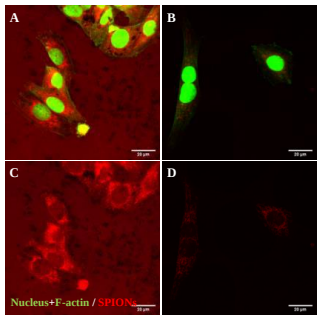

Figure 1 - Laser scanning confocal microscopy images for SPIONs intracellular localization in WM983b melanoma cells untreated with inhibitors (A, C) and pretreated with methyl-β-cyclodextrin (B, D). Scale bar: 20 μm.

CONCLUSION

Here, we confirm the hypothesis that a pharmacological approach to block the SPIONs cellular uptake improves magnetic fluid hyperthermia efficiency. This work could lead to great advancements in magnetic hyperthermia as a cancer treatment and opens a range of alternative combined therapies.

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Multifunctional polydopamine nanoparticles as a platform for treating colorectal cancer

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INTRODUCTION: Colorectal cancer (CRC) represents one of the most common forms of cancer, being at the second place worldwide among cancers for mortality and the third most common form of malignancy.[1] Currently, the treatment of CRC poses major issues mainly due to the high heterogeneity of the disease and the relatively high abundance of drug resistance among the various forms of CRC.[2,3]
Polydopamine nanoparticles (PDNPs) are a class of nanomaterials obtained through the polymerization of dopamine.[4] One of the most interesting properties of PDNPs is their ability to convert near-infrared (NIR) radiation into heat, making them an ideal candidate for photo-thermal therapy (PTT).[5] In this work, we proposed the use of PDNPs loaded with the chemotherapy drug sorafenib (Sor-PDNPs) as a potential tool to achieve drug delivery and thermal ablation of CRC. The final aim is to obtain a platform able to overcome the current limitations present in the treatment of CRC and also to provide a potential targeted therapy with minimal adverse effects on healthy colon tissues.

EXPERIMENTAL METHODS: Sor-PDNPs were obtained through Stöber process. Sor-PDNPs were characterized through dynamic light scattering (DLS), zeta potential analysis, and scanning electron microscopy (SEM) imaging (Figure 1). Moreover, the drug loading and release profile in various conditions (different pH, different temperatures) was analyzed by using high-performance liquid chromatography (HPLC). Once fully characterized, PDNPs and Sor-PDNPs were tested on both colon cancer cells (Caco-2) and colon healthy cells (CCD-18Co). In particular, the effects on viability on both cells lines and their uptake levels of PDNPs were measured and compared. Viability was tested through PicoGreen® assay, being the output fluorescence levels of the test proportional to the number of living cells. Finally, the possibility to stimulate cancer cell thermal ablation through NIR stimulation of Sor-PDNPs was measured and analyzed by stimulating both cell lines subjected to different treatments (control cells, cells treated with sorafenib, cells treated with PDNPs, and cells treated with Sor-PDNPs).

RESULTS AND DISCUSSION: Sor-PDNPs presented an average diameter of 183 ± 17 nm, an average zeta potential of -49.1 ± 0.9 mV, and a drug loading efficiency of 2.18 w%. Biocompatibility assay showed how plain PDNPs did not have any significant effect upon the viability of either Caco-2 or CCD-18Co healthy colon cells. However, the treatment with Sor-PDNPs caused a significant dose-dependent viability reduction in Caco-2 cells, and, most interestingly, healthy CCD-18Co cells seemed less affected by Sor-PDNPs with respect to Caco-2 cells, suggesting a higher sensitivity of cancer cells to the proposed treatment. NIR stimulation of Caco-2 and CCD-18Co cells at various conditions was also investigated. The treatment with NIR of control cells or cells treated with plain sorafenib did not cause any significant increment in cellular death. Conversely, the NIR stimulation of cells treated with plain PDNPs caused a significant increment in cellular death. and the combination of NIR stimulation and Sor-PDNPs treatment caused an even higher reduction in cell viability, probably due to the synergistic effects of drug and thermal stimulation. Interestingly, also in these conditions healthy cells were less affected by the combinatory treatment of NIR and Sor-PDNPs with respect to cancer cells (Figure 2).

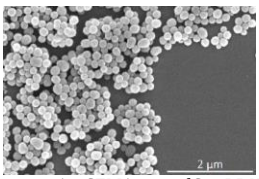

Figure 1. Representative SEM image of Sor-PDNPs suggesting spherical morphology and uniform size.

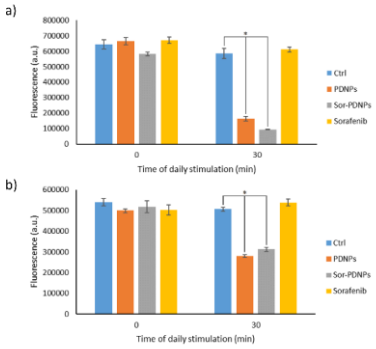

Figure 2. Viability assay performed via PicoGreen® test on cells after 3 days of daily NIR stimulation. Results obtained on a) Caco-2 cells and b) CCD-18Co cells (∗$p < 0.001$; $n = 3$).

CONCLUSION: Collected data suggest that Sor-PDNPs are a promising tool in the treatment of CRC, presenting minimal adverse effects on healthy cells. In particular, Sor-PDNPs proved to be able to induce cellular death on CRC cells with a synergistic effect of chemotherapy and thermal ablation, with a moderate cytotoxic effect on healthy colon cells.

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Ultrasound responsive polymer microbubbles for a targeted treatment of thrombotic diseases

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INTRODUCTION

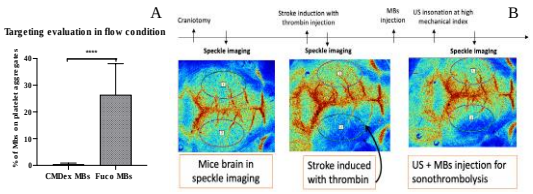
Our team focuses on the first worldwide mortality rate, cardiovascular diseases, and more specifically on thrombotic diseases. The major unmet medical need in the treatment of pathological blood clot, is the systemic injection of the thrombolytic drug (rtPA) causing collateral hemorrhages and showing a short therapeutic window[1]. Our objective is to develop a safe, targeting, visualizable and treating carrier to overcome surgical complications of blood clot related events. Our technology consists in the innovative synthesis of polymer microbubbles (MBs) via acoustic cavitation. Unseen in the literature, this method allows an improved yield and reduced time of synthesis compared to regular hydrodynamic cavitation. Those MBs are composed of a co-polymer shell of poly-isobutyl cyanoacrylate and a sulfated polysaccharide, fucoidan. Our strategy is based on the affinity of fucoidan for activated platelet and activated endothelial cells via P-selectin proteins[2]. MBs are filled with perfluorobutane gas known for its echogenic properties allowing their visualization in optical microscopy but also in ultrasound (US) imaging. Thereby, we aim to use MBs as a carrier for the treatment of thrombotic diseases. In addition, the ultrasound insonation can also induce sonothrombolysis, a mechanical perturbation of the MBs that can accentuate the clot disruption[2].

EXPERIMENTAL METHODS

MBs synthesis is performed via acoustic cavitation of an aqueous phase containing our targeted agent (fucoidan) with a surfactant. The phase containing the monomer of isobutyl cyanoacrylate is injected into the aqueous solution and the mix is insonated with continuous US. During this process, perfluorobutane is injected into the solution to be imprisoned inside the MBs. The obtained foam is washed and filtered to 5 µm. MBs size characterization was performed with granulometry analysis, and the morphology of the MBs was observed by Scanning Electron Microscopy (SEM). Targeting experimentations are obtained with a microfluidic system[3] that consists in coating microfluidic channels with collagen type I in order to promote platelet aggregation. Whole human blood is then injected at arterial flow speed. When platelet aggregates are formed, the channel is rinsed and the MBs suspension is injected. Microbubbles attachment to the platelet aggregates is observed by fluorescence microscopy. Echogenicity evaluation is performed in static and fluidic conditions with an US scanner to evaluate the response of MBs to different US intensities. Preliminary *in vivo* experiments have been performed on a stroke mice model to observe the effect of US treatment accompanied with the injection of targeted MBs on the recanalization of the occluded artery. Image analysis of the speckle imaging allows recanalization quantification.

RESULTS AND DISCUSSION

The innovative synthesis allowed us to obtained reproducible, stable (> 6 weeks on shelf stability), and non-toxic microbubbles (< 4% of hemolysis at 10^8 MBs /ml). Targeting evaluation (Fig. A) on activated platelet in flow condition was demonstrated to be effective ($p<0,0001$) compared to a group of MBs with a non-targeting polysaccharide (CMDex = carboxymethyl dextran). Preliminary *in vivo* experiments encourage the treatment of US insonation with the injection of targeted MBs in a stroke mouse model (Fig. B).



Figures A-B: (A) Targeting efficiency of fucoidan MBs compared to CMDex MBs on platelet aggregate. (B) Stroke model from brain before surgery (left), brain post stroke (center), brain after MBs injection and US treatment (right).

CONCLUSION

We are proud to present our work on an innovative tool for the treatment of thrombotic diseases. We have demonstrated a reproducible synthesis of MBs able to target *ex vivo* activated platelet. Encouraging on going work focuses on the incorporation of the thrombolytic protein (rtPA) with various chemical reactions like adsorption or EDC/NHS. The quantification of this grafting is performed with the evaluation of the amidolytic and fibrinolytic property of the protein, to obtain a full theragnostic tool. *In vivo* validation is also established to validate the live targeting in fluorescence macroscopy and final sonothrombolysis experiment will be performed on the mouse stroke model.

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Cytotoxicity of nitric oxide releasing Pluronic F-127 hydrogel containing silica nanoparticles loaded with cisplatin towards breast cancer cell

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INTRODUCTION

Several papers describe promising uses of nitric oxide (NO) in cancer therapies, mainly because NO actuates as a chemotherapeutic sensitizer.[1] However, its action is limited by time, concentration and application site. Due to its short half-life (0.5 s), NO donors with highest stability are used to carry and deliver NO, such as S-nitrosothiols (RSNOs). RSNOs have been incorporated into polymer matrices to enhance the sustained NO release.[2] Furthermore, to improve cancer-targeting therapies, hyaluronic acid (HA) has been used due to its strong affinity to the CD44 glycoprotein, overexpressed in cancer cells membrane.[3] In addition, silica nanoparticles (SiO_2 NPs) have been shown to be an excellent drug carrier, due to their porosity that allows the incorporation of chemotherapeutics, such as cisplatin (SiO_2@CisPt NPs).[4] Thus, the aim of this study was to synthesize, characterize and evaluate the cytotoxicity of NO-releasing Pluronic F-127 hydrogel containing HA and SiO_2@CisPt NPs against breast cancer cells. We hypothesize that the combination of NO and cisplatin in a biomaterial would enhance its toxicity against cancer cells

EXPERIMENTAL METHODS

SiO_2@CisPt NPs: SiO_2 NPs were prepared by sol-gel method.[5] The loading with cisplatin was made as previously reported,[3,5] dispersing 45 mg of SiO_2 NPs and mixing with cisplatin (1:1) for 24 h.

GSNO (RSNO): GSH (1.23 mol/L) was dissolved into HCl (1 mol/L), followed by the addition of an equimolar amount of $NaNO_2$. The nitrosation reaction was carried out for 20 min, in an ice bath, under magnetic stirring and in the dark, leading to the formation of a pink precipitate of GSNO.[6]

Pluronic F-127 hydrogels: All hydrogels were prepared with 25% (w/v) Pluronic F-127 and 0.05% (w/v) of hyaluronic acid (HA).[6] Briefly, 1.25 g of solid Pluronic F-127 (PL) was added to 5.0 mL of PBS, kept at 10 °C overnight, leading to the complete PL dissolution. Then, 2.5 mg of HA was added into PL solution, as well as GSNO (25 mM) and/or silica NPs (0.5 % w/v), in an ice bath, followed by gentle homogenization.

Characterization of materials: Several techniques were used to evaluate the morphology and physical-chemistry of the prepared materials, such as X-ray diffraction (XRD), Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), and Kinetics of NO release from GSNO-containing PL.

In vitro cytotoxicity: The cytotoxicity of the biomaterials was evaluated by MTT assay, using epithelial breast cancer adenocarcinoma MDA-MB-231 cell line. Statistical difference between groups was determinate using Tukey's multiple comparisons test.

RESULTS AND DISCUSSION

SiO_2@CisPt showed an average hydrodynamic size of 318 ± 2.6 nm, polydispersity index of 0.353 ± 0.04 and a zeta potential of -20.2 ± 0.75 mV, indicating stability in aqueous suspension. XRD reveled an amorphous structure for silica NPs and TEM showed that these NPs has spherical shape morphology and are well-distributed, with an average size in solid-state of 158 ± 20 nm. The kinetics of NO release from hydrogel matrix revealed a spontaneous and sustained release of NO at millimolar range for at least 24 h at room temperature. The cytotoxicity of the hydrogel was evaluated against MDA-MB-231, which are highly aggressive, invasive and poorly differentiated, with different groups and concentrations. Results showed a concentration-dependent cytotoxicity (100 to 200 µg/mL in terms of NPs). Interestingly, the combination of GSNO and SiO_2@CisPt halved cell viability when compared to treatments without these compounds over 100 µg/mL, decreasing cell viability by 20% more than others. At 200 µg/mL, this combination led to a critical cell viability of 30%, indicating a synergistic effect between GSNO and SiO_2@CisPt NPs.

CONCLUSION

The hydrogel was prepared successfully, such as its components (NO donor – GSNO and SiO_2@CisPt NPs). The combination between these compounds led to a formation of a biomaterial with a great antitumor action, since it was found to exist a synergistic effect between GSNO and SiO_2@CisPt NPs, highlighting the potential uses of this drug delivery system for anticancer therapies.

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Calcium phosphate nanoparticles as carrier of peptides and plasmid for the treatment of cystic fibrosis and colorectal cancer related inflammations.

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INTRODUCTION

Synthetic calcium phosphates (CaPs) are the most widely accepted bioceramics for the repair and reconstruction of bone tissue defects. The recent advancements in materials science have prompted a rapid progress in the preparation of CaPs with nanometric dimensions, tailored surface characteristics, and colloidal stability opening new perspectives in their use for applications not strictly related to bone. In particular, the employment of CaPs nanoparticles as carriers of therapeutic and imaging agents has recently raised great interest in nanomedicine.[1]

In this work, two different types of molecules for the treatment of cystic fibrosis and colorectal cancer (CRC) associated infection have been conjugated to CaPs nanoparticles (NPs) to improve their efficacy. These molecules are (i) plasmide vectors carrying gene-specific activatory CRISPR/Cas9 (CRISPRa) system for the resolution of CRC induced inflammation[2], and colistin, i.e. one of the most active antimicrobials against Gram-negative bacteria involved in cystic fibrosis related infections.[3] The molecules were conjugated to CaP-NPs in two different ways, namely adsorption for colistin, and encapsulation for CRISPRa.

EXPERIMENTAL METHODS

CaP-NPs were prepared by a wet precipitation as reported by Delgado-Lopez et al.[4] Two solutions of (i) 0.1 M $CaCl_2$ + 0.4 M Na_3(Cit) and (ii) 0.12 M Na_2HPO_4 + 0.1 M Na_2CO_3 were mixed at 4 °C. The pH of the mixture was adjusted to 9.0 with HCl. The mixture was then introduced in a round-bottom flask, sealed with a stopper, and immersed in a silicone oil bath under magnetic stirring. After precipitation, the particles were repeatedly washed with ultrapure water by centrifugation (10000 RPM, 5 minutes), and the resultant pellet was resuspended in ultrapure water and stored at 4 °C. Before use, CaP-NPs suspension was dispersed by sonication for 2 min in a sonic bath.

For the conjugation with colistin, a water solution containing 2.5 mg mL^{-1} of peptide was mixed to a solution with 8.0 mg mL^{-1} of preformed CaP-NPs in a 1:1 ratio. The adsorption was carried out at room temperature for 150 min, after which the conjugate was recovered by centrifugation. For the encapsulation with the plasmid, the biomolecule was added in the solution containing $CaCl_2$ before the addition of the phsophate solution. The mixture was then heated at 37°C for 5 minutes.

Both the conjugates were then tested on the respective *in vitro* and *in vivo* models.

RESULTS AND DISCUSSION

To investigate the functional role of MFSD2A in experimental CRC, we delivered MFSD2A-OE EPCs in mice orthotopically injected with the colorectal cancer cell line CT26. Preliminary results showed that EPCs are recruited to tumor sites, where they integrate either into preexisting vessels or into perivascular regions.[5] Notably, MFSD2A-OE EPCs-treated mice display a significant reduction in tumor growth in comparison with control animals.

On the other hand, the in vitro tests results obtained with colistin loaded CaP-NPs on pulmonary cells demonstrated that these NPs are not cytotoxic up to a concentration of 125 µg mL-1. Moreover, the antimicrobial and antibiofilm activity of Col loaded CaP-NPs tested on Pseudomonas aeruginosa RP73, a clinical strain isolated from a CF patient, was similar to that of free Col demonstrating that the therapeutic effect of Col adsorbed on CaP-NPs was retained.

CONCLUSION

This study provides the proof of concept for an innovative inhalable CaP-NPs based delivery system potentially able to counteract PA infection in CF. On the other hand, the results obtained from CaP-NPs conjugated with CRISPRa are still under investigation.

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Peptide Mediation of Nanoparticles To Cross The Blood-Brain Barrier – A Platform For Brain Drug Delivery

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INTRODUCTION

Poly(lactic-co-glycolic) acid (PLGA) nanoparticles enhance drug pharmacodynamics and bioavailability, and when loaded with superparamagnetic iron oxide nanoparticles (SPIONs) they can act as contrast agents for magnetic resonance image (MRI)[1]. These characteristics make them attractive for brain imaging and therapy. However, the application of nanoparticles (NPs) for brain drug delivery is hindered by the presence of the blood-brain barrier (BBB). The BBB is a natural defense against circulating toxic and infection agents that also prevents most therapeutic compounds from reaching the brain. BBB peptide shuttle (BBBpS) are small peptides that engage adsorptive mediated transport (AMT) across the BBB and allow brain uptake[2]. In this work, we propose SPIONs-loaded PLGA nanoparticles functionalized with a BBBpS as a platform for brain drug delivery.

EXPERIMENTAL METHODS

SPIONs-loaded PLGA NPs were produced through simple-emulsion solvent evaporation according to a modified procedure reported by Kandasamy et al.[3]. The size of the nanoparticulate system was confirmed by DLS and TEM techniques, while the zeta potential was measured by ELS. Iron content of SPIONs encapsulated in NPs was determined using 1,10-phenanthroline colorimetric method through UV/VIS spectrometry. To test the activity of NP we first investigated the interaction with human brain endothelial cells (BEC) that make up the BBB. NP internalization in BEC was evaluated through flow cytometry and fluorescence microscopy.

RESULTS AND DISCUSSION

The nanoparticles produced have a size range of 110-145 nm (Figure 1 (A)) and iron content of SPIONs of 80%. In the functionalization step, 15% of fluorescently labeled BBBpS was conjugated to NPs surface, resulting in alteration of size and charge. The size increasing in 60 nm and zeta potential from -21.2 ± 0.6 before functionalization, to -4.2 ± 1.2 after functionalization. The increase in charge was expected due to the presence of cationic BBBpS at NPs surface. Internalization of NPs by BEC reveal that BBBpS promotes internalization, with an increase of 6-fold in BBBpS modified NP, in comparison with naked NP, at 24 h (Figure 1 (B)). Also, time-course evaluation of NP internalization reveals a plateau at 12 h, suggesting an equilibrium between endocytosis and exocytosis. Given the results obtained, we are now studying the translocation efficiency of these NPs when conjugated, or not, with the BBBpS through the BBB *in vitro* model.

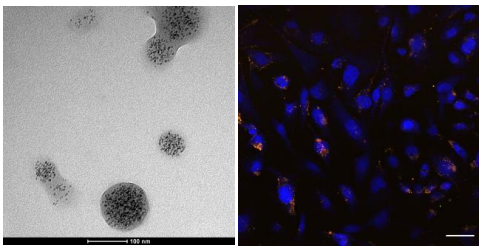

Fig. 1. (A) TEM image of SPIONs-loaded PLGA NPs and **(B)** Confocal microscopy of NPs conjugated with BBBpS and internalized in BEC. Nuclei are stained with Hoechst (blue), labeled NPs appear in yellow and labeled BBBpS appear in red.

CONCLUSION

We have successfully synthesized magnetic-polymeric nanoparticles tailored with a BBBpS for brain uptake. Morphological and stability characterization allowed us to move for *in vitro* evaluation of NPs through flow cytometry and fluorescence microscopy where we could find that NPs-BBBpS conjugation improves brain cells uptake. We will further test these NPs in *in vitro* models of the BBB and *in vivo* brain uptake with the purpose of supporting those novel hybrid nanoparticles as a new strategy for brain drug delivery.

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>> 9:45 - 11:15 | SYMP-13 - BIOHYBRID CELL-LADEN POLYMER MESHES FOR TISSUE REGENERATION

Chairpersons: Paul Wieringa & Yannis Missirlis
Locations: Room F

9:45 | KL1 Biohybrid materials - Electrowriting of functional biomaterials
Miguel CASTILHO, Dept. of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, The Netherlands; Inst. for Complex Molecular Systems, Eindhoven University of Technology, Eindhoven, The Netherlands ; Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands, The Netherlands

10:15 | O1 Biohybrid materials - Yarn of Human Amniotic Membrane Can Be Woven into a Vascular Graft with Clinically-Relevant Mechanical Properties.
Nicolas L'HEUREUX, Univ. Bordeaux, INSERM, Laboratory for the Bioengineering of Tissues - BioTis, UMR1026, Bordeaux, France

10:30 | O2 Biohybrid materials - Organoids generation in human decellularized extracellular matrix
Anastasia PAPOZ, Univ. Grenoble Alpes, CEA, Inserm, IRIG, Biomics, Grenoble, France

10:45 | O3 Biohybrid materials - Ice-Templated Collagen-Elastin Scaffolds As Potential Substrates For Lung Alveolar Organoids
Gengyao WEI, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

11:00 | FP01 Biohybrid materials - Quantitative analysis of distribution paxillin and vinculin in osteoblasts and fibroblasts binding to electrospun PMMA fibers based on super-resolution fluorescent images.
Krzysztof BERNIAK, Faculty of Metals Engineering and Industrial Computer Science, AGH University of Science and Technology, Cracow, Poland

11:05 | FP02 Biohybrid materials - Development Of A Cardiac Bio-prosthesis
Jean-Philippe JEHL, Institut Jean Lamour UMR 7198 CNRS / Université de Lorraine, Nancy, France

11:10 | FP03 Biohybrid materials - Poly(L-lactic acid) and Ceramic Composite Structures for Fully Resorbable Cranial Implants
Ana GRZESZCZAK, Department of Materials Science and Engineering, Uppsala University, Uppsala, Sweden

Electrowriting of functional biomaterials

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INTRODUCTION

Electrowriting is process that has gained the spotlight in regenerative medicine due to its potential to engineer highly-ordered fibrous scaffolds, capable of replicating extracellular microenvironment (ECM) functions. Highly resolved porous constructs fabricated in this manner have great utility for both *in vitro* and *in vivo* applications and represent the cutting-edge of 3D printing in biomedical applications. This keynote talk will highlight some of latest developments in such high-resolution 3D processing technique.

RESULTS & DISCUSSION

Results include: functional designs for cardiac tissue regeneration (Fig 1A). We showed that microfiber scaffolds with reversible stretchable properties allowed for the generation of artificial muscle fibers. By combining hexagonal-shaped fiber scaffolds with clinically relevant human iPSC-CMs, we have produced a heart patch that allow maturation of contractile myocytes for cardiac tissue engineering; Actuating structures: We showed that highly ordered microfiber scaffolds based on PCL/rGNP@ blends can be reproducibly fabricated by electrowriting, allowing for controlled deposition of customized geometries. This facilitated the design of actuating scaffolds that support skeletal cell growth and differentiation in vitro, as well as magnetically triggered mechanical stimulation (Fig 1B); hydrogel-based scaffolds based on natural polymers like silk; as well as, multilayered microfibrous scaffold that can capture the graded composition and organization of native bone-to-periodontal ligament (bone-PDL) interface tissue and result in long-term, stable, PDL regeneration (Fig 1C).

CONCLUSIONS

Our findings provide new perspectives for the development of structured fiber scaffolds that can better reproduce native tissues ECM organization and further instruct and respond to biology. A particular focus is placed on discussing examples from *in vivo* studies where electrowritten constructs with functional designs and bioactive compositions allow to guide structural organization of the newly developed tissues.

ACKNOWLEDGMENTS

The author would like to thank his Biomaterials & design team and funding from Dutch Research Council (024.003.013 and OCENW.XS5.161) and EU's H2020 program (874827).

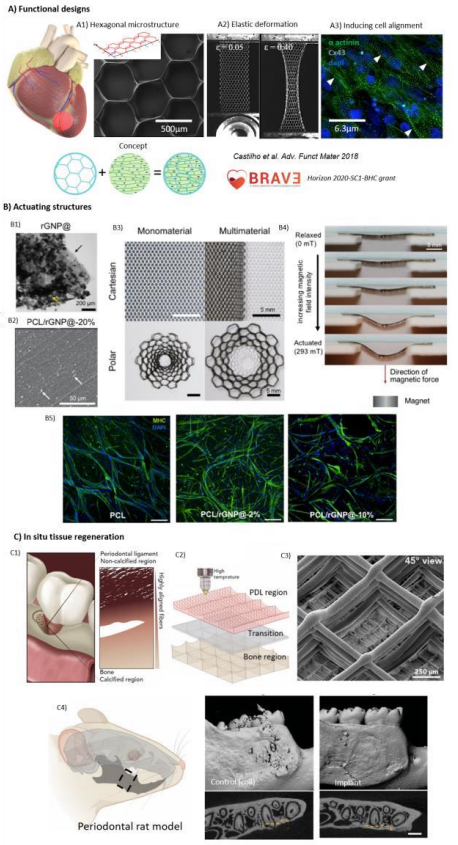

Figure 1: Overview of functional electrowritten scaffolds that instruct and respond to biology. A) Functional hexagonal shaped fibers scaffolds for cardiac tissue regeneration. B) Magneto-active scaffolds that support muscle cell growth. B1) TEM of reduced graphene oxide nanoplatelets (rGNP@); rGNP (black arrow); ION (yellow arrow). B2) SEM of PCL/rGNP@-20% filament cross-section; rGNP@ (arrows). B3) Modular scaffolds based on PCL (white) and PCL/rGNP@ (black). B4) Deformation of PCL/rGNP@-10% scaffold under external magnetic fields. B5) Confocal imaging of C2C12-seeded scaffolds after 8 days of in vitro culture. MHC (myosin heavy chain); DAPI (nuclei). C) Graded scaffold for bone-PDL interface regeneration. C1) Schematic of bone-periodontal ligament interface and C2) multilayered implant. C3) Microstructure of printed fiber implant. C4) In vivo model and micro-CT assessment of bone formation at 6 weeks of both collagen (control) and bone-PDL implant. Transverse views highlight the visual differences between the area and density of bone regenerated within the defect (Scale bar = 1 mm).

Yarn of Human Amniotic Membrane Can Be Woven into a Vascular Graft with Clinically-Relevant Mechanical Properties.

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INTRODUCTION

Since synthetic vascular prosthesis perform poorly in small diameter revascularization, biological vascular substitutes are being developed as an alternative. Although their *in vivo* results are promising, their productions involve tissue engineering methods that are long, complex and expensive.[1-3] To overcome these limitations, we propose an innovative approach that combines the human amniotic membrane (HAM), which is a widely available and cost-effective biological raw material, with a rapid and robust textile-inspired assembly strategy.[4]

EXPERIMENTAL METHODS

Fetal membranes were collected after cesarean deliveries at term. Once isolated by dissection, HAM sheets were cut in ribbons that could be further processed, by twisting, into threads. Ribbons were decellularize by trypsin (1.25 g/l) and ethylenediaminetetraacetic acid (EDTA, 0.625 g/l) treatment for two minutes at 37°C. After rinsing in PBS (3x5 min) and distilled water (1 × 5 min), HAMs were placed on an orbital shaker at room temperature for 7h with a decellularization solution (8 mM 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate hydrate (CHAPS), 25 mM EDTA, 0.12 M NaOH, and 1 M NaCl in PBS), and then washed with PBS (1 × 5 min) and distilled water (3 × 30 min). HAMs were then cut, air-dried, spooled, and stored at -80°C. Dry or hydrated (distilled water) ribbons were sterilized at room temperature by gamma irradiation (25 kGy, BGS, Germany).

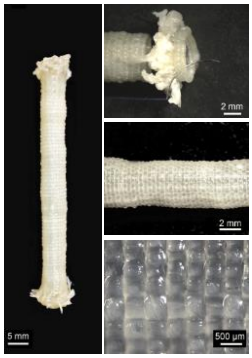
Tensile tests were performed as previously described. [1]

The TEVGs were produced using a basic weaving technique (plain 1/1) with a custom-made circular loom, as previously described.[1]

Significant differences were assessed by two-tailed t-test (two groups compared) or one-way analysis of variance (more than two groups compared) if data had a normal distribution. Otherwise, they were assessed by Mann-Whitney test (two groups compared) or by Kruskal-Wallis test (more than two groups compared). Differences were significant when $p < 0.05$.

RESULTS AND DISCUSSION

Characterization of HAM yarns (both ribbons and threads) showed that their physical and mechanical properties could easily be tuned. Since our clinical strategy will be to provide an off-the-shelf, allogeneic implant, we studied the effects of decellularization and / or gamma sterilization on the histological, mechanical, and biological properties of HAM ribbons. Decellularization had little effect of HAM yarn mechanical properties other than a small increase in strain at failure. However, gamma sterilization of the dried and decellularized HAM caused a decrease in rehydrated yarn diameter, an increase in ultimate tensile strength and a decrease in strain at failure. Gamma irradiation of hydrated (and decellularized) HAM largely avoided these mechanical changes. Furthermore, the process did not interfere with the ability of the matrix to support human umbilical vein endothelial cells to form a confluent monolayer *in vitro*. Finally, HAM-based, woven, tissue-engineered vascular grafts (TEVGs) showed clinically relevant mechanical properties with a burst pressure of over 8000 mmHg (at a diameter of 4.4 mm), suture retention strength of over 5 N, and a transmural permeability of 1 $ml \cdot min^{-1} \cdot cm^{-2}$ (Fig).



CONCLUSION

This study demonstrates that human, completely biological, allogeneic, small diameter TEVGs with excellent mechanical properties can be produced from HAM, thereby avoiding costly manufacturing strategies based on cell culture and complex bioreactors.

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Organoids generation in human decellularized extracellular matrix

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INTRODUCTION

For optimal development, organoids need a physiological 3D microenvironment and an adapted conditioned media. Usually, the Matrigel derived from Engelbreth-Holm-Swarm murine sarcomas, is the most used hydrogel for organoids generation[1]. This matrix provides a permissive microenvironment for the development of almost all organoids types[2,3]. However, Matrigel origin prevents its use in regenerative medicine. Thus, the transplantation of organoids or tissue precursor like Langerhans islets (our objectives) are not feasible. To overcome these limitation, others hydrogels (natural or synthetic polymers) have been developed to control matrix properties and optimized organoids formation[4]. However, the lack of native key factors (growth factors, cytokines, and chemokines) in these hydrogels remains essential for a complete differentiation and organoids growth.

In recent years, the use of decellularized extracellular matrix (dECM) derived from various organs have also been developed. Through the process of tissue decellularization, nuclear and cellular components are eliminated while ECM proteins and soluble factors such as growth factors, cytokines, and even non-coding nucleic acids remain relatively preserved in terms of quality and quantity. Consequently, it retains richness of the ECM and provides specific structural support and signalling clues for cell growth and differentiation. Furthermore it presents the advantage to be good manufacture practices compliant[5].

Our laboratory, developed a human extracellular matrix hydrogel, derived from adipose tissue, (atdECM) that could be transplantable. Adipose tissue is a soft tissue that surrounds many organs such as digestive organs or skin. Its main functions are energy regulation and carbohydrate homeostasis balance, but it is considered also as an endocrine organ, which provides cytokines and growth factors usefull for organoids development.

EXPERIMENTAL METHODS

Human adipose tissue of two donors have been decellularized and delipidated to obtain hydrogels for 3D cell culture. Histological labellings have been made to characterized the decellularization and delipidation efficiency. Futhermore, protein profile of atdECM and Matrigel have been studied and compared by mass spectrometry and cytokines array. Finally, pancreatic and prostatic organoids and tumoroids have been generated on atdECM coating as well as Matrigel coating and their morphology and functionality was characterized by immunofluorescence stainings and microscopy.

RESULTS AND DISCUSSION

Mechanical and biological properties (proteomic signature) of the human atdECM has been characterized and compared to Matrigel. Total decellularization and delipidation have been histologically shown. The proteomic profile revealed that atdECM is mostly composed by collagens whereas Matrigel is more a glycoproteins (laminin, nidogen) composed hydrogel. Furthermore, the presence of cytokines and growth factors in atdECM hydrogel after the decellularization process has been proven. Finally, we showed that atdECM support organoids formation as well as Matrigel even if their proteomic profile are different. The functionality and polarity of pancreatic and prostatic organoids have been characterized as illustrated by E-cadherin (green) and Giantin (red) labellings, respectively (Fig 1).

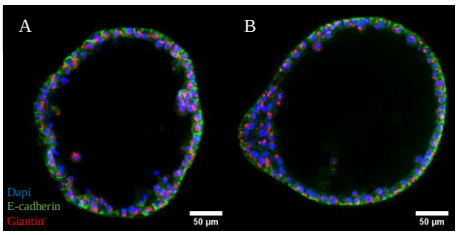

Fig 1. H6c7 derived pancreatic organoids cultured under atdECM coating (A) or Matrigel coating (B).

CONCLUSION

While Matrigel could never be used in regenerative medicine due to its origin, our team has developed a human-derived, non-tumoral hydrogel with the objective to perform human pancreatic islet transplantation. Even if Matrigel and atdECM proteomic profile are very different, this not seems to affect significantly organoids generation and functionality. Thus, atdECM could be an alternative to Matrigel in drug screening, disease modelling as well as regenerative medicine.

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Ice-Templated Collagen-Elastin Scaffolds As Potential Substrates For Lung Alveolar Organoids

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INTRODUCTION

There is increasing interest in the development of accurate models for lung tissue, since the healthcare issues associated with lung disease represent a major financial burden. Lung alveolar organoids are in vitro self-organised aggregates of alveolar epithelial cells. They are intended to mimic the structure and function of the lung alveolus and have applications in drug testing and regenerative medicine therapies.[1] However, in order to develop optimal alveolar models, there is a need for improved three dimensional models to act as substrates to recapitulate the natural lung environment. Here, we propose the use of ice-templated collagen-elastin composite scaffolds and show that careful choice of lyophilisation parameters can result in the fabrication scaffolds with defined pore structures.[2] We have used X-ray microtomography to image natural lung tissue and have explored the effects of different ratios of collagen and elastin (the main lung structural proteins in the alveolar tissue) on the structure and mechanical properties of the scaffolds produced.

EXPERIMENTAL METHODS

Porcine lung tissue was fixed with 2% glutaraldehyde 1% paraformaldehyde solution for 5 hours. Lung tissue samples approximately 1 cm^3 were scanned using a Skyscan 1172 tomographer, and 3D datasets were reconstructed. Fibrillar bovine dermal type I collagen (Devro medical) and soluble bovine neck ligament elastin (Sigma Aldrich) were hydrated in 0.05 M acetic acid and homogenised. 1 wt.% (1 g protein in 100 mL acetic acid) suspensions a range of elastin contents (given by weight) were prepared: 0% (C100E0), 25% (C75E25) and 50% (C50E50). The samples were then freeze-dried, as follows, to create cylindrical collagen-elastin composite scaffolds (35 mm diameter, 5 mm thickness). First, the suspensions were frozen at -30°C at a cooling rate of -0.83°C min^{-1} and they were dried in a vacuum of 80 mTorr. The scaffolds were cross-linked with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxysuccinimide (NHS) in a molar ratio of 5:2:10 EDC:NHS:Collagen. X-ray microtomography was undertaken using a similar methodology as for the natural tissue. Quantitative information including pore size, porosity, pore interconnectivity, pore anisotropy, and percolation diameters were extracted from 1 mm^3 volumes of interest (VOI). Compression testing of hydrated collagen-elastin scaffolds samples were performed at a strain rate of 5 mm min^{-1}.

RESULTS AND DISCUSSION

The pore architecture of collagen scaffolds (Figure 1a) was found to be similar to that of fixed porcine lung (Figure 1b). The average pore size of the scaffolds varied from 81 to 102 µm and the average pore size of the fixed porcine lung was 79 µm. The percolation diameters measured for all samples were in the range 35 − 45 µm where that of the porcine lung was 38 µm. The stiffness of the scaffolds was found to increase with elastin content (from 0 − 50%) and was found to range from 1.3 kPa to 2.8 kPa. These values are of the same order of magnitude as the reported values of the lung.[3] While the elastin content did not result in major differences in pore architecture of the scaffolds, of the compositions tested, the 25% elastin samples showed largest pore size, interconnectivity and percolation diameter. Given that 25% elastin coincides with the natural elastin composition in the lung parenchyma,[4] it appears that the formulation of 75% collagen-25% elastin the optimum composition for application as a lung organoid substrate.

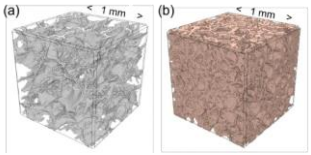

Figure 1. Micro-computational tomography revealed the similarity between the porous structures of a 1 wt.% collagen scaffold (a) and that of porcine lungs (b).

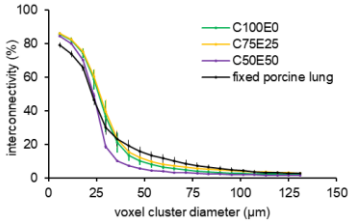

Figure 2. The interconnectivity of collagen-elastin scaffolds of varying collagen:elastin ratios (0% elastin, C100E0, to 50% elastin, C50E50) followed similar trend to that of the fixed porcine lung. The interconnectivity is given as percentage of pore volume accessible when increasingly larger probes (voxel cluster diameter) are scanned across the VOI.

CONCLUSION

The Conclusion section presents the outcome of the work by interpreting the findings at a higher level of abstraction than the Discussion and by relating these findings to the motivation stated in the Introduction. Explain the significance of your findings / outcomes and future implications of the results

ACKNOWLEDGMENTS

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Quantitative analysis of distribution paxillin and vinculin in osteoblasts and fibroblasts binding to electrospun PMMA fibers based on super-resolution fluorescent images.

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INTRODUCTION

The study of interactions between electrospun fibers and human cells is one of the most crucial elements in assessing potential application in bioengineering. Electrospun fibers are one of the very often used scaffolds in n tissue engineering, which should have desired strength and surface properties. Modifying the surface properties of the fibers has a direct effect on cells' adhesion to the scaffold and their proliferation. The dynamics of the cell adhesion process to the scaffold are regulated by the internal multi-protein complex's focal adhesions. They are responsible for forming mechanical links between intracellular actin bundles and the extracellular matrix or substrate. A description of the distribution of adhesion sites in a cell can provide information on the dynamics of the binding process of cells to the scaffold1. Quantification of the special distribution of proteins requires highly specific fluorescent labeling techniques and optical fluorescent microscopy.

In this study, we focus on analyzing the spatial distribution of proteins that are included in focal adhesions formation to the scaffold using two types of cells: osteoblasts and fibroblasts. Vinculin and paxillin are used as a marker of focal adhesion for both types of cells. To produce electrospun fibers Poly(methyl methacrylate) (PMMA) was used. It is one of the most commonly used polymers with a very high degree of biocompatibility with human cells[3], and they have already been used for cell dynamics studies for tissue engineering[2]. To assess potential correlations between the distribution of focal adhesions in two different types of cells to electrospun PMMA fibers, a quantitative analysis of localizations of particular protein foci in 3D multicolor microscopy images is required. Additionally, we show the potential application of high-resolution confocal microscopy with Airyscan2 to study cell-matrix interaction on electrospun polymer fibers.

EXPERIMENTAL METHODS

To obtain a 12 wt. % solution, PMMA was dissolved in DMF. The solution was stirred at 700 rpm for two h on a hot plate set at 55°C. PMMA fibers were produced via electrospinning using the apparatus EC-DIG with climate control. The studies were performed on PMMA electrospun fibers using human osteoblast-like cell line MG63 and fibroblasts NIH 3T3 cell line. Cells were seeded in the PMMA scaffolds in culture media under standard conditions. After three days of cell growth, samples of the PMMA scaffolds were fixed and permeabilized. To visualize actin filaments, cells were incubated with Alexa Fluor™ 488 Phalloidin. Nuclear DNA was stained with DAPI for 5 min. Selected proteins involving in focal adhesion (vinculin and paxillin) were labeled by immunofluorescence. Multicolor 3D microscopy images of cells connecting to PMMA fibers were acquired using confocal microscopy with the high-resolution concept Airyscan2 (Zeiss LSM 900), and the image analysis was performed by using ImageJ.

RESULTS AND DISCUSSION

The application of the high-resolution confocal microscopy with Airyscan2 allowed to register the distribution of vinculin and paxillin to polymer fibers with high resolution. Spatial analysis reveals significant changes in the density of proteins foci along with the cell-binding places to PMMA fibers between osteoblast and fibroblast. It indicates the different architecture of binding mechanisms for osteoblast and fibroblast to the PMMA electrospun fibers. Comparison distribution accumulation of vinculin and paxillin between PMMA fibers and glass as a control for both types of cells shows that the type of substrate with which cells interact has a direct impact on the dynamics of the process of creating binding sites to them.

CONCLUSION

The use of Airyscan2 revealed the distribution of vinculin and paxillin in the areas of cell-fiber interaction in osteoblasts and fibroblasts. Cluster analysis on images with the super-resolution localization of the adhesion proteins in osteoblast and fibroblast clearly highlights substrate-related correlations. It enabled a quantitative description of the architecture of the process of cell-PMMA interaction.

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Development Of A Cardiac Bio-prosthesis

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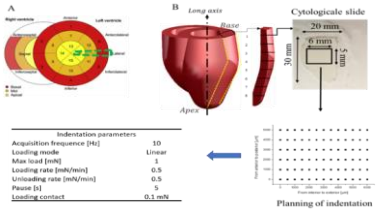
INTRODUCTION

New therapeutic approaches, such as regenerative medicine and tissue engineering are being developed to compensate or even replace damaged tissues. One of the attempts of tissue engineering is to reproduce as closely as possible the mechanical behavior of the tissue to be treated[1]. Biomaterials applied to the epicardium have been studied intensively in recent years for different therapeutic purposes[2]. Their mechanical influence on the heart, however, has not been clearly identified. Most biomaterials for epicardial applications are manufactured as membranes or cardiac patches that have isotropic mechanical behavior, which is not well suited to myocardial wall motion. In this work, an approach linking the mechanical characterization of healthy heart tissue with its microstructure was carried out. To this end and in order to avoid the degradation of the material properties due to its drying out and/or de-vascularization, an experimental protocol was defined to perform the measurements in a context close to its physiological environment. The cardiac tissue was thus characterized through the estimation of Young's modulus in two main directions by spherical indentation.

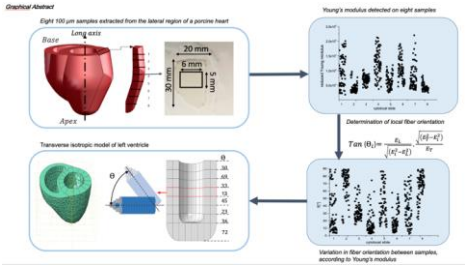
EXPERIMENTAL METHODS

The samples used in this study were extracted from five healthy pig hearts provided by the School of Surgery of the University of Lorraine. Ethical approval for the study was obtained from our university's ethics committee (committee number: 201922016556026) and then further validated by the French Ministry of Higher Education. Heart samples cut to 10-µm thickness were stained with DAPI (D9542,Sigma-Aldrich), for the nuclei, and Phalloidin (P5282, Sigma-Aldrich), for the actin, as permanufacturer's instructions. Imaging was performed using fluorescent microscopy (Moticam 3000, Motic). The mechanical properties of the specimen were determined using an ultra nanoindenter (ANTON-PAAR, Peseux, Switzerland).



RESULTS AND DISCUSSION

The principal novelty of this study is that we found a simple method describing representative fiber orientation calculated within detected Young's modulus. In fact, the existing computational models describing fiber orientation are based on imaging observation (CT, RMI or histology), while the mechanical behaviour detection was apart. On the basis of the mechanical characterization of the myocardium, a model of the mechanical behaviour of a bio-prosthesis has been defined. A first membrane prototype has been produced and tested on small animals.



CONCLUSION

The mechanical performance of cardiac tissue is related to local fiber structure. The formula that we propose outlines, for the first time, a way to describe the elasticity of cardiac tissue and underlying fiber orientation. Building off CT imaging, we created a computational model that can accurately simulate the diastole phase of the LV. By modelling LV wall motion during passive diastole filling, our understanding of volume changes and the local deformation of the epicardium will be vastly improved, which will guide advances in the manufacturing of biomaterials for epicardial applications.

AKNOWLEDGMENTS

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Poly(L-lactic acid) and Ceramic Composite Structures for Fully Resorbable Cranial Implants

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INTRODUCTION

Several neurosurgical procedures are followed by a cranioplasty, which currently carries a high complication rate (7-20%[1,2]). This rate was found to decrease (2%[3]) when using cranial implants composed of a titanium mesh and calcium phosphate ceramic tiles, the latter allowing for partial replacement of the implant by the patient's own bone[3]. However, the titanium interferes with follow-up imaging techniques and is non-resorbable. A fully resorbable implant would allow for a complete regeneration of the bone defect and thus provide several patient benefits, such as a reduced need for revision surgeries, reduced potential sites for infections, and potentially greater use in young patients. In this study, poly(L-lactic acid) (PLLA) was evaluated as a candidate resorbable material to replace the titanium in such composite implants. This material is biocompatible, 3D-printable, offers suitable mechanical properties, and degrades at a rate ranging up to several years. It will be used as a starting point towards the development of a material adapted to the specific need, with a suitable and non-harmful degradation[4]. With this study, we aimed to evaluate ceramic and polymer composite structures, and their interaction, to assess the suitability of using PLLA for cranial implants.

EXPERIMENTAL METHODS

The PLLA structure was manufactured by fused deposition modeling (FDM) on a Prusa i3 MK3S+ (Prusa Research a.s., Prague, Czech Republic). The ceramic part was manufactured in silicone molds, using β-tricalcium phosphate (Sigma-Aldrich, Missouri, USA), monocalcium phosphate monohydrate (Scharlau, Scharlab S.L., Sentmenat, Spain), and 0.5M citric acid solution. The samples were designed as beams with square cross-sections (3x3x45mm), embedded and centered in a ceramic beam (6x6x35mm). Mechanical characterization was performed by 4-point bending tests (n=6 per group), similarly to ISO 5833 guidelines on a Shimadzu AGS-X universal testing machine (Shimadzu, Kyoto, Japan). Scanning electron micrographs of cross-sections were taken at an accelerating voltage of 3kV with a Zeiss 1550 SEM (Carl Zeiss AG, Oberkochen, Germany), equipped with an InLens detector.

RESULTS AND DISCUSSION

SEM images showed that the crevices of the polymer structure are well filled by the ceramic, and the two materials bond together (Figure 1 left). After tearing, retention of ceramic was observed on the PLLA wall (Figure 1 right). After 4-point bending tests, the average bending modulus obtained was 2274±316MPa. The ceramic in the composite structure exhibited a first crack at 139±23N, but retained structural integrity and carried load until a final failure at 249±42N, while the controls composed of only ceramic failed catastrophically already at 115±10N.

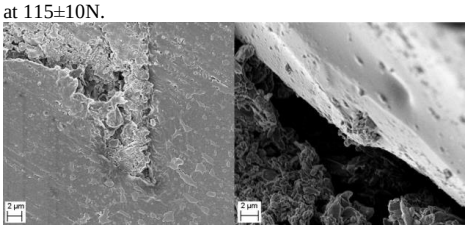

Figure 1: SEM micrograph of ceramic filling a hollow area of the polymer structure (Left), and of ceramic particles retained on the PLLA wall after tearing (Right).

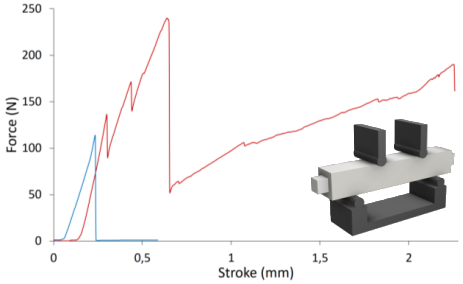

Figure 2: Typical 4-point bending test results for the PLLA-ceramic structures (red) and ceramic alone (blue).

CONCLUSION

SEM images and mechanical tests showed that the PLLA structure supports the molded ceramic, together with promising observations of attachment between the two materials. Further studies will be conducted on the degradation behavior of the material under physiological conditions.

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>> 14:45 - 16:15 | PSOP-13 - TISSUE MODELS
Chairpersons: Audrey Ferrand & Khoon Lim
Location: Room B

14:45 | KL Tissue models - Writing 3D In Vitro Models of Human Tendon Within a Biomimetic Fibrillar Support Platform
Rui DOMINGUES, 3B's Research Group, I3Bs—Research Institute on Biomaterials, Biodegradables and Biomimetics of University of Minho, Braga/Guimarães, Portugal

15:15 | O1 Tissue models - Design of 3D bioengineered cardiac tissue models for the evaluation of chemical cardiotoxicity
Gianluca CIARDELLI, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

15:30 | O2 Tissue models - A Humanized In Vitro Model of Innervated Skin for Transdermal Analgesic Testing
Paul WIERINGA, Complex Tissue Regeneration Department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

15:45 | O3 Tissue models - An in vitro model as a drug testing platform for glaucoma
Hannah LAMONT, Institute of Clinical Sciences, University of Birmingham, Edgbaston, Birmingham, UK; School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, UK

16:00 | FP01 Tissue models - Microphysiological systems for the study of neurodegenerative diseases in vitro
Eleonora DE VITIS, CNR NANOTEC – Institute of Nanotechnology, Lecce, Italy; Università Del Salento, Dipartimento di Matematica e Fisica E. de Giorgi, Lecce, Italy

16:05 | FP02 Tissue models - Decellularized Fibrilar Matrix for Engineering Organotypic Tumor-stroma 3D Biomodels
Vitor GASPAR, CICECO-Aveiro Institute of Materials, Aveiro University, Aveiro, Portugal

16:10 | FP03 Tissue models - Evaluating the use of synthetic self-assembling peptides to 3D bioprint in vitro cartilage tissue models
Patricia SANTOS BEATO, Biochemical Engineering, University College London (UCL), London, UK

Writing 3D *In Vitro* Models of Human Tendon Within a Biomimetic Fibrillar Support Platform

Rosa F. Monteiro[1,2], Syeda M. Bakht[1,2], Manuel Gomez-Florit[1,2], Rui L. Reis[1,2], Rui M. A. Domingues[1,2*], Manuela E. Gomes[1,2]

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INTRODUCTION

Tendon pathologies are highly debilitating diseases for which current treatments have poor recovery outcomes. Therefore, relevant *in vitro* models allowing to study tendinopathies and test new regenerative approaches to develop better treatments are highly needed. We have recently proposed an innovative strategy allowing the automated 3D writing of microphysiological systems (MPS) embedded into its own biomimetic fibrillar support platform based on self-assembled of cellulose nanocrystal,[1] which we believe is promising for tendon modeling. However, commonly used macromolecular bioink polymers have limited ability to mimic a microenvironment as rich as tendon, whose extracellular matrix (ECM) is responsible for activating and regulating several signaling pathways controlling cell behavior. Bioinks based on decellularized ECM (dECM) have emerged as an alternative biomaterial that better represent the signaling complexity of their native microenvironments. Building on these concepts, here we explored our CNC platform as support for writing *in vitro* tendon models using tendon dECM as bioink hydrogels to closely recapitulate the biophysical and biochemical cues of tendon cell niche and thus self-induce the tenogenic differentiation of stem cell.

EXPERIMENTAL METHODS

The dECM bioink hydrogel was prepared from decellularized porcine flexor tendons. To enable the fabrication of human MPS, the easily accessible human adipose derived stem cells (hASCs) were used as cell source. The bioink was directly printed within the CNC fluid gels used as support media for freeform bioprinting of embedded constructs with the desired 3D patterns. To evaluate the effects of cellular crosstalk with endothelial cells, tendon constructs were co-printed with compartmentalized microvascular structures. The subsequent induction of CNC self-assembly post-printing locks the living 3D structures within a permissive ECM mimetic fibrillar material support their long-term *in vitro* cell maturation and function (Fig. 1A and B). The maintenance of this MPS in culture was tested under static or dynamic conditions.

RESULTS AND DISCUSSION

This MPS showed high cell viability, proliferation, and alignment during culture up to 21 days, demonstrating that the synergy between dECM cues and printed patterns induce cells organization similar to tendons tissues ((Fig. 1C and D). Gene and protein expression assays demonstrate that the biophysical and biochemical cues of the dECM induce hASCs commitment toward tenogenic phenotype and that the device culture condition have impact on these cell fate. Remarkably, endothelial cells could migrate toward the tendon compartment showing the existence of chemoattraction effect, but do not invade it (Fig. 1E). Unexpectedly, these cells seem to support the tenogenic differentiation of hASCs. Further cell and molecular biology analysis are being performed to better understand the cellular crosstalk mechanisms occurring in this tendon MPS.

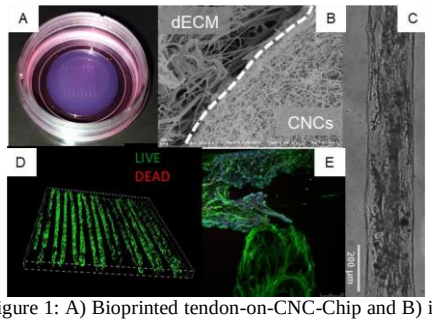

Figure 1: A) Bioprinted tendon-on-CNC-Chip and B) its microstructure; C) transmitted light microscopy and D) Live Dead image of embedded cell structure at D21 and D11, respectively. E) Co-culture with endothelial cells at D21.

CONCLUSION

Overall, the result obtained so far suggest that the proposed system might be promising for the automated fabrication of organotypic tendon-on-chip models that will be a valuable new tool to study tendon physiology and pathologies or the effect of drugs for the treatment of tendinopathy.

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ACKNOWLEDGMENTS

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Design of 3D bioengineered cardiac tissue models for the evaluation of chemical cardiotoxicity

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[2] Department of Surgical Sciences, Università di Torino, Torino, Italy
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INTRODUCTION

Humans are continuously exposed to a huge amount and a variety of chemicals. Animal tests are the gold standard for toxicity testing. However, they often fail in finely replicating the real physio-pathological scenario and their use is associated with ethical issues. 3D *in vitro* tissue models more efficiently mimic the native human environment and bring clear ethical advantages.[1] In order to design a 3D bioengineered tissue model, the 3D matrix used to guide cell behavior, extracellular matrix (ECM) production and new tissue formation should replicate the architecture and composition of the native tissue. In this context, the proper selection of the biomaterial, the fabrication method and the functionalization protocol plays a pivotal role. In cardiac tissue engineering (TE), elastomeric polymers are required as constituents of porous structs replicating *in vitro* the myocardium architecture and mechanical properties. Moreover, surface functionalization with cardiac ECM proteins replicates *in vitro* the biochemical cues present in the native tissue. In this work the versatility of poly(urethane) (PU) chemistry was exploited to design a plethora of polymers with a wide range of physico-chemical properties. The most promising material for cardiac TE was then microfabricated through melt extrusion additive manufacturing (AM). Scaffolds were surface functionalized with cardiac ECM proteins (e.g., laminin, LN) and seeded with cardiac progenitor cells (CPCs) to establish cardiac tissue models.

EXPERIMENTAL METHODS

PUs were synthesized[2] using the same macrodiol (poly(ε-caprolactone) diol, 2000Da) and aliphatic diisocyanate, and different chain extenders (e.g., 1,4-butanediol, 1,8-octanediol, 1,12-dodecanediol, L-lysine ethyl ester, N-Boc serinol). As synthesized PUs were characterized by Infrared (IR) spectroscopy and Size Exclusion Chromatography (SEC). Thermal characterization was carried out through Thermogravimetric analysis (TGA), Differential Scanning Calorimetry (DSC) and rheology to assess polymer suitability for processing in the melt state. Finally, tensile tests were performed on PU dense films to evaluate their mechanical performances. The selected PU according to the measured mechanical properties was then microfabricated into scaffolds by melt-extrusion AM. The structs were surface plasma treated in the presence of acrylic acid vapor to expose -COOH groups and then grafted with ECM proteins through the carbodiimide chemistry.[3] Scanning electron microscopy, IR spectroscopy and X-ray photoelectron spectroscopy (XPS) were performed. CPC adhesion and proliferation were then assessed, while cardiac markers expression was tested by Real-Time Quantitative Reverse Transcription-Polymerase Chain Reaction.

RESULTS AND DISCUSSION

PU successful synthesis was assessed by IR spectroscopy and SEC. Aliphatic linear chain extenders gave stiff PUs (Young's Modulus (E) ≈ 350MPa) with elongation at break (ε%) ranging from few to tens %. In particular, 1,8-octanediol gave a PU with higher ε% (≈ 30-40%) compared to both 1,4-butanediol and 1,12-dodecanediol that resulted in highly brittle polymers. Conversely, N-Boc serinol gave a PU with around 150MPa Young's Modulus and ε% of approx. 150%. L-lysine ethyl ester instead provided the resulting PU with an elastomeric-like behavior (E and ε% of around 10MPa and 700%) that made it the optimal one for the fabrication of structs replicating the cardiac tissue. Rheological temperature ramp tests evidenced a solid-to-liquid transition temperature around 160°C, in agreement with DSC thermograms. Isothermal TGA analysis proved PU suitability for processing in the melt state. Scaffolds with a 0°/90° lay-down pattern were thus fabricated via melt-extrusion AM. Successful surface functionalization was proved by XPS and IR spectroscopy. LN functionalization promoted CPC proliferation and the expression of differentiation markers for endothelial and smooth muscle cells, and cardiomyocytes.

CONCLUSION

In this contribution we have demonstrated that PU versatile chemistry can be exploited to *ad-hoc* engineer a polymer best matching the mechanical requirements for cardiac TE. Our results proved the potential of the developed structs as cardiac tissue models with tunable structural, mechanical, and biochemical features. Such models will allow the investigation of physio-pathological processes and cardiotoxicity testing.

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A Humanized *In Vitro* Model of Innervated Skin for Transdermal Analgesic Testing

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INTRODUCTION

This work establishes a fully human three-dimensional (3D) *in vitro* platform of human skin equivalents (hSE) innervated by induced pluripotent stem cell-derived nociceptors spheroids (hNSs). The skin is densely innervated by nociceptor nerve fibers which play a major role in detecting noxious stimuli, such as irritant chemicals (e.g. capsaicin). Interestingly, the application of capsaicin can treat chronic dermal pain through nociceptor desensitization and nerve ending ablation.[1] Recent engineered human skin equivalents (hSE) have evolved from fibroblasts and keratinocytes to more advanced models containing also vasculature[2], hair follicles[3] and melanocytes.[4] However, innervated hSE platforms to study pain management are rare. In this work, we show the development of an innervated hSE model with a particular focus on mimicking the native innervation pattern and investigating topical analgesics to pass the skin barrier and trigger axonal ablation.

EXPERIMENTAL METHODS

A fibrin/collagen hydrogel platform was created using customized PDMS molds to form a gel containing two separate compartments, one for a sensory neuron population and the other for a hSE construct. The hSE was constructed from fibroblasts in a collagen/fibrin hydrogel blend, followed by seeding keratinocytes on top. Sensory neurons were differentiated from iPSC neurospheres, differentiated to neural crest progenitors via dual SMAD inhibition, CHIR99021 and retinoic acid exposure, Notch inhibition, and exposed to NGF for a mature nociceptor phenotype.
Compartmentalized collagen/fibrin platforms formed with two juxtaposed mini-compartments. Differentiated neurospheres were added to the 'neuron' compartment and, after 7 days of neurite growth, a circular section of a mature hSE was placed in the 'skin' compartment. After 21 days, a clinically available capsaicin patch (Qutenza™) was placed in direct contact with the skin section and incubated for 1 or 24 hrs. Samples were fixed, stained for neural markers (βIII tubulin, TRPV-1) and skin markers (involucrin), and fluorescent images were acquired. Images were assessed for axonal degeneration triggered by capsaicin.

RESULTS AND DISCUSSION

Both the hSE and nociceptor population exhibited morphological and phenotypical characteristics resembling their native counterparts, such as epidermal and dermal layer formation and nociceptor marker expression, respectively.

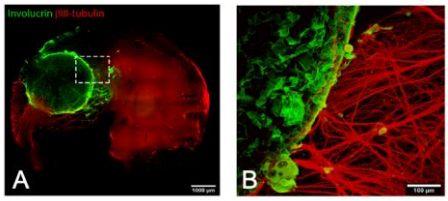

Fig. 2. A) A device with nerve growth (red) and skin (green). B) Innervation of the skin.

In the co-culture platform, neurites developed from the hNSs and navigated the 3D gel to achieve distal innervation of the hSE. Application of the capsaicin patch caused a dose-dependent neurite regression and degeneration. In platforms without the hSE, axonal degeneration was further increased, validating the barrier function of the skin construct.

CONCLUSION

In sum, the work here described constitutes an advancement in the development of fully human and functional innervated hSE constructs that permit convenient testing and tissue analysis.

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ACKNOWLEDGMENTS

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An in vitro model as a drug testing platform for glaucoma

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INTRODUCTION

Glaucoma is a leading cause of irreversible blindness worldwide, with cases estimated to increase globally by 74% by 2040 (1). Primary Open Angle Glaucoma (POAG), the most prevalent subset, occurs highly in patients that express pathological increases in intraocular pressure (IOP), subsequently causing optic neuropathy and vision loss. IOP maintenance is regulated by the trabecular meshwork (TM) and loss of tissue homeostasis leads to ocular hypertension due to the induction of a fibrotic response, which stiffens the matrix (2). The drivers of TM fibrosis are still unknown, and there is a lack of reliable *in vitro* and *in vivo* models to accurately mimic the disease pathology for future drug screening (3). The aim of this project was to develop and characterise a 3D *in vitro* culture model of the TM that can emulate the sites of POAG pathogenesis for future drug targeting. This will be conducted using plastically compressed type I collagen as a support and to explore how the compression process influences the phenotype of the embedded cells.

EXPERIMENTAL METHODS

In the first instance, the collagen fibre architecture created through confined plastic compression was assessed imaging fluorescently labelled collagen fibres after they had been subjected to a 2.5-minute compression using a 20-gram weight. After, primary human trabecular meshwork cells (HTMC) were embedded in a type I collagen scaffolds before being subjected to confined plastic compression by applying a similar load. All control HTMC-collagen samples were formed without prior compression. All HTMC-collagen constructs were incubated at 37°C, free floating for 7 days previously before measuring for relevant protein expression associated with an in vivo TM tissue (α-SMA, fibronectin, TGFβ-2, elastin, α-β-Crystallin, and myocillin) and analyses of cellular phenotype (vinculin and phalloidin) associated with HTMC characteristics.

RESULTS AND DISCUSSION

We demonstrated that there was an increase in collagen fibre density, anisotropy properties and maintenance of collagen fibre alignment within the compressed collagen constructs, compared to fully hydrated type I collagen controls (results not shown). HTMC cultured in the collagen without compression exhibited an elongated, spindle-type morphology with relatively little positive staining for focal adhesions. The HTMC cultured in the plastically compressed collagen exhibited a more fibroblastic phenotype, with an increase in focal adhesion expression and pronounced actin cytoskeleton (Figure 1). Changes in cellular phenotype influenced a change in nuclear morphology, inducing a change in protein expression and cellular characteristics associated with the in vivo TM (results not shown). It was observed that there was heightened protein expression and deposition alike that of the native TM in comparison to fully hydrated collagen gel controls (α-SMA, fibronectin, TGFβ-2, elastin, α-β-Crystallin, collagen type IV, and laminin).

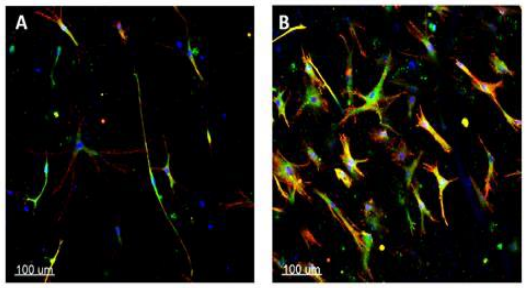

Figure 1: Difference in HTMC morphology 7 days post incubation, when cultured in (A) fully hydrated type I collagen hydrogels (control) displaying an elongated morphology and (B) compressed collagen constructs, showing enhanced cellular spreading and pronounced focal adhesion expression (green), actin cytoskeleton (red), and nuclei (blue). 100μm bar (20X magnification).

CONCLUSION

These results demonstrate the importance of mechanical and geometrical properties of supporting materials in controlling HTMC phenotype. Here, confined plastic compression of type I collagen hydrogels influenced phenotypic changes in the HTMC necessary for the induction of protein and cellular characteristics comparable to that of an innate TM, developing a biomimetic TM in vitro model as a potential drug testing platform.

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Microphysiological systems for the study of neurodegenerative diseases *in vitro*

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INTRODUCTION

Understanding the complex communication between different cell populations and their interaction with the microenvironment is fundamental in neuroscience research. Due to the lack of suitable animal models capable of faithfully reproducing the physio-pathological mechanisms of many human diseases, the development of appropriate *in vitro* approaches and tools, able to selectively analyze and probe specific cells and cell portions (e.g., axons and cell bodies in neurons) has become therefore crucial in this direction. From one hand, the rising technology of organ-on-a-chip (OoCs) offers the possibility to overcome these problems. OoCs are microengineered systems that aim to replicate key units of living organs and organisms, and in particular to reproduce higher-order anatomical and functional features[1]. From the other hand, the discovery of human induced pluripotent stem cells (hiPSCs) represents a revolutionary tool for developing personalized medicine approaches starting from cells directly collected from a patient and reprogrammed to obtain specific cells, even those hardly obtained from living patients (*i.e.* neurons, cardiac and pancreatic cells)[2]. For this reason, hiPSC technology has found application especially in the *in vitro* modelling of neurological diseases (NDDs)[3] also integrated into microfluidic devices. During the past two decades, many platforms have been fabricated in order to study NDDs, focusing the attention on the neuromuscular junction (NMJ) that is damaged in these disorders and, in particular, in Amyotrophic Lateral Sclerosis (ALS). The NMJ is a specialized region composed of presynaptic lower motor neuron, postsynaptic muscle myofiber and terminal Schwann cell, involved in the control of vital body process, such as voluntary movements and breathing[4]. However, most of the platforms focus on the interaction between motor neurons and muscle cells since, to date, protocols to generate pure populations of terminal Schwann cells still need to be developed. Here, we propose a microfabricated *in vitro* model for studying the NMJ where different neuronal populations, glial and skeletal muscle cells can grow and communicate in a perfusable environment. This platform could be also useful to investigate the ALS pathological mechanism.

EXPERIMENTAL METHODS

Microfluidic multi-compartmentalized devices were fabricated by SU-8-based multi-level optical lithography and PDMS replica molding, displaying a series of microchannels that connect three different compartments (hosting three different cell types) and promote neurite elongation unidirectionally from one cell compartment to another one. hiPSC were differentiated toward motor neurons (MNs) and Schwann cells (SCs) and the interactions between them were investigated.

RESULTS AND DISCUSSION

We proposed a microfluidic device with three different perfusable compartment (250 µm high, 500 µm wide and 6 mm long) interconnected through a series of narrow microchannels (2.5 µm high, 5 µm wide and 50 µm long) in which the three main components of the NMJ will be hosted. We developed hiPSC differentiation, on-chip protocols towards MNs and SCs and co-cultured them to investigate possible interactions. After carrying out preliminary experiments in order to improve manual skills with this cell type, to optimize the protocol and to characterize them at different differentiation stages, we differentiated the iPSCs into MNs evaluating their behaviour such as axonal elongation in the adjacent compartment in presence of chemical cues (Brain-derived neurotrophic factor or ciliary neurotrophic factor) or other cell types (iPSCs-derived SCs), investigating in this case potential connections and physiological phenomena such as myelin formation.

CONCLUSION

We designed a robust microfabricated platform for studying the motor circuit components, performing hiPSCs differentiation on chip towards MNs and SCs and investigating possible interactions between these cell types.

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Decellularized Fibrillar Matrix for Engineering Organotypic Tumor-stroma 3D Biomodels

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INTRODUCTION

Currently available 3D in vitro breast cancer models focus mostly on the independent recapitulation of either cell population interactions or singular ECM-specific moieties[1]. Within this context, decellularized scaffolds provide a new pathway to recapitulate major tumor hallmark characteristics in complex cell-representative coculture models capable of integrating ECM-specific stimuli and bioactive components[2]. Seeking to overcome these disadvantages, herein we developed fibrillar dECM breast tumor-stromal models which recapitulate the cellular and matrix landscape of the human tumor.

EXPERIMENTAL METHODS

ECM-derived from porcine adipose mammary tissues was decellularized, characterized and homogenized into fibrillar micro-fragments suitable for inclusion in the process of microtumor assembly as we recently described[3]. As an initial characterization of the decellularization process, DNA, Collagen GAGs and fibrillar elements size distribution was evaluated. Breast cancer cells (MDA-MB-231) and Breast Cancer Associated Fibroblasts (BCAFs) were directly cultured with conjugated dECM microfibrillar fragments.

RESULTS AND DISCUSSION

Physiomimetic culture of tumor and stroma elements in fibrillar ECM supporting matrix led to the assembly of spherical 3D tumors with, fibrillar dECM spatial distribution in microtumors volume (**Figure 1**).

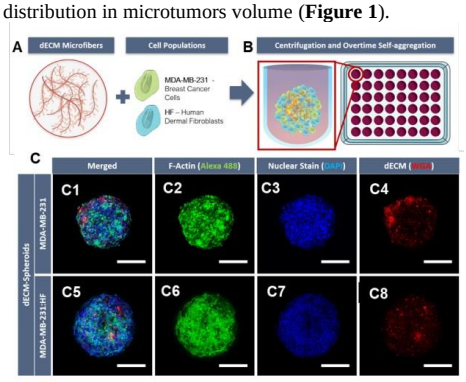

Figure 1. Engineering physiomimetic 3D microtumor-stroma models in fibrillar dECM matrix via superhydrophobic surfaces. Green channel: F-actin. Red channel: Fibrilar dECM. Blue Channel: Nuclei.

Endometabolomics analysis indicated a clear impact of fibriblar dECM inclusion in microtumor-stroma models, with a marked increase in lactate excretion and pyruvate consumption being observed, simulating the metabolic pathways activated in the *in vivo* scenario.

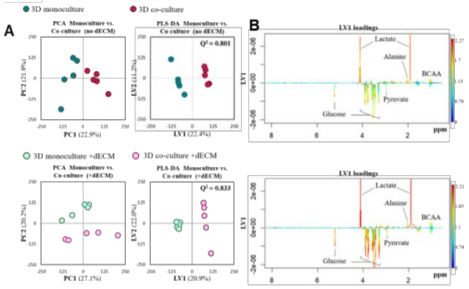

Figure 2. Exometabolomics analysis. (A and B) PCA scores scatter plots, PLS-DA scores scatter plots (middle) and LV1 loadings plots, respectively obtained through multivariate analysis of 1H NMR spectral profiles.

CONCLUSION

Overall, our finding evidence the importance of fibrilar dECM in promoting microtumor-stroma models recapitulation of key disease hallmarks. The herein proposed approach provides a universal methodology for developing more physiomimetic in vitro tumors models that are amenable for high-throughput drug screening. We envision that the modularity of this methodology will further allow the inclusion of other tumor building blocks including tumor-associated macrophages in the future, contributing for the so desired immunization of such models.

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ACKNOWLEDGMENTS

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Evaluating the use of synthetic self-assembling peptides to 3D bioprint in vitro cartilage tissue models

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INTRODUCTION

Cartilage pathologies remain a challenge in the field of orthopedic medicine, with diseases such as osteoarthritis remaining without a cure. Development of reliable disease models is a key to improve our understanding of these pathologies. However, conventional tissue engineering techniques, such as scaffold top cell seeding, to develop cartilage constructs *in vitro* have encountered multiple issues such as lack of structural control and heterogeneous cell distribution. 3D bioprinting has been used as an alternative approach to overcome these limitations, enabling the manufacturing of intricate structures with a controlled and homogeneous cell deposition. Most cell-laden materials used to 3D bioprint cartilage, such as gelMA[1] or hyaluronic acid[2], are animal-derived. With the goal of taking the next step towards a more sustainable and ethical scientific approach, synthetic polymers are an alternative to biologically derived materials to 3D bioprint cartilage.

In this research, we present the use of a commercial self-assembling peptide as a potential material for 3D bioprinting cartilage tissue *in vitro* models, and its initial evaluation in terms of how the 3D bioprinting process affects the ability of primary human chondrocytes to produce cartilage with this material.

EXPERIMENTAL METHODS

Alpha 1 Peptigel (Manchester Biogel, UK) was mixed with human primary chondrocytes and 3D bioprinted into cylindrical structures (1 mm tall, 5 mm diameter) using the BIOX 6 bioprinter (Cellink, Sweden). These were cultured using chondrogenic growth media (Cell Applications, San Diego, CA) for 21 days, incubated at 37°C in a humidified atmosphere with 5% pCO_2.

Cell viability, cell proliferation, and histological assessments were performed. Specific cartilage markers such as Sox-9, collagen II, and aggrecan expression were assessed through immunochemistry and quantitative ELISA methods. The results were compared to a 3D chondrocyte cell pellet control.

RESULTS AND DISCUSSION

Human primary chondrocytes embedded in Alpha 1 Peptigel showed high cell viability (>75%) after LIVE/DEAD staining; as well as maintenance of cell numbers after day 7 and up to 21 days of culture, assessed through DNA quantification, showing a cartilage-like behavior where cells do not proliferate. The 3D control on the other hand showed a decrease in cell viability and a decrease in cell number over 21 days potentially due to the hypoxic conditions experienced in the cell pellet core.

Regarding cartilage-specific markers, Alpha 1 based *in vitro* cartilage models showed high Sox-9 expression from as early as day 0, indicating that chondrocytes presented a chondrogenic phenotype, which is lost in 2D cell culture expansion[3]. Collagen II and aggrecan expression was firstly observed at day 7 and strongly expressed up to day 21. The expression of collagen II and aggrecan indicated that these cells had matured and produced extracellular matrix later in the culture post-printing. These markers were also observed in the 3D pellet control, which confirmed expected 3D control behavior[4]. These cell viability results as well as the specific cartilage marker expressions shown in Alpha 1 based constructs are similar to the results observed with other hydrogels, such as gelMA, in cartilage 3D bioprinting[1,2].

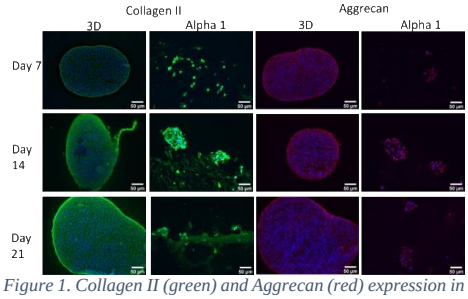

Figure 1. Collagen II (green) and Aggrecan (red) expression in human primary chondrocyte cell laden Alpha 1 and 3D cell pellet control images across days 7, 14 and 21; cell nuclei shown in blue (DAPI). Scale bar show 50 µm.

CONCLUSION

This study highlights the potential of the Alpha 1 self-assembling peptide as a non-animal derived bioink for 3D bioprinting of *in vitro* human cartilage tissue models. Human primary chondrocytes embedded in 3D bioprinted structures show specific cartilage marker expression as well as high cell viability. Future application of this model could include cartilage-specific disease modelling, such as osteoarthritis.

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>> 14:45 - 16:15 | PSOP-14 - PROTEIN SURFACE INTERACTIONS

Chairpersons: Elzebieta Pamula & Julien Gautrot
Location: Room C

14:45 | KL Protein surface interactions - Competitive Binding and Molecular Crowding Regulate the Cytoplasmic Interactome of Non-Viral Polymeric Gene Delivery Vectors
Julien GAUTROT, Queen Mary, University of London

15:15 | O1 Protein surface interactions - Correlation between protein adsorption onto Cu-doped sol-gel coatings and their antibacterial potential
Julio SUAY, Department of Industrial Systems and Design Engineering, Universitat Jaume I, Castellón, Spain

15:30 | O2 Protein surface interactions - Elastomeric, Bioadhesive and pH-responsive Copolymer Hydrogels Based on Poly(glycerol sebacate) and Poly(ethylene glycol)
Mina ALEEMARDANI, Biomaterials and Tissue Engineering Group, Department of Materials Science and Engineering, Kroto Research Institute, The University of Sheffield, Sheffield, UK

15:45 | O3 Protein surface interactions - "Stealth graft polymers for therapeutic mRNA vaccines"
Coral GARCIA FERNANDEZ, Group d'Enginyeria de Materials (GEMAT), Intitut Químic de Sarrià, Universidad Ramon Llull, Barcelona, Spain

16:00 | FP01 Protein surface interactions - Injectable hydrogels for microRNA delivery to promote direct cell reprogramming in cardiac regeneration
Elena MARCELLO, Department of Mechanical and Aerospace Engineering, Politecnico di Torino, Torino, Italy

16:05 | FP02 Protein surface interactions - A protein-based multiplex assay to screen osteogenic properties of calcium phosphate biomaterials with inorganic additives
Maria EISCHEN-LOGES, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

16:10 | FP03 Protein surface interactions - Milk-Derived Extracellular Vesicles for siRNA Delivery into Intestine Mimicking Caco-2 Cells
Josepha ROERIG, Pharmaceutical Technology, Medical Faculty, Leipzig University, Germany

Competitive Binding and Molecular Crowding Regulate the Cytoplasmic Interactome of Non-Viral Polymeric Gene Delivery Vectors

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INTRODUCTION

Although polycationic vectors display excellent performance in vitro with many cellular systems, their clinical use remains very restricted. To some level, this is due to the poor compatibility of such systems with biological fluids and tissues. In addition, in contrast to the processes controlling the complexation, targeting and uptake of polycationic gene delivery vectors, such as poly(ethylene imine) and poly(dimethylaminoethly methacrylate), the detailed molecular mechanisms regulating their cytoplasmic dissociation remains poorly understood. Upon cytosolic entry, gene delivery vectors become exposed to a complex, concentrated mixture of molecules and biomacromolecules.

EXPERIMENTAL METHODS

To explore cytosolic release mechanisms, we characterised the cytoplasmic interactome associated with a polycationic vector based on poly(dimethylaminoethyl methacrylate) (PDMAEMA) brushes grafted from nanoparticles (using proteomics and gel electrophoresis). Such cationic brushes were found to be particularly effective at trapping small RNAs, resulting in high knock down efficiencies (1-4). However, how such stable association is disrupted in the cytosol was not clear. To quantify the contribution of different classes of low molar mass molecules and biomacromolecules to RNA release, we used fluorescence microscopy and developed a kinetic model based on competitive binding.

RESULTS AND DISCUSSION

We propose that the molecular structure and architecture (in particular the high surface density) of cationic brush-decorated nanoparticles, together with the cytosolic molecular crowding, modulate competitive binding and, in turn, the long term release of RNA. Based on these observations, we chemically designed polymer brushes with improved RNA retention in the cytosol, avoiding burst release, and enabling to achieve long term (at least 10 days) knock down (>70%) with one single transfection.

CONCLUSION

Our data demonstrate the importance of cytosolic interactions in regulating the disassembly of RNA-polycation complexes upon internalisation. Understanding the mechanism regulating cytosolic dissociation will enable the improved design of cationic vectors for long term gene release and therapeutic efficacy. In addition, our proteomics data identifies the association with a number of protein and complexes regulating translation, suggesting a new mechanism for off-target effects and cytotoxicity of RNA delivery systems. Understanding such processes will enable the rational design of more efficient and safer RNA delivery platforms.

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ACKNOWLEDGMENTS

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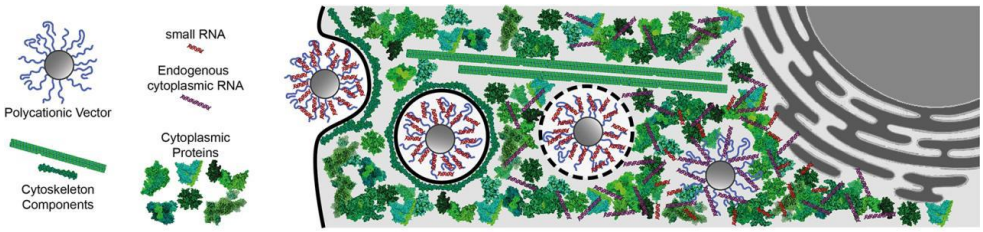

Figure 1. Competitive binding defines the cytoplasmic interactome associated with polycationic gene delivery vectors and regulates cytosolic RNA delivery.

Correlation between protein adsorption onto Cu-doped sol-gel coatings and their antibacterial potential

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INTRODUCTION

Bacterial infection can have fatal results in the implantology field. Moreover, the arising multidrug bacterial resistance makes the development of new biomaterials with advanced antibacterial properties a key goal. Cu exhibits antibacterial, angiogenic and osteogenic functions, representing an ideal option to bioactivate prostheses and help to limit the development of antibiotic resistance[1]. However, the exact antimicrobial mechanism of Cu-doped biomaterials remains poorly understood[2].

The aim of this study is to evaluate how the addition of Cu affects biocompatibility in biomaterials and their antibacterial potential. In order to obtain a better understanding about the interaction mechanism of Cu-biomaterials with biological systems proteomic characterization was performed. For that, sol-gel coatings with increasing amounts of Cu were developed, their physicochemical and biological properties characterized and their interaction with proteins analyzed via proteomics.

EXPERIMENTAL METHODS

Methyltrimethoxysilane (M) and tetraethyl orthosilicate (T) were used as precursors to synthetize the sol-gel mixtures with 0.1, 0.5, 1 and 3 %wt $CuCl_2$. The Cu-doped sol-gel compositions were applied as coatings onto Ti discs and were cured at 80ºC for 2 h. The physicochemical characterization was carried out using SEM, EDX, FT-IR, NMR and XRD, and the Cu^{2+} release kinetics measured through ICP. *In vitro* tests with both HOb and THP-1 cell lines were performed to evaluate the coating osteogenic and inflammatory potentials, respectively. The antibacterial activity of Cu-doped coatings was measured with *E. coli* and *S. aureus*. The protein adsorption onto the coatings were studied by incubating the samples with human serum. The adsorbed proteins were eluted and analyzed using nLC-MS/MS and proteomic tools.

RESULTS AND DISCUSSION

The sol-gel materials with $CuCl_2$ were successfully synthetized and applied as coatings. The addition of Cu showed no effect on the crosslinking degree in the silica networks. The presence of Cu in the materials was detected by EDX and XRD. The ICP results showed a controlled release of Cu^{2+} in the coatings dependent of the initial amount of salt incorporated. The materials resulted not cytotoxic. Moreover, the addition of Cu increased the expression of osteogenic genes (BMP-2, ALP and OCN). The Cu-doped coatings also displayed a higher inflammatory potential as an increased expression of INF-δ, TNF-α and IL-1β and an elevated secretion of TNF-α were measured with THP-1. A significant reduction in the viability of both *E. coli* and *S. aureus* was detected in the coatings from concentration of 0.5 and 1 %wt $CuCl_2$, respectively.

Proteomic studies revealed that Cu-doping increased the adsorption of proteins associated with the immune system (CO5, C4BPA, C4BPB, CO8B, SAMP ENOA), coagulation, angiogenesis and fibrinolysis functions (FA9, FA10, PROS, HRG and PLMN), and antibacterial properties (ENOA, HRG). In parallel, protease inhibitors such as A2MG, which is able to neutralize immune products with a protective effect on bacteria, were found less adsorbed onto the coatings in a Cu dose-dependent manner.

CONCLUSION

The developed Cu-doped sol-gel coatings displayed osteogenic and antibacterial properties, as well as, an increased inflammatory potential. This fact was correlated with an increased adsorption of proteins associated with the complement system activation due to Cu-doping. These proteins could lead to the membrane attack complexes formation, promoting the antibacterial effect. Therefore, our results deepen the knowledge about the antibacterial mechanisms associated with Cu-enriched biomaterials.

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The authors would like to thank MINECO [PID2020-113092RB-C21], Generalitat Valenciana [APOSTD/2020/036, PROMETEO/2020/069], Universitat Jaume I [UJI-B2021-25] and the University of the Basque Country under [GIU18/189] for providing financial support to this project.

Elastomeric, Bioadhesive and pH-responsive Copolymer Hydrogels Based on Poly(glycerol sebacate) and Poly(ethylene glycol)

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INTRODUCTION

Synthetic polymer hydrogels have proven applications for biomedical purposes, including cell culture, tissue engineering and drug delivery. Synthetic hydrogels with amphiphilic structures can possess useful properties, such as adjustable mechanical features and biodegradation, responsiveness to physicochemical dynamics, and bioadhesiveness, all of which have great potential in biomedical applications[1].

Poly(glycerol sebacate) (PGS) is an example of a synthetic polymer that has exhibited elasticity, cytocompatibility, and biodegradability in a biomedical context. An interesting aspect of PGS is its resemblance to fatty acids, and its modulus, which is comparable to that of human soft tissues; however, its hydrophobic characteristics have hindered its use in advanced applications, such as hydrogel fabrication, bioink development, and cell encapsulation[2,3]. We sought to address this limitation by developing novel biodegradable, biocompatible, bioadhesive, highly elastomeric and pH-responsive copolymer hydrogels by direct crosslinking of the PGS pre-polymer and two types of polyethylene glycol (PEG), without using crosslinking agents. By combining hydrophilic PEG with hydrophobic PGS, we have demonstrated that it is possible to create hydrogels with outstanding bioadhesiveness, elastomeric properties, and pH-responsive swelling.

EXPERIMENTAL METHODS

In this study, the effect of PEG type and quantity in copolymers scaffold production was systematically evaluated with regards to physicochemical properties and biocompatibility. The copolymerisation of PGS-co-PEG pre-polymer was conducted via a two-step process: (1) polycondensation of sebacic acid (SA) and PEG with different types and weight ratios to yield the SA/PEG pre-polymer, and (2) addition of glycerol and synthesis of PGS-co-PEG pre-polymers.

RESULTS AND DISCUSSION

Hydrogels made of copolymers were shown to be highly flexible and stretchable, and with complex knotting and stretching deformations. These hydrogels can adhere firmly to different substrates, such as glass, polycaprolactone, polytetrafluoroethylene, silicone, wood and aluminium, with maximum lap-shear strength and adhesion strength reached at 336 kPa and 84 kPa, respectively. Their Young's moduli were in the range of 0.004–0.521 MPa, and hydrogels with higher amounts of PEG (PEG≥40%) showed stretching beyond 4-5 times their initial length. The copolymer hydrogels had pH-responsive behaviour. Swelling ratios were higher in basic conditions (pH=9.1), ratios were greater at pH 9.1>7.4>5. Therefore, these hydrogels can be used for pH-dependent drug release. Moreover, due to the structure of copolymers, tunable biodegradation can be achieved by changing parameters related to crosslinking and PEG content. This work shows that the degradation kinetics accelerated with the addition of PEG, with a direct correlation with the amount of PEG added. Copolymer hydrogels were also found to be cytocompatible with human keratinocyte (HaCat) cell lines through testing with a resazurin assay for the cell metabolic activity.

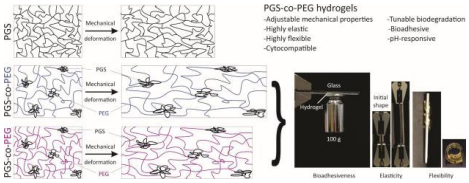

Figure 1. A schematic representation of the structure of copolymer hydrogels and their response to mechanical deformation, along with a description of their significant features.

CONCLUSION

With their multiple functions, these new copolymer hydrogels show great potential for biomedical applications and controlled drug delivery systems. It is also expected that the development of these copolymer hydrogels will provide new ideas for developing future tissue engineering biomaterials.

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"Stealth graft polymers for therapeutic mRNA vaccines"

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INTRODUCTION

The current pandemic has evidenced the significant and growing contribution of mRNA as biotherapeutic molecule to tackling manifold health problems[1]. However, the urgency of the situation has precipitated its approval, leaving a plenty of room for improvement. Research in this field now focuses on developing new materials to improve the mechanisms described, facilitate the storage, and open the door to the treatment of other diseases.

The efficiency of these vaccines is inherently related to the efficiency of the formulation used for their administration. Nanoparticles stabilized with polyethylene glycol (PEG) are traditionally the preferred option. However, recent studies have shown evidence of anaphylaxis and immunogenicity in treated patients, seriously compromising the efficiency of future doses[2]. On the other hand, the knowledge of immune cells and their interaction with nanoparticles is of great interest to the pre-clinical/clinical study scenario. The lack of specificity of the current platforms results in undesired inflammatory responses. Modification of nanovehicles' synthetic properties due to their interaction with the physiological medium, i.e. the protein corona, is an uncontrolled phenomena that leaves the nanoparticle's fate random[3].

This work aims to describe the design and synthesis of a new polymer family. The obtained "stealth" formulation is not only able to avoid opsonization and the undesired action of macrophages, but also selectively target dendritic cells.

EXPERIMENTAL METHODS

Synthesis of stealth polymer. Synthesis of poly(beta aminoester) backbone was synthesized by addition of 5-aminopentanol, hexylamine and 1,4-butanediol. Lateral chains were modified with a Chain Transfer Agent to perform a final zwitterionic grafting on the initial backbone by RAFT polymerization of sulfobetain monomers. Further modification with a complementary peptide to dendritic cells was done.

Formulation and characterization. Polyplexes were formulated by addition of the polymer to mRNA sodium acetate solution. Characterization was conducted by Dynamic Light Scattering, Nanotracking Analysis and Transmission Electronic Microscopy. Long-term stability of the formulation was evaluated after lyophilization.

Proteomics. Nanoparticles were incubated overnight in Human Serum and protein corona was isolated by centrifugation and sonication. Samples were analysed by BCA kit and MS/MS.

***In vitro* and *in vivo* assays.** Cytotoxicity was assessed on immune primary cell lines. Transfection efficiency and specificity were evaluated by flow cytometry and confocal microscopy. Biodistribution of polyplexes was evaluated in BALB/c mouse by intravenous and intramuscular administration routes.

RESULTS AND DISCUSSION

Obtained polyplexes were characterized after previous determination of the optimal mRNA/polymer ratio for encapsulation. Nanoparticles are lower than 210 nm diameter and +15 mV of ζ-potential. Physicochemical properties remained stable after lyophilization, enhancing their stability and easing the storage and distribution process.

Interaction of the delivery system with proteins was then evaluated. Currently, the presence of a stabilizing coating to extend the circulation time is required. Without stabilization, the main barrier is the activation of the immune system when macrophages detect circulating nanoparticles. This detection occurs due to attachment of opsonin proteins on the surface of the particles. These proteins promote phagocytosis by blood-circulating myeloid leukocytes and tissue-resident macrophages compromising the efficacy of the therapy. Our zwitterionic polyplexes showed less total protein attached and the absence of opsosins and ubiquitins on the corona, confirming their stealth properties (**Figure 1).**

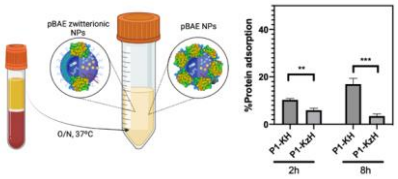

Figure 1. *Scheme of evaluation of total protein corona by colorimetric BCA assay and representative results.*

In vitro assays in primary immune cells confirmed the biocompatibility of the formulation. A selective and efficient transfection to immature dendritic cells was achieved, thanks to the active targeting. Finally, *in vivo* biodistribution assays confirmed the migration of the polyplexes to the lymph nodes, where the activation of lymphocytes occurs.

CONCLUSIONS

In this communication, we present a new family of stealth polymers. Stealth properties were widely demonstrated by proteomics and *in vitro* assays. .Furthermore, an active targeting was achieved to dendritic cells, powering the efficacy of treatment together with good stability results for storage.

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Injectable hydrogels for microRNA delivery to promote direct cell reprogramming in cardiac regeneration

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INTRODUCTION

Cardiac regeneration post- myocardial infarction (MI) represents a worldwide clinical challenge due to the poor regenerative capabilities of heart tissue. After MI, cardiac extracellular matrix undergoes significant remodeling leading to the formation of a fibrotic scar populated by cardiac fibroblasts (CFs), non-contractile cells. Regenerative medicine approaches are currently being explored to replenish the population of cardiomyocytes (CMs) and re-establish the functionality of injured myocardium. In particular, the direct reprogramming of CFs into induced CMs using a combination of four micro-RNA (miRNA), named miRcombo, has shown encouraging results both *in vitro* and in mouse models[1]. MiRcombo encapsulation in lipoplexes (LPs) can highly improve miRNA cell uptake and transfection efficacy. Furthermore, LPs embedding into an injectable hydrogel could improve their *in situ* delivery to the pathological cardiac tissue. Algysil LVR, an alginate-based material, has been investigated in clinical trials to favor cardiac tissue remodeling after MI[2]. However, Algysil LVR is not cell adhesive and is a permanent device due to alginate poor *in vivo* degradability. To overcome these hurdles, an oxidised formed of alginate, alginate dialdehyde (ADA), was herein explored as a delivery system[3].

In this work, an ADA-based injectable hydrogel was developed able to encapsulate and release miRNA-loaded LPs. To improve hydrogel cell adhesive properties, hydrogel composition was optimized through functionalization with a chemically-modified gelatin (Gel-M).

EXPERIMENTAL METHODS

ADA was prepared by partial oxidation of alginate with sodium metaperiodate and characterized using MAS solid-state NMR spectroscopy and via the iodine–starch test to determine the degree of oxidation[3]. ADA hydrogels were obtained by ionic crosslinking with calcium ions using a double syringe mixing system and characterized rheologically. Novel LPs containing miRNA were prepared *via* spontaneous electrostatic interaction and physically encapsulated in the ADA hydrogels[4]. Release studies of a Cy5-siRNA-loaded LPs from ADA hydrogels were conducted in distilled water at 37°C and estimated by plate reader analysis.

A modified gelatin (Gel-M), able to react with ADA was produced and characterized for its functionalization degree by 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) assay[5]. ADA/Gel-M hydrogels were prepared varying the polymers weight ratios (%w/w) (from 70-30 to 30-70, ADA: Gel-M), keeping constant the final polymer concentration (%w/v). Adult human CFs (AHCFs) were cultured on ADA/Gel-M hydrogels for 1 and 7 days, and the adhesion of DAPI-stained cells was evaluated by fluorescence microscopy. The analysis of the release of miRNA-loaded LPs from ADA/Gel-M hydrogel is in progress.

RESULTS AND DISCUSSION

ADA with an oxidation degree of 40% was produced from alginate with an average yield of 75%. The presence of aldehyde groups was confirmed by ATR-FTIR and ^{13}C MAS NMR spectroscopy. Tailored calcium ions and polymer concentrations allowed to obtain physical hydrogels with viscoelastic properties similar to Algysil LVR. Novel miRNA-loaded LPs were entrapped into ADA hydrogels without altering rheological properties. Cy5-siRNA, a model fluorescent oligonucleotide loaded in LPs was completely released from the hydrogel in 24h. To improve cell adhesive properties of ADA hydrogels, Gel-M was prepared with an average yield of 80% and with an average degree of functionalization of 14%. Injectable hydrogels with different viscoelastic properties were developed exploiting the possibility to create chemical crosslinking between ADA and Gel-M. Biocompatibility studies with AHCFs showed that the incorporation of Gel-M significantly increased CFs adhesion compared to ADA-based materials.

CONCLUSION

A novel strategy for cardiac regeneration was investigated based on the *in situ* delivery of miRNA-loaded LPs through ADA-based injectable hydrogels to promote myocardial regeneration. The incorporation of Gel-M into the hydrogel matrix supported CFs adhesion. We are currently investigating the release of miRNA-loaded LPs from this novel ADA/Gel-M matrix.

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ACKNOWLEDGMENTS

The authors would like to thank the European Research Council (ERC) under EU H2020 research and innovation program (BIORECAR; 772168) for providing financial support to this project.

A Protein-based Multiplex Assay to Screen Osteogenic Properties of Calcium Phosphate Biomaterials with Inorganic Additives

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INTRODUCTION: The intrinsic healing capacity of bone tissue often falls short in critical-sized bone defects. In this context, off-the-shelf–available and synthetic bone graft substitutes such as calcium phosphate (CaP)–based biomaterials are already widely used in clinical applications.[1] The properties and clinical performance of these biomaterials remain, however, inferior to those of an autologous bone graft, which is still considered the gold standard treatment for these defects.[2] Doping CaPs with inorganic additives such as strontium, copper or magnesium is considered a promising method to improve their performance.[3] Here, we propose a targeted protein multiplex assay as a screening tool to improve the in vitro evaluation of such biomaterials and to gain a better understanding of their effect on osteogenic differentiation.

EXPERIMENTAL METHODS: Cell culture plates were coated with CaP doped with a range of inorganic additives including Sr^{2+} (CaP+Sr), Mg^{2+} (CaP+Mg), Mn^{2+} (CaP+Mn), Cu^{2+} (CaP+Cu), and Zn^{2+} (CaP+Zn) as previously described and characterized.[4] Human mesenchymal stem cells (hMSCs) were seeded on the coatings. HMSCs in basic medium (BM) and osteogenic induction medium (OM) were used as controls. Cell morphology and adhesion were assessed, and an MTT assay was done to determined cell metabolic activity. A multiplex protein assay to detect analytes related to osteogenesis, angiogenesis, and immunomodulation was performed in medium supernatant and in the cell lysate. Osteogenic differentiation was confirmed by an alkaline phosphatase (ALP) assay and a qPCR panel.

RESULTS AND DISCUSSION: Multiplex analysis showed differences in protein expression in hMSCs on the different CaP coatings with inorganic additives (Fig. 1). The effect of different inorganic additives was described in terms of hMSC stemness, osteogenic, angiogenic, immunomodulatory, and other markers. Differences in angiogenesis-related markers in hMSCs on CaP+Mn as compared to the control in BM and on CaP without additive incorporation indicated a beneficial effect of CaP+Mn on angiogenesis, which needs to be analyzed further. Gene expression analysis showed an upregulation of the osteogenic transcription factor RUNX-2 in hMSCs in OM, and on CaP+Mn and CaP+Cu at day 7. BMP-2 was upregulated in hMSCs on all coatings at day 7 and on CaP+Mn at day 21 as well. ALP activity was diminished in hMSCs on all coatings at day 7, except in CaP+Zn where no difference was detected. At day 21, ALP activity was increased in hMSCs in OM and decreased in hMSCs on CaP+Cu. BMP-2 was upregulated in hMSCs on all coatings at day 7 and on CaP+Mn at day 21 as well. ALP activity was decreased in hMSCs on all coatings at day 7, except in CaP+Zn where no difference was detected. At day 21, ALP activity was increased in hMSCs in OM and decreased in hMSCs on CaP+Cu.

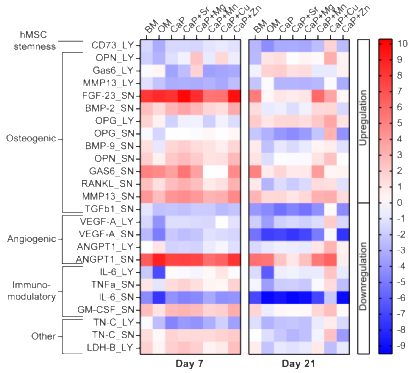

Figure 1. Multiplex protein analysis of hMSCs on CaP coatings measured in the supernatant (SN) and lysate (LY).

CONCLUSION: Here, we presented our efforts on screening osteogenic, angiogenic, and immunomodulatory properties of CaP-based biomaterials with inorganic additives using a protein multiplex-based tool. The protein multiplex assay, combining several relevant analytes, generated a substantial amount of biological data and allowed a better insight in the functional capacity of CaP-based biomaterials with inorganic additives. Protein profiles of CaP-based biomaterials were detected and biomaterials of interest were highlighted. The potential angiogenesis inducing effect of the CaP+Mn biomaterial will be explored further.

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ACKNOWLEDGMENTS: This research has been made possible with the support of the Netherlands Organisation for Scientific Research Vidi grant (15604), the Dutch Province of Limburg (LINK project) and the Interreg Vlaanderen/ Nederland project 'BIOMAT-on-microfluidic-chip'. PH gratefully acknowledges the Gravitation Program 'Materials-Driven Regeneration', funded by the Netherlands Organization for Scientific Research (NWO) (024.003.013). ZTB gratefully acknowledges the NWO grant for women in STEM (Project 'Biotetris').

Milk-Derived Extracellular Vesicles for siRNA Delivery into Intestine Mimicking Caco-2 Cells

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INTRODUCTION

Extracellular vesicles (EVs) are cell-derived nanostructures, which gain increasing interest for their role as natural RNA carriers. We would like to exploit EVs for the oral delivery of therapeutic RNA. Therefore, EVs must fulfill: (1) stability in the gastrointestinal system, (2) bioavailability, and (3) loading with RNA.

We have chosen bovine raw milk as a source for EVs, as these survive the harsh conditions of the gastrointestinal system[1]. To assess the suitability of EVs for oral RNA delivery further, this work aims to study EV uptake and transport across the intestinal barrier *in vitro*. Currently, low RNA loading efficiencies limit the therapeutic use of milk EVs. Therefore, we explore novel strategies to load EVs with siRNA and test the functional siRNA delivery in Caco-2 cells.

EXPERIMENTAL METHODS

EV Isolation and Analysis

Raw milk was centrifuged and loaded onto a Sephacryl S-500 (Cytiva, USA) column. The presence of EVs was evaluated by antibody detection of marker proteins, electron microscopy, and nanoparticle tracking analysis.

EV and Liposome Uptake into Caco-2 Cells

Enterocyte-like Caco-2 cells were incubated with stained EVs (5 µM Vybrant DiO, 20 µM CellTrace CFSE, or 10 µM SYTO®). For comparison with liposome uptake, cells were incubated with the same particle number of DPPC-Chol (70/30 mol/mol) liposomes. Cell uptake was analysed by confocal microscopy (Leica, Germany).

EV Transport across the Caco-2 Monolayer

Caco-2 cells grown on 3 µm PET inserts were used for transport studies. Stained EVs were added to the donor compartment and fluorescence measured in the acceptor compartment to calculate the transport rate (P_{app}).

siRNA Loading and Functional Delivery

Fusion with non-cationic liposomes, dehydration-rehydration, and dual asymmetric centrifugation were compared for EV loading with either siRNA complexed with calcium phosphate nanoparticles[2] or free siRNA. Encapsulation efficiency and loading capacity were calculated using fluorescently labelled siRNA (Quiagen, Netherlands). To test the functional delivery in Caco-2 cells, AllStars Hs Cell death siRNA (Quiagen, Netherlands) was used instead. Cell viability was measured three days after transfection using RotiTest® Vital (Roth, Germany).

RESULTS AND DISCUSSION

Milk EVs were isolated using size-exclusion chromatography and analysed according to the MISEV2018 guidelines[3]. Cell uptake was compared to liposomes (DPPC/Chol) in intestinal cells. Three different EV labelling approaches showed comparable results by microscopic evaluation after 15 min to 6 hours, indicating a fast EV internalization into Caco-2 cells (Fig. 1, white arrows). Liposomes rather remained attached to the cell surface (Fig. 1, blue arrows). As well, the EV transport rate across the Caco-2 barrier was quantified as $P_{app} = 2.4 – 6.5 \cdot 10^{-6}$ cm/s.[4]
Strategies to load EVs were adapted from liposome loading including fusion, dehydration-rehydration, and dual asymmetric centrifugation. Cationic transfection reagents that alter the EV membrane were avoided. Loading efficiencies were sufficient to functionally deliver siRNA. As a proof-of-concept, cell-death siRNA decreased the cell viability of Caco-2 cells dose-dependently.

CONCLUSION

In conclusion, size-exclusion chromatography offers a reproducible method to isolate milk-derived EVs. The fast uptake of EVs in intestinal Caco-2 cells as well as their beneficial properties compared to liposomes make them promising for oral drug delivery purposes. The systematic investigation of siRNA loading approaches and the functional siRNA delivery in Caco-2 cells will set a basis to further investigate milk EVs for oral delivery of therapeutic nucleic acids.

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We are grateful to C. Vissiennon and L. Schiller (Medical Physics and Biophysics, Leipzig) for their expertise with Caco-2 cells, to H. Kalwa (Clinical Pharmacology, Leipzig) for access to CLSM imaging, and to G. Hause (Biozentrum, Halle) for the TEM images.

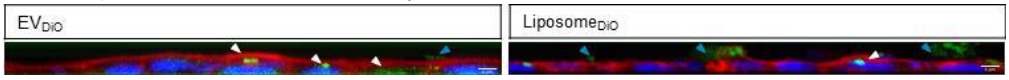

Figure 1. z-stacks of Caco-2 cells incubated with DiO (green) labelled EVs (left) and liposomes (right) are shown.

>> 16:45 - 17:45 | PSOP-15 - HYDROGELS II
Chairpersons: Catarina Custodio & Jos Olijve
Location: Room B

16:45 | O1 Hydrogels II - Click Chemistry Crosslinked Hydrogel Sealant for Repairing Corneal Perforations
Patrick SHAKARI, Department of Chemistry - Ångström, Macromolecular Chemistry, Uppsala University, Uppsala, Sweden

17:00 | O2 Hydrogels II - Influence of hyaluronic acid within biomimetic pulmonary niche on the mesenchymal stem cells differentiation toward mature Type II pneuumocytes
Francesca DELLA SALA, Institute of Polymers, Composite and Biomaterials, National Research Council of Italy, Viale J.F. Kennedy 54, 80125 Naples, Italy

17:15 | O3 Hydrogels II - An innovative phyllosilicate-based hydrogel for skin decontamination against chemical warfare agents
Kardelen DURMAZ, IBCP, LBTI, CNRS UMR 5305, Lyon, France

17:30 | O4 Hydrogels II - Hyaluronan-O-Carboxymethyl Chitosan-based Hydrogels as Delivery Platforms of Osteogenic Factors and Mesenchymal Stem Cells
Daniel FERNÁNDEZ-VILLA, 1 Instituto de Ciencia y Tecnología de Polímeros (ICTP-CSIC), Spain; 2 Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Spain

Click Chemistry Crosslinked Hydrogel Sealant for Repairing Corneal Perforations

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INTRODUCTION

For patients with a corneal perforation, caused mainly by infection or trauma, urgent treatment is needed. Current treatment includes sealing the perforation using cyanoacrylate or fibrin glue. However, these materials have several drawbacks such as toxicity, promoting neovascularisation, or weak sealing. Therefore, new types of materials which are biodegradable, non-toxic, and can provide adhesion on wet surfaces are needed. In the present work, this has been achieved by a hydrogel sealant which is chemically crosslinked using a cell-compatible ligand-accelerated copper-catalysed click chemistry between alkyne modified gelatine and azide modified polyethylene glycol (PEG).

EXPERIMENTAL METHODS

Gelatine was modified with alkyne groups through a reaction with glycidyl propargyl ether. Hydrogels with tuneable gelation were prepared by altering the catalyst (Cu(II) and ligand) concentration, and with tuneable storage modulus by altering the alkyne to azide stoichiometry. Hydrogels were characterised by rheology for their viscoelastic properties, and by tensile lap-shear tests and corneal burst pressure tests for their adhesive and sealant properties, respectively. *In vitro* biocompatibility of the developed hydrogels was demonstrated by culturing corneal epithelial cells in 2D and HepG2 cells in 3D.

RESULTS AND DISCUSSION

The gelation time can be fine-tuned between 1-10 minutes by changing the catalyst concentration (Fig. 1a, b). With increasing catalyst concentration not only, the G´/G´´ crossover happens earlier, but also the resulting hydrogel reaches its maximum modulus quicker. Moreover, the stiffness of the hydrogels can be fine-tuned between 700 – 2300 Pa by altering the alkyne to azide ratios (Fig. 1c) at a given catalyst concertation (0.25 mM Cu (II) in this case). This is highly beneficial since most attempts in the literature for obtaining fine-tuneability in mechanical properties of hydrogel come with a compromised control on the gelation time. In the reported case, we are able to independently control the gelation time and modulus of a crosslinked hydrogel by independently altering the catalyst concentration and reactive group stoichiometries. Furthermore, tensile lap shear test with the developed hydrogels revealed adhesive strengths comparable to fibrin adhesive (Fig. 1d) but much lower than cyanoacrylate adhesive. On the other hand, burst pressure test with cadaveric porcine corneas showed promising results with bursting pressure varying between 15 to 70 mmHg for penetrating perforations, while normal intraocular pressure is between 11 to 24 mmHg. Also, the developed hydrogels could support attachment and proliferation of human corneal epithelial cells in 2D culture with more than 90% viability on day 1 and 7 (Fig. 1e). Moreover, the cytocompatibility of the crosslinking reaction was demonstrated by encapsulating HepG2 cells in the hydrogel at the time of crosslinking and gel formation. Over 85% viability was obtained after 24 hours of 3D culture (Fig. 1f).

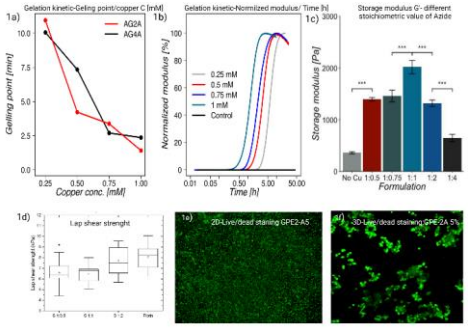

Figure 1) A representation of the gelling point plotted against Cu (II) concentration (a). Gelling kinetics in which G' achieves its maximum value after a certain amount of time for varying Cu (II) concentrations (b). Fine-tuneability in G´ for different hydrogels by altering the reactive group stoichiometry (c). Tensile lap shear data (d). Cell viability assessed after live-dead staining for corneal epithelial cells in 2-D and HepG2 in 3-D (e-f).

CONCLUSION

In this study, a gelatine-based hydrogel sealant for repairing corneal perforation was developed using a cytocompatible copper catalysed click chemistry for crosslinking. The gelation time and stiffness of the developed hydrogels could be independently fine-tuned by altering the catalyst concentration and reactive group stoichiometries. The adhesive strength of these hydrogels was comparable to fibrin glue and perforated corneas could be sealed with developed hydrogels with burst pressures higher than the normal ocular pressures. In future, these hydrogels could be potential alternatives of clinically used cyanoacrylate adhesives for patching penetrating corneal perforations.

Main topic: Adhesives and anti-adhesives(c-01)
Subtopic: Hydrogels (A-06)
Keywords: Bio-orthogonal, copper-catalysed azide-alkyne cycloaddition (CuAAC), and regenerative medicine

June 2022

Influence of hyaluronic acid within biomimetic pulmonary niche on the mesenchymal stem cells differentiation toward mature Type II pneuumocytes

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INTRODUCTION

In lung pathologies, the alteration of the extracellular matrix (ECM) makes the promising therapies based on mesenchymal stem cells (MSCs) ineffective, resulting in a reduced attachment and homing of the MSCs, precluding their differentiation and viability[1]. Pulmonary tissue regeneration, until now, has been devoted to mimic the architectural unit of the alveoli by developing "alveolar-like structures" based on collagen or gelatin scaffold to be seed with lung cells[2], missing the complex interplay between the components of the lung niche. Indeed, this niche dynamically orchestrates the signals, such as proliferation or differentiation of MSCs, which induces lung tissue repair. Lung niche includes ECM constituents such as hyaluronic acid (HA) and Collagen (COLL), and several types of stem cells[3]. In particular, HA is an anionic polysaccharide, playing in lung ECM the main role of water regulation in the interstitium, and acting in alveolar surface structure stabilizing the surfactant proteins (SP). Moreover, HA seems to enhance MSCs differentiation into Alveolar type II (ATII) cells[4]. The aim of this work was to develop pulmonary biomimetic niche microenvironment based on HA/COLL hydrogel and to study the effect of HA on MSCs differentiation in ATII cells. This role was elucidated by comparing different HA molecular weight (MW), 200 kDa (L), 500 kDa (M), and 1435 (H), both like structural component of the HA/COLL hydrogel and like trophic factor in solution, implementing the cell culture media.

EXPERIMENTAL METHODS

HA solutions at 0.5% w/v (LMWHA), 0.25% w/v (MMWHA) and 0.1% w/v (HMWHA) have been set by cell viability tests. Rheological characterization and Scanning electronic microscopy (SEM) have been performed. MSCs derived from Wharton jelly umbilical cord (hUCMSCs) were cultured in hydrogels based on HA/COLL with L, M and HMW HA and on COLL only as control. Moreover, the cells in the hydrogels were incubated with HA at different MW in lung cell culture media named SAGM (S). ATII cells differentiation was evaluated qualitatively by confocal microscopy. Elisa kit was performed to quantify the expression of SPC, specific marker of pulmonary differentiation, along with SPA, B, and D.

RESULTS AND DISCUSSION

The viscosity of HA in solution was within the range of $1x10^{-3}$ Pa s -1Pa s, while HA/COLL solution before fibrillogenesis have a range between of $4x10^{-3}$ Pa s and $1x10^{-1}$ Pa s. These solutions were in the range of physiological solutions, suitable for intraracheal or nebulization routs of administrations. Time sweep experiment showed that HA seminterpenetration within COLL had the same mechanical behavior of the COLL gel. LMWHA and MMWHA interpenetrated more efficiently the COLL entanglement network, and after COLL fibrilillogenesis, the presence of HA improved its viscoelastic properties. SEM images of the HA/COLL hydrogels proved qualitatively the presence of a mesh structure with higher size compared to COLL only. Fig. 1 shows representative confocal images, the imunoreactive SPC cells were observed clearly with green fluorescence spots detected in a homogeneously diffused distribution at cytoplasmic level. Quantitative expression of SPC (Figure 1) demonstrated that when in the culture media HA significantly improved SPC expression (~2 ng/mL), without showing difference in the MW tested, compared to control only (~1 ng/mL). Furthermore, LMWHA in the hydrogel promoted SPC expression (~2 times) compared to COLL, MMWHA/COLL and HMWHA/COLL hydrogels.

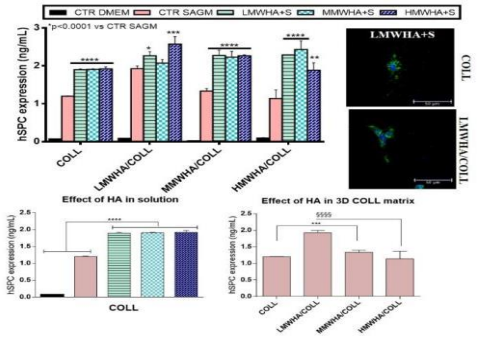

Fig. 1: Quantitative expression of SPC evaluated by Elisa kit test and the representative Maximum projection of Z-stack sections, obtained by confocal microscopy of differentiated ATII cells in presence of LMWHA. Scale bar: 50 µm.

CONCLUSION

These outcomes suggested that pulmonary mimetic niche microenvironment based on HA is a promising tunable platform for the MSCs sustenance, able to overcome the actual limits of MSCs based therapy and to improve the lung tissue repair.

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An innovative phyllosilicate-based hydrogel for skin decontamination against chemical warfare agents

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INTRODUCTION

Organophosphate neurotoxins are chemical agents that can enter the body through skin absorption. Decontamination is therefore crucial to prevent significant uptake and avoid a cholinergic crisis[1].If the use of phyllosilicate (PS) suspensions as skin decontamination tool has been recently highlighted[2], it cannot be applied on damaged skin. Hence, we hypothesized that a composite hydrogel allowing (i) an easy application and removal on the skin through the formation of a film, and (ii) the incorporation of a phyllosilicate, would ensure an efficient adsorption, sequestration and disposal of an organophosphorus compound (paraoxon, POX), simulating the neurotoxic VX. To assess this hypothesis and develop biocompatible hydrogels, different formulations of hydrophilic polymers (HP), PS and surfactants (S) have been investigated and evaluated in regard of their applicability, film-forming/removal ability and decontamination properties.

EXPERIMENTAL METHODS

Hydrogel were prepared by mixing various concentrations of HP, PS and S (HP 12 S 3% w/w, HP 20 S 3% w/w, HP 12 PS 15 S 3% w/w and HP 20 PS 9,1 S 3% w/w) in a solvent composed of 10% ethanol at 90 degrees. The film-forming capacity of the hydrogels and their drying times (RT and 32°C) were evaluated after spreading 150 µm-thick layers on glass slides. The mechanical properties of the resulting films (traction and peeling tests) were evaluated on a tensile test machine (10-100 N force cell). Peeling tests were performed on glass slides and pig ear skin. The ability to decontaminate was assessed by depositing POX on glass slides, followed by a covering with the hydrogel. After drying, the quantity of POX present in the hydrogel and on the surface were quantified by HPLC-UV.

RESULTS AND DISCUSSION

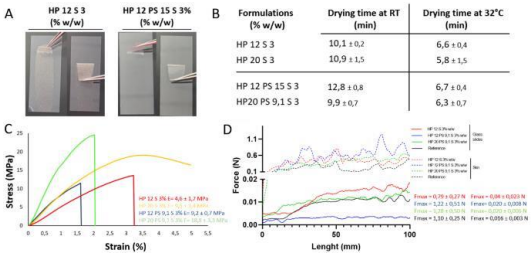

Figure 1: A) Film-forming appearance of HP + S, HP + PS + S hydrogels after drying on slides, B) Drying times of each hydrogel at RT and 32°C (n=4), C) Tensile tests for each hydrogel and determination of Young's Modulus (n=6), D) 180°C peeling test on glass slides and pig ear skin (n=6 for glass slides and n=4 for pig ear skin). Values are presented as mean ± SD.

All hydrogels, with and without PS, were easy to apply and formed cohesive films in 10 minutes at RT, which could easily be removed from the glass slides and manipulated (Figure 1A). Drying time was correlated to the amount of PS incorporated (Figure 1B), possibly due to a higher viscosity of the hydrogels. However, at 32°C (physiological temperature of the skin) the drying time was considerably decreased to 6 minutes, without influence of the PS concentration. This drying time allows to envisage a potential use even in emergency situations. Mechanically, an increase in HP concentration of the formulations improved the resulting film stiffness with a young's modulus of 9.5 MPa for HP 20 S 3% compared to 4.6 MPa for HP 12 S 3% (Figure 1C). However, higher concentrations of PS resulted in a decrease of tensile strength (9,2 against 18,8 MPa for formulation HP 12 PS 15 S 3% and HP 20 PS 9,1 S 3%). Peeling tests (180°) showed that hydrogels adhere better to pig skin than to glass slides with maximum adhesion forces up to 1,28 N for the HP 20 PS 9.1 S3% w/w formulation (Figure 1D). This stronger adhesion did not prevent their easy removal, without removal of the stratum corneum.

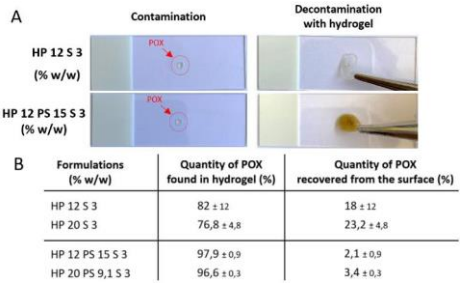

Figure 2: A) Procedure for contamination of glass slides (1 µl of POX) and decontamination (72 µl of each hydrogels), B) Determination of the quantity of POX (in %) found in the hydrogels and on the surface by HPLC-UV (n=4). Values are presented as mean ± SD

During the procedure of contamination and decontamination we can notice that the removal of the hydrogel could be done simply while sequestrating the contaminant (Figure 2A). Strikingly, formulations containing PS clearly demonstrated their ability to very efficiently decontaminate POX from surfaces, allowing up to 97.9% of retention by the films, versus 82% for hydrogels without PS (Figure 2B).

CONCLUSION

We describe here new formulations of hydrogels, easy to obtain, potentially compatible with a skin application, and with high decontamination capabilities. Validation of these results on skin explants is currently in progress.

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Hyaluronan-O-Carboxymethyl Chitosan-based Hydrogels as Delivery Platforms of Osteogenic Factors and Mesenchymal Stem Cells

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INTRODUCTION

Osteopenia is a condition present in many bone diseases, characterized by a reduced bone density which ultimately leads to an increased risk of bone fractures, and results in enormous social and economic consequences.[1] Advanced regenerative therapies are focused on the guided delivery of bone-promoting factors in combination with human mesenchymal stem cells (hMSCs). However, there is still a need for injectable vehicles that combine biomimicking properties and an effective supply of bioactive factors.[2] In this work, we present a delivery platform of osteogenic agents based on a dual-component hydrogel consisting of two bio-based and biodegradable polymers, designed to be injected through minimally invasive techniques at the site of application into the osteopenic bone.

EXPERIMENTAL METHODS

Materials. Developed formulations were composed of two solutions of o-carboxymethyl chitosan (OCC, deacetylation degree: 91%; carboxymethylation degree: 83%, Santa Cruz Biotechnologies) and aldehyde-functionalized hyaluronic acid (AHA) in PBS. AHA was obtained by partial oxidation (25 and 50%) of hyaluronic acid (HA, Mw: 0.8-1.2 MDa; Bioibérica S.A.) using $NaIO_4$ as an oxidant. Molecular weights of AHAs were analyzed by GPC. Selected bioactive cargoes were Strontium folate (SrFO), model proteins (bovine serum albumin (BSA) and ribonuclease A (RNase A)).

Injectable formulations. Hydrogels were synthesized by mixing ice-chilled solutions of AHA, at a fixed final concentration of 2%, and OCC, in different aldheyde:amine molar ratios (CHO:NH_2 of 1:0.5 (Low), 1:1 (Med), and 1:1.5 (High)), and incubating the reaction at 37 ˚C.

Characterization of samples. The chemical structure of the precursors and the resulting hydrogels was analyzed by ATR-FTIR. Rheological analyses were performed in oscillatory mode at 37 ºC using an ARG2 rheometer equipped with a solvent trap. Swelling/degradation studies of hydrogels immersed in PBS were carried out for 20 days at 37 ˚C, measuring the weight of the samples overtime. Release kinetics of the loaded compounds were monitored for 14 days by UV-VIS measurements.

Biological assays. hMSCs were added to the OCC precursor solution and the formulations were injected into Alvetex™ strata scaffolds, simulating a 3D microporous environment. Cell viability was monitored with Alamar blue measurements at different times and the cellular morphology was assessed by staining with Hoechst and phalloidin and visualizing the samples by confocal microscopy.

RESULTS AND DISCUSSION

AHAs of 25 and 50 % oxidation degrees were synthesized in yields over 70%. GPC analysis showed a reduction of their molecular weights to 350 and 325 kDa respectively. Hydrogels were instantaneously formed when combining AHA and OCC, and the CHO:NH_2 ratio was varied to tailor different mechanical properties, as assessed by oscillatory rheology (**Fig. 1**).

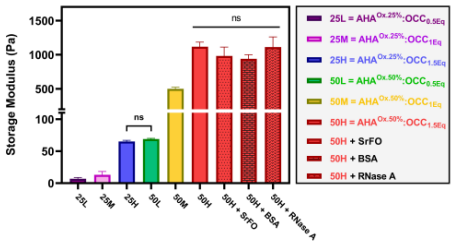

Figure 1. Storage modulus of assayed formulations calculated by oscillatory rheology.

Hydrogels were further characterized in terms of swelling and degradation. While a similar tendency was observed for the swelling behavior, gels with a higher degree of crosslinking lasted longer (15 days). Accordingly, the "50H" formulation was selected for release studies. A 50% release of SrFO, BSA and RNase A was achieved after 1, 2 and 8 days, respectively, and cargoes were sustainedly delivered until the hydrogel was completely degraded. Moreover, the incorporation of bioactive molecules did not alter neither the gelation time of the formulation, nor its mechanical properties (**Fig. 1**). Lastly, biological studies confirmed the suitability of both "25H" and "50H" formulations to encapsulate living cells and released them into a 3D environment, being the gelation process cell permissive. Microscopy studies revealed that cells were shown to be attached to the surface of the scaffold.

CONCLUSION

This work presents for the first time AHA-OCC hydrogels as a promising delivery platform for regenerative agents, ranging from small molecules to proteins; as well as a vehicle to improve the outcome of current hMSC-based cellular therapies.

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ACKNOWLEDGMENTS

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>> 16:45 - 17:45 | PSOP-16 - ADDITIVE MANUFACTURING
Chairpersons: Nicolas Dunne & Vera Tiodorovic
Location: Room F

16:45 | O1 Additive manufacturing - Ceramics Dynamic Molding: a new technology of additive manufacturing for large-size Cranio-Maxillo-Facial orthopedic implants
Ambra PATERLINI, 3Deus Dynamics, Villeurbanne, France

17:00 | O2 Additive manufacturing - Drop-On-Demand Bioprinting From Micro To Macro Scale Using The Acoustic Droplet Ejection Principle
Stefan JENTSCH, Department of Dental Materials and Biomaterials Research, RWTH Aachen University Hospital, Aachen, Germany

17:15 | O3 Additive manufacturing - Engineering of a Bioartificial Filtration Unit of the Kidney using a Natural Polymer
Syed Mohammad Daniel SYED MOHAMED, Department of Materials Science and Engineering, Faculty of Engineering, University of Sheffield, Sheffield, UK

17:30 | O4 Additive manufacturing - Giving Life to Materials: Tuning bacterial behavior in Pluronic based bioinks for living therapeutic applications
Shardul BHUSARI, Leibniz Institute for New Materials (INM), Saarbrücken, Germany, Chemistry Department, Saarland University, Saarbrücken, Germany

Ceramics Dynamic Molding: a new technology of additive manufacturing for large-size Cranio-Maxillo-Facial orthopedic implants

Ambra Paterlini[1*], Célia Halimi[1], Julien Barthès[1], Edwin-Joffrey Courtial[1,2]
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INTRODUCTION

Ceramics present a long history in the biomedical field – and especially in the osteoarticular tissue engineering – thanks to their biocompatibility, the bone-like structure and the excellent mechanical and tribological properties. With the advent of Additive Manufacturing (AM), during recent years ceramic medical implants are facing a continuous innovative revolution due to the wide range of applications these new technologies can include. The main advantages are related to the freeform design, including highly complex geometries, and controllable porous and semi-porous structures that are not obtainable by conventional processes[1].

Despite the significant progresses of the last decades, ceramics AM most performing technologies (i.e., robocasting and stereolithography) still present some limitations, two overall:

- the limited size of printable objects (< 1 dm^3),
- the need of supports in case of complex geometries that can affect not only the surface finishing of final parts, but also their mechanical performance[2].

For various applications requiring high performances and large components, the use of AM is still a challenge. This is the case of large bone replacements or massive orthopedic implants, as the case of cranio-maxillo-facial implants.

Our goal in this project is the evaluation of ceramic materials printability by Dynamic Molding, in order to overcome the limitations of existing AM technologies. Dynamic Molding is a new AM technology whose jammed environment is composed of a partially ordered system by using powder, behaving like a dynamic molding system, in which viscoelastic materials (inks) are dispensed[3]. The hypothesis is that the support given to printed parts by the surrounding powder would allow for heavier and larger objects without using extra support structures, while the compressive force acting during the entire printing process – due to the powder weight – can compact and integrate the powder in the printed green part and result in denser objects.

The ceramic materials targeted in this study are alumina and zirconia-toughened alumina, well-known in the orthopedic field for the excellent mechanical performance and tribological properties (*i.e.*, bending strength until 800 MPa, Vickers hardness >18 GPa, friction coefficient <0.2 in lubricated conditions[4]).

EXPERIMENTAL METHODS

Firstly, injectable inks are prepared with different formulations of poloxamer binder, a carbonic acid-based dispersant and a ceramic load of 30 to 40 wt.% including alumina and zirconia micrometric powders (D50 = 40-60 µm). Dynamic Molding is performed by using a cartesian 3D printer, equipped with 30 cm^3 cartridges and 400 µm nozzles for inks injection and a 30l powder tray. Both powders and inks are tested in terms of flowability and rheology, respectively. A debinding and sintering treatment follows with a maximal temperature of 1600°C. Density and tomography measurements on sintered samples allow for the detection of residual porosities after printing. Evaluations of bending resistance and cytotoxicity are carried out as well.

RESULTS AND DISCUSSION

The good flowability of alumina and zirconia powders is proved by a flow index between 5-7 (*i.e.*, a Carr's index in the range of 11-13%). Ceramic inks present a rheological behavior following the Herschel-Bulkley model. The optimization of printing parameters as speed, and layer height in ranges of 1-100 mm/s and 300-350 µm, respectively, permit to successfully print a mandibular implant prototype (Figure 1A). Thermal treatments follow with no particular defects on the printed parts, despite the significantly thick walls (>1 cm), known to generate cracks due to debinding and sintering gas out.

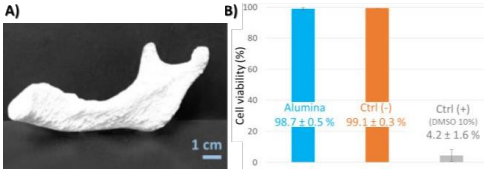

Figure 1 – A) Mandibular implant prototype produced by dynamic molding; B) cytotoxicity test results

Dynamic Molding allow us to achieve relative density values >80% on sintered samples and bending strength >200 MPa. An important influence of printing parameters such as vat printing depth and extrusion rate on final samples properties is noticed. Cytotoxicity tests show a cell viability >98% (Figure 1B).

CONCLUSION

Cytotoxicity tests proved the biocompatibility of printed samples showing a high cell viability. Sample density and bending strength still need improvements to be compared to conventional process state-of-the-art values (>90%, 600 MPa)[4]. However, the promising results of the printability analysis, as well as the successful proof of concept, are an important launching pad in the AM of ceramics by Dynamic Molding, overcoming the dimensional and weight limits imposed by most existing AM ceramic technologies.

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Drop-On-Demand Bioprinting From Micro To Macro Scale Using The Acoustic Droplet Ejection Principle

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INTRODUCTION

Bioprinting technology enables spatial pre-arrangement of cells in tissue engineered constructs. The established bioprinting techniques typically use a nozzle to deposit droplets or extrude material. However, the nozzles are a critical component because they must be very narrow for high-resolution printing and quickly become clogged during printing. As the nozzle diameter is reduced and the speed in the nozzle is increased, shear stress increases. Critical shear stresses lead to damage of the printed cells. The acoustic droplet ejection (ADE) does not use a nozzle at all. Thereby, no nozzle-related wall shear stress occurs. ADE, previously associated primarily with single cell printing[1], has recently been exploited for macroscopic 3D bioprinting[2].

EXPERIMENTAL METHODS

A custom-made bioprinter based on the ADE principle was built, meeting the specific requirements for 3D bioprinting. The structure of the acoustic bioprinter includes six separately controllable and movable axes, in particular two sets of three orthogonally arranged axes, one for the movement of the building platform and the other for the movement of an ultrasonic transducer. The transducer, placed beneath the bioink reservoir, is aligned in height so that the ultrasonic wave is focused to the air-liquid interface. The bioink reservoir is mounted in a water tank that serves as coupling medium to transmit the ultrasonic wave from the transducer to the cell-laden bioink. With this setup, the emitted ultrasonic signal can eject single droplets from the cell-laden bioink reservoir towards a building platform placed above. The droplets attach gently onto the platform. To build a three-dimensional cell-laden hydrogel structure, the position of the platform can be adjusted in three directions to catch the droplet at predefined positions (**Figure 1**). A sine wave is generated using a waveform generator (33622A, Keysight). The high-frequency signal is amplified using a power amplifier. The inverse piezoelectric effect generates an ultrasonic field through a HIFU transducer. By simply adjusting the ultrasound, droplets the size of a single cell can be printed as well as large droplets containing cell agglomerates. The ejected droplets are recorded in flight using a 5-megapixel camera (VCXU-51M, Baumer) with a microscope objective and can be analyzed with respect to their size, velocity, and direction of flight. A second camera was used to measure droplet patterns consisting of 100 droplets at different ultrasound parameters for their printing accuracy. Complex 3D structures were printed based on cell-laden Matrigel and Pluronic hydrogels and were analyzed using confocal microscopy.

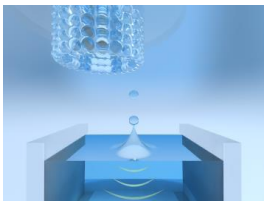

Figure 1: Principle of acoustic droplet ejection exploited for 3D bioprinting.

RESULTS AND DISCUSSION

By combining the camera approach to capture the droplets in flight and the print patterns, we can determine the optimal ultrasound parameters for the best print precision. With the best parameter combinations, 3D constructs with a size of several millimeters were successfully realized. A cylinder with a diameter of 7 mm (**Figure 2**) and other biologically relevant 3D structures were printed. Analysis of the printed constructs at the cellular scale revealed that discrete boundaries of individual droplets can be detected on the microscale.

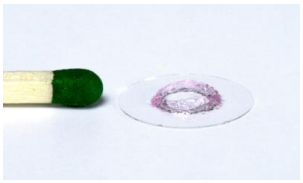

Figure 2: A cylinder printed with Pluronic® F-127.

CONCLUSION

This novel acoustic 3D bioprinting technique enables the manufacturing of highly accurate 3D cell-laden constructs. During printing, the cells are not exposed to wall shear stress because the technique does not require a nozzle. Moreover, the droplet formation can be easily controlled regarding droplet sizes by modulating the frequency, amplitude, and corresponding signal duration. Therefore, the presented multiscale and cell-preserving bioprinting method holds great potential for the field of 3D tissue engineering.

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ACKNOWLEDGMENTS

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Engineering of a Bioartificial Filtration Unit of the Kidney using a Natural Polymer

Syed Mohammad Daniel Syed Mohamed[1*], Jack Tuffin[2], Elbaraa Elghazy[1], Gavin Welsh[2], Ipsita Roy[1]

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[2]Bristol Renal, Translational Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
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INTRODUCTION

Kidney failure happens due to two conditions; acute kidney injury (AKI) and chronic kidney disease (CKD). Both of these lead to the deterioration of the glomerulus, the filtering unit in the kidney.[1] Haemodialysis and kidney transplantation are the treatments available at the moment. The former cannot provide a replacement for the physiological activities of a normal kidney, and the latter depends on the availability of donor organs. Hence, there is great interest in developing a bioartificial filtration barrier utilising actual kidney cells.

This research aims to engineer a bioartificial glomerular filtration unit using bacteria-derived polymers called Polyhydroxyalkanoates (PHAs). It is biocompatible and has been widely utilised in biomedical applications.[2] A medium-chain length PHA (mcl-PHA) has been selected for this research based on its mechanical properties and processibility, which has enabled the adoption of a 3D-printing approach using Fused Deposition Modelling (FDM).

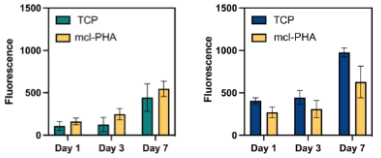
EXPERIMENTAL METHODS

The mcl-PHA was produced by anaerobic bacterial fermentation. Chemical characterisations were conducted on the polymer, including FTIR and Gas Chromatography (GC), to confirm the monomer composition. Gel Permeation Chromatography (GPC) was used to determine the molecular weight (M_w). Material characterisations were performed using Differential Scanning Calorimetry (DSC) to determine the thermal characteristics and tensile testing for its mechanical properties. Two types of glomerular cells were used in the tissue engineering approach; conditionally immortalised human glomerular endothelial cell line (ciGEnCs) and human conditionally immortalised podocytes (CIHP) (Figure 1).[3, 4] The mcl-PHA was subjected to 3D printing by FDM to engineer a kidney bioartificial filtration barrier scaffold. Bioprinting of CIHP and ciGEnC cells was achieved using alginate[5] as the encapsulating agent. The cells were bioprinted onto the polymer scaffold to introduce the required spatial separation.

RESULTS AND DISCUSSION

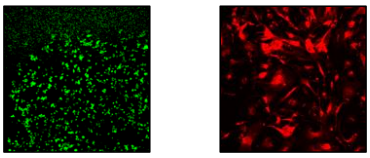
The M_w of the mcl-PHA using GPC was around 73,000 g/mol. The monomers present are 3-hydroyoctanoate (3HO) and 3-hydroxydecanoate (3HD), obtained from GC analysis. The melting point of the mcl-PHA was around 50 °C, which promoted its processability through FDM. It has been printed as tensile testing grid samples, and the resulting Young's modulus was 5.7±0.8 MPa.

The initial cytocompatibility results proved that the polymer was highly biocompatible with the kidney cells and supported their growth and differentiation, comparable with tissue culture plastic (TCP) (**Figure 1 and 2**).



(a) (b)

Figure 1: Cytocompatibility result of (a) CIHP and (b) ciGEnC, comparison between TCP and mcl-PHA.



(a) (b)

Figure 2: Confocal microscopy of the attachment and growth of (a) CIHP (green) and (b) ciGEnC (red) on mcl-PHA on Day 7 of growth.

CONCLUSION

The Bioartificial Filtration Unit is being designed to accurately mimic the filtration barrier of the glomerulus by adopting polymer printing by FDM and bioprinting of cells, which will be assessed for functionality. In the future, we aim to create a fully functional filtration barrier *in vitro* from the engineered construct. This eventually will address the blood filtration problem using a biological approach instead of synthetic dialysis filters.

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ACKNOWLEDGMENTS

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Giving Life to Materials: Tuning bacterial behavior in Pluronic based bioinks for living therapeutic applications

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INTRODUCTION

Engineered living materials (ELM) is an emerging technology in materials research which blends live cells with non-living materials to create devices with life-like properties. Rapid progress has been made in the recent years with living devices specialized for biosensing, bioadhesives and on-demand drug delivery with replenishable repositories.[1] These devices can serve as ideal systems for drug encapsulation and delivery systems containing genetically modified living organisms performing enhanced functions. However, much is yet to be understood about the effects of encapsulation on bacterial performance. To address this issue, we used a bacterially orthogonal hydrogel system based on Pluronic F127[2], in which network stabilization could be established with both non-covalent and covalent interactions that could be proportionally tuned.[3] The interplay between the dynamic viscoelastic properties of the pluronics and the functional performance of the encapsulated bacteria was studied. We demonstrate the development of this tunable hydrogel system to encapsulate genetically engineered *E. coli* bacterial strain and take it a step closer to device fabrication by developing core-shell 3D bioprinted scaffolds capable of light-regulated, localized, tunable and prolonged drug/protein release.

EXPERIMENTAL METHODS

The bioink chosen for the study was Pluronic F127 polymer. The dynamic mechanical properties were studied using rheology. A light-regulated dVio drug-producing *E. coli* strain was engineered by incorporating the genes related to the metabolic synthesis of the drug into a light responsive optogenetic plasmid. Bacteria was encapsulated in 3D-bioprinted core-shell constructs and the effect of change in the crosslinking density and the resulting mechanical properties on the bacterial growth rate, drug/protein production and viability was studied using light-sheet and confocal laser scanning microscopy.

RESULTS AND DISCUSSION

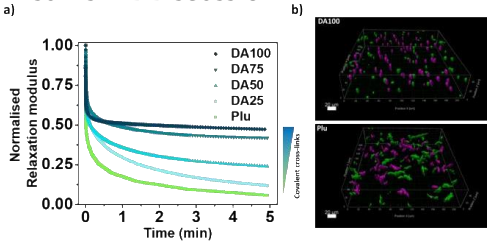

Figure 1: a) Stress relaxation curves of the Pluronic (Plu) and Pluronic Diacrylate (DA100) hydrogels mixed in varying proportions; **b)** The differences in the morphology of the bacterial colonies within DA100 and Plu hydrogel constructs. (Scale = 20 µm)

Hydrogel matrices with a range of viscoelastic properties (stiffness from 15-50 kPa and relaxation of modulus by 50-90 %) were obtained by mixing pluronic (Plu) and pluronic diacrylate (DA100) in varying proportions (Figure 1a). In a controlled nutrient environment, bacteria in physically crosslinked Pluronic hydrogels (Plu) grew unidimensionally, as a chain with cells predominantly arranged along their longitudinal axis (Figure 1b). In contrast, in covalently crosslinked pluronic diacrylate hydrogel (DA100), the growing chain buckled already in the 2nd or 3rd division cycle, and the dividing bacterial population formed rounded colonies. These results evidence that the nature of the crosslinks of the hydrogel network influences the growth of the encapsulated microorganisms. The compressive forces imposed by the network as the bacterial colony grows act as physical modulators of bacteria proliferation in the confined state.

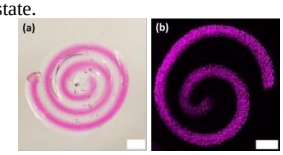

Figure 2: Spiral 3D printed core-shell living therapeutic fiber containing light responsive violet colored dVio. a) Optical image and b) epifluorescence image highlighting the dVio drug production. (Scale = 2 mm)

Also, 3D printed core-shell fibres with violet colored dVio drug were fabricated (Figure 2). The bacteria were viable in the 3D scaffolds stored at various temperature conditions for over 14 months, making it a good candidate for long-term storage.

CONCLUSION

We demonstrate that the bacterial functions can be regulated by careful design of the encapsulating matrix and understanding such behavioral changes in model matrices can enable the optimization of ELM matrix designs to maximize functionality of the embedded organisms. The pluronic encapsulation not only allowed for a conducive environment for the bacteria to grow in, protecting them from antagonistic external entities like immune cells or competitive microbes, but also provided for diffusion of nutrients, metabolites, and drugs.

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>> 16:45 - 17:45 | PSOP-17 - CELL TISSUE BIOMATERIAL INTERACTIONS I
Chairpersons: Marie-Christine Durrieu & Lorenzo Moroni
Location: Room C

16:45 | O1 Cell & Tissue interactions I - A Tissue Engineering Model of Non-Syndromic Craniosynostosis for Identifying Potential Therapeutic Targets that Accelerate Bone Repair
Mariangela MEYER, (1) Tissue Engineering Research Group (TERG), Department of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland (RCSI), Dublin 2, Ireland. (2) Advanced Materials Bio-Engineering Research Centre (AMBER), RCSI and Trinity College Dublin (TCD), Dublin 2, Ireland

17:00 | O2 Cell & Tissue interactions I - High-Throughput Screening to Elucidate Biomaterial-Associated Infection
Lisa TROMP, Department of Biomedical Engineering, W.J. Kolff Institute for Biomedical Engineering and Materials Science, University of Groningen/University Medical Center Groningen, Groningen, The Netherlands

17:15 | O3 Cell & Tissue interactions I - Transcriptomics Analysis During Early Healing of Rabbit Calvarial Defects; Influence of Scaffold's Microarchitecture
Julien GUERRERO, University of Zurich, Center of Dental Medicine, Oral Biotechnology & Bioengineering, Zürich, Switzerland

17:30 | O4 Cell & Tissue interactions - Learning from Cell-Material Interactions to Improve MSC Therapeutic Potential
Nathan LAGNEAU, Nantes Université, Oniris, INSERM, Regenerative Medicine and Skeleton, RMeS, UMR 1229, F-44000, France

A Tissue Engineering Model of Non-Syndromic Craniosynostosis for Identifying Potential Therapeutic Targets that Accelerate Bone Repair

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INTRODUCTION: Cranial sutures are fibrous joints that separate the skull bone plates, allowing brain growth during development. In the developmental condition of craniosynostosis (CS), premature ossification of the sutures occurs, causing skull deformation and eventually brain damage. At the moment, the only treatment available for CS is a surgical method called cranial vault remodelling (CVR), a highly complicated procedure with significant potential risks, involving the reshape and reposition of the skull plates. Among the different types of CS, non-syndromic (NS) has been described as the most common type, being directly linked to microenvironmental causes. However, due to a lack of appropriate research models, little is known about the associated signalling pathways that govern the accelerated cranial suture ossification. Therefore, we focus on investigating the role of microenvironmental cues in NS-CS, in order to identify novel therapeutic targets that could be utilised to develop new biomaterials-based treatments for children with CS and bone healing related conditions in adults.

METHODOLOGY: After ethical approval and parental consent were obtained, tissue samples were collected from patent (unfused) sutures (PSCs), fused sutures (FSCs) and calvarial bone of children (5-28 months old) diagnosed with NS-CS, undergoing CVR procedures[1]. Initially, cells were characterised by flow cytometry including positive and negative markers for mesenchymal stem cells (MSCs), to determine their intrinsic differentiation capacity. Subsequently, in order to establish the effect of biochemical cues in their native behaviour, the osteogenic potential of cells cultured for 7-21 days in growth (GM) and osteogenic medium (OM) was determine based on alkaline phosphatase (ALP) activity and extracellular matrix mineralization. In addition, to understand how biophysical cues affect premature ossification, cells were cultured on soft (10 kPa) and stiff (300 kPa) collagen-coated polyacrylamide substrates[1]. Then, their osteogenic potential and morphological responses were evaluated. The differences in the mechanoresponse of these cells, when combining variations on biochemical and biophysical cues, were further investigated with a 96 gene PCR array to identify potential therapeutic targets[1].

RESULTS: Cell characterization revealed that PSCs expressed higher levels of positive surface markers for MSCs (CD90 and CD44) and lower levels of negative markers (CD45, CD34, CD11, CD19 and HLA-DR), suggesting that PSCs retained features of multipotent progenitors and greater differentiation capacity than FSCs and cells from calvarial bone. On the other hand, PSCs and FSCs expressed similar ALP activity and extracellular matrix mineralisation at the different time-points, when cultured with GM. However, when cultured with OM, FSCs expressed the highest mineralisation and ALP activity levels. These findings demonstrated that FSCs have stronger osteogenic response than PSCs when biochemically stimulated. Furthermore, when cultured with GM on soft and stiff substrates, cells from both patent and fused sutures exhibited morphological changes, such an increase in their spreading area, in a stiffness-increasing manner. Particularly, FSCs showed a bigger and rounded shape, resembling osteoblasts, while PSCs exhibited an elongated shape, resembling MSCs[1]. Finally, when combining variations in the substrate stiffness and OM, FSCs showed a stiffness-dependent upregulation of genes mediating bone development (TSHZ2, IGF1) and activation of inflammatory response (IL1β) as well as those involved in the breakdown of extracellular matrix (MMP9) and in the control of osteogenic differentiation (WIF1, BMP6, NOX1). These results suggest that the increased osteogenic potential of FSCs might be associated to the activation of the BMP6, IGF1 and/or MAPK-associated non-canonical WNT pathways[1]. Currently, the role of extracellular matrix variations on driving premature ossification of NS-CS-derived cells is been assessed by culturing the clinically relevant cells on bone-like or suture-like collagen-based 3D scaffolds.

CONCLUSIONS: This study identified key mechano-pathways involved in suture ossification, suggesting that NS-CS may be linked to an abnormal mechanical environment. Understanding the changes in the regulation of genes associated with the premature suture ossification in CS opens up avenues to not only understand better this developmental condition but also will help us to design novel therapeutic strategies to accelerate bone healing in adults.

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ACKNOWLEDGEMENTS: This work was funded by the Children's Health Foundation Temple Street (RPAC-2013-06 and RPAC-19-01), and by a European Research Council Advanced Grant (ReCaP project #788753).

High-Throughput Screening to Elucidate Biomaterial-Associated Infection

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INTRODUCTION

Despite the successes of biomedical implants, which are able to solve many biomedical problems, major drawbacks remain. Implant-associated complications frequently arise, such as biomaterial-associated infection, fibrosis, and implant rejection.[1, 2] Therefore, there is a need for innovative approaches that enhance biomedical device function. To achieve control over cellular behavior, it is crucial to understand the interplay between the biomaterial, microbes, and host cells. It is well-known that all cells respond to their microenvironment and that their behavior can be modulated by, for example, altering physicochemical material properties.[3] Although cell-material interactions are studied widely, conventional approaches do not reach the level of complexity as seen in vivo, where cells respond to multiple biophysical and biochemical cues simultaneously. Studies often focus on the effect of individual parameters and only assess specific values, leaving out a significant number of variables and data points. Closing this knowledge gap is essential to fully understand how the body responds to biomaterials and will aid us in further developing medical implant technology. We have developed a double orthogonal gradient (DOG) platform that enables us to effectively screen the cell response to an extremely broad range of combined physicochemical material properties in a high-throughput screening (HTS) manner. The platform offers a long-awaited change in approach, as it offers the ability to screen thousands of combined parameters in a single experiment. Currently, the platform is being used to screen material properties of silicone rubber to prevent infection.

EXPERIMENTAL METHODS

Polydimethylsiloxane DOGs are formed that consist of two linear parameter gradients under a 90º angle on the same surface. The DOGs are prepared by a specific set of plasma oxidation treatments following protocols of previously published single linear gradients.[4–6]

RESULTS AND DISCUSSION

The developed platform is based on double orthogonal gradients, on which every position on the surface has a unique combination of the parameters topography, stiffness, and wettability (Figure 1). The wrinkled topography gradients range from wavelength (λ) = ~1–12 µm and amplitude (A) = ~100–1500 nm, with wavelength and amplitude increasing in a coupled fashion. The stiffness gradients range from Young's Modulus (E) = 20-500 MPa and the wettability gradients range from water contact angle (WCA) = 10-90º. Our current work focuses on the effect of combined material properties on the behavior of fibroblasts, which play a major role in the development of implant-associated complications. We will identify regions of interest (ROIs) with specific combinations of material properties that show the most pronounced effect in enhancing or reducing fibroblast adhesion, spreading, proliferation, and vinculin expression. In the future, this screening approach will also be used to investigate the behavior of bacteria and macrophages to provide an extensive overview of how material properties can contribute to the development of an infection.

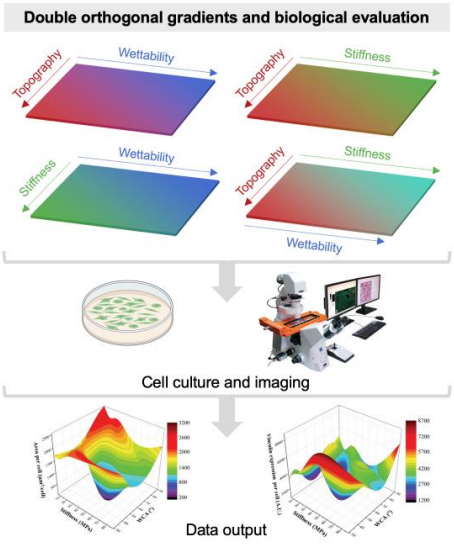

Figure 1 HTS approach. The screening platforms (top) provide all parameter combinations between topography, stiffness, and wettability. The influence on cell behavior is analyzed through immunofluorescence staining and automated imaging.

CONCLUSION

To achieve enhanced implant design, it is crucial to understand the interplay between the biomaterial, microbes, and host cells. The DOG platform developed enables us to effectively screen thousands of cell-material responses in a high-throughput fashion. This will generate knowledge that can be used to enhance biomaterials for medical implant technology.

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Transcriptomics Analysis During Early Healing of Rabbit Calvarial Defects; Influence of Scaffold's Microarchitecture

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INTRODUCTION

Bone regeneration is a physiological process closely regulated by different growth factors, transcription factors, hormones, and cytokines[1]. It takes place in four overlapping phases, which include blood clot formation, inflammation, proliferation, and remodeling. The process of bone regeneration has been extensively investigated in fracture studies, where both intramembranous and endochondral ossification are normally observed. However, most of the key molecules, genes, and signaling pathways involved in the complex cascade of events occurring during osseous regeneration, as well as the influence of scaffold's microarchitecture on it, remain elusive.

The goal of this study was to investigate the gene expression and biological/molecular pathways implicated in the regulation of the early osseous healing process and the influence of high and low osteoconductivity of the implanted scaffolds microarchitecture on it.

EXPERIMENTAL METHODS

The scaffolds were designed using Solidworks software and fabricated using stereolithography-based 3D printing (CeraFab 7500, Lithoz) from a tri-calcium phosphate-based (TCP) slurry LithaBone TCP 3400. Scaffolds with two types of microarchitecture were produced and named Fil125G and Fil050G. Those two architectures have been chosen according to previous results from our lab showing a significantly higher rate of bone formation and osteoconductivity for Fil050G related scaffold after 4 weeks of implantation into rabbit calvaria defects.

To study *in vivo* the gene expression, transcriptomic analyses were performed after 10 days of implantation on bone defect site with our two types of fabricated scaffolds. Briefly, samples were extracted from the calvaria defects after 10 days and mRNA extraction was performed for further transcriptomic analysis.

RESULTS AND DISCUSSION

At the end of the analysis of four pairwise samples from four animals, more than 20 000 genes were differentially regulated. After 10 days, adaptive immune response, regulation of cell adhesion, and cell-matrix adhesion biological processes were present and clearly upregulated. However, looking at genes expressed in our two TCP-based scaffolds presenting two different microarchitectures of high and low osteoconductivity (Fil125G and Fil050G), Gene Ontology terms related revealed other information. In our study angiogenesis, regulation of ERK1 and ERK2 cascade, regulation of cell adhesion, and cell differentiation were overexpressed in high osteoinductive Fil050G related scaffold compared to low osteoinductive Fil125G related scaffold. We also observed several genes linked to mesenchymal stromal cells differentiation highly expressed in our osteoinductive Fil050G implanted scaffold.

Moreover, CDH13 (T-Cadherin) gene has been detected in a high amount of transcripts per million for Fil050G-related scaffolds, correlating with several publications showing a critical role of T-Cadherin in angiogenesis and bone formation. Remarkably, a regulation of ERK1 and ERK2 cascade, a pathway essential for skeletal development and homeostasis[2], was already observed at 10 days.

However, no expression of bone formation-related genes was detected at this early time point post-implantation.

CONCLUSION

According to our initial results, change in the degree of osteoconductivity tuned by the microarchitecture of our implanted scaffolds influences several key biological pathways at early time points after implantation, even though both scaffolds are built from the same amount of material, and are identical in macro- and microporosity and transparency.

Angiogenesis, involved at an early stage of bone healing and being a key component of bone repair[3], seems to be drastically influenced by the osteoconductivity determined by the microarchitecture/design of the implanted scaffold and could indeed impact significantly the bone formation and degree of defect bridging at later time points.

This study improves our understanding of the signaling pathways regulating early stages of bone regeneration and suggested a number of genes differentially expressed according to the different microarchitecture of TCP-based scaffolds distinct in the degree of osteoconductivity.

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ACKNOWLEDGMENTS

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Learning from Cell-Material Interactions to Improve MSC Therapeutic Potential

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INTRODUCTION

Owing to their capacity to secrete immunomodulatory factors in response to a pro-inflammatory environment, mesenchymal stem cells (MSCs) are widely investigated as therapeutic cells. The MSC secretome is known to vary depending on the physical and biochemical properties of the cell microenvironment. Therefore, tailored biomaterials, especially hydrogels, are increasingly used to deliver MSCs and tune their therapeutic potential. Yet, much remains to be done to decipher the role of cell-material interactions on MSC secretome; and such investigations require biomaterials with independently tunable physical and biochemical properties. In this context, using two new hydrogel crosslinking platforms recently developed in our laboratory, we envisioned to simultaneously investigate the effects of a broad variety of mechanical properties (i.e., stiffness, viscoelasticity) and biochemical properties (i.e., polymer type, peptides) on MSC secretory ability.

EXPERIMENTAL METHODS

The effects of the mechanical properties and composition of hydrogels were assessed by quantifying the secretion of various immunomodulatory factors (e.g., IDO, PGE2, IL-6) upon the pro-inflammatory stimulation (TNF-α and INF-γ) of encapsulated adipose-derived stromal cells (hASCs). Using two new chemical crosslinking strategies, we produced a series of hydrogels from a variety of polysaccharides (e.g., hyaluronic acid (HA), alginate, chondroitin sulfate (CS)), all nonswelling, long-term stable, and cytocompatible. One of the two platforms allowed the synthesis of purely elastic hydrogels, and the other the synthesis of viscoelastic hydrogels. To investigate the effect of hydrogel mechanical properties on MSC secretory ability, hASCs were encapsulated in purely elastic hydrogels with various stiffness (1 kPa vs 5 kPa; relaxation time > 1000 seconds), and in viscoelastic hydrogels (relaxation time ≈ 1 second). To evaluate the sole influence of polymer type, hASCs were encapsulated in elastic hydrogels with similar mechanical properties (E = 1 kPa), composed of either HA or alginate. Hydrogel composition was also tuned by incorporating CS as a natural glycosaminoglycan of interest. We further investigated the effects of specific biochemical interactions by immobilizing either a fibronectin mimicking peptide (RGD) or a N-Cadherin mimicking peptide (HAVDI).

RESULTS AND DISCUSSION

Regarding the influence of mechanical properties on MSC secretion under pro-inflammatory conditions, we showed that varying the stiffness of elastic HA-based hydrogels, from 1 to 5 kPa did not change the secretion of IDO, PGE2 and IL-6. Interestingly, we further showed that encapsulating hASCs in viscoelastic hydrogels, compared to purely elastic ones, did increase the secretion of PGE2, emphasizing the role of hydrogel viscoelasticity on MSC therapeutic potential. Regarding the influence of biochemical cues, we showed that, compared to hASCs encapsulated in alginate gels, hASCs encapsulated in HA hydrogels increased the secretion of IDO, PGE2 and IL-6. These results suggest for the first time a beneficial effect of HA on MSC anti-inflammatory properties, possibly through specific receptor activation such as CD44 or RHAMM. In contrast, the co-crosslinking of CS in HA gels did not alter the hASC secretory capacity. Finally, we showed that the immobilization of the RGD peptide led to a decrease in PGE2 secretion by encapsulated hASCs, while that of the HAVDI peptide increased PGE2 secretion. This seems to indicate that mimicking cell-cell interactions, through the immobilization of a biomimetic peptide, is an effective way to increase MSC immunomodulatory properties.

CONCLUSION

This study put into perspective for the first time the effects of a variety of independently tuned biochemical and mechanical properties of hydrogels on the hASC secretome in a pro-inflammatory environment. We showed that viscoelastic HA gels modified with an N-cadherin-inspired peptide are suitable candidates to increase MSC immunomodulatory properties. We are now broadening the scope of the investigated cytokines (e.g., HGF, IL-10, IL-4, bFGF, VEGF) to fully understand and control cell-material interactions to drive MSC therapeutic potential.

ACKNOWLEDGMENT

The authors are grateful to the Fondation pour la Recherche Médicale (VD), the Nantes Excellence Trajectory program (VD), and the Marie-Sklodowska Curie Actions (VD) for their financial support.

>> 16:45 - 17:45 | PSOP-18 - DRUG DELIVERY I
Chairpersons: Sei Kwang Hahn & Catherine Picart
Location: Room E

16:45 | O1 Drug delivery I - Nanoparticles drug delivery systems to counteract inflammation on microfluidic-based neurovascular bone unit
Estrela NETO, INEB - Instituto Nacional de Engenharia Biomédica, Universidade do Porto, Porto, Portugal; i3S – Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Porto, Portugal

17:00 | O2 Drug delivery I - Regioselectively Oxidized Hyaluronate for Enhanced Cisplatin Delivery
Jan VICHA, Centre of Polymer Systems, Tomas Bata University in Zlín, Zlín, Czech Republic

17:15 | O3 Drug Delivery I - Drug-eluting metals by cold sintering processing: high strength and prolonged drug release
Aliya SHARIPOVA, Department of Material Science and Engineering, Technion – Israel Institute of Technology, Haifa, Israel

17:30 | O4 Drug delivery I - Stimulation of Neovascularisation by Controlled Delivery of VEGF using an Implantable Hydrogel Loaded Soft Robotic Drug Delivery System
Eimear WALLACE, 1. Anatomy and Regenerative Medicine Institute (REMEDI), School of Medicine, College of Medicine Nursing and Health Sciences, National University of Ireland Galway, Ireland, 2. Aurum Laboratories, Explora-Biotech, Rome, Italy

Nanoparticles drug delivery systems to counteract inflammation on microfluidic-based neurovascular bone unit

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INTRODUCTION

Bone diseases, such as osteoarthritis, comprise the action of inflammatory mediators leading to a de-regulation of sensory innervation and angiogenesis. Although there are models to mimic bone vascularization or innervation, *in vitro* platforms merging the complexity of bone, vasculature, innervation and inflammation are missing. Organ-on-a-chip *in vitro* platforms offer the ability to recapitulate and dissect mechanisms of physiological and pathological settings, as well as an accurate tool for drug screening and development of new therapeutic targets.

In this study, we propose a microfluidic-based NeuroVascularized Bone chip (NVB chip) to 1) model the mechanistic interactions between innervation and angiogenesis in the inflammatory bone niche, and 2) explore as a screening tool of novel strategies targeting inflammatory diseases, using a nano-based drug delivery system.

METHODS

We set a novel microfluidic-based design to incorporate different cells in culture concerning their *in vivo* interaction. The design comprised three different compartments to accommodate neuronal (sensory neurons), endothelial (human umbilical vein endothelial cells (HUVEC)) and bone cells (osteoclasts). Ibuprofen-loaded nanoparticles were tested in bone compartment under inflammatory Il-1b stimuli.

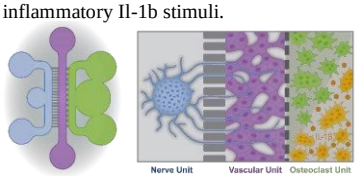

Figure 1 – Model for NeuroVascular Bone chip (NVBchip).

RESULTS: We were able to set the optimized conditions (cell density and time of culture) for the different cell types, starting with endothelial cells, then osteoclasts and finally sensory neurons. We showed the formation of lumen-like structures inside the chip and a reduction in the permeability along the culture time.

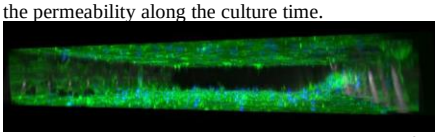

Figure 2 – Lumen-like structure at day 1 in microfluidic.

Osteoclasts were cultured under non-inflammatory and inflammatory conditions and characterization of pro-inflammatory and angiogenic profiles was performed. Neuronal cells were then added to the system and axonal growth was quantified. Under inflammatory conditions, it was observed a higher axonal growth and an increase in the endothelial barrier permeability. The axonal growth was reverted in the presence of ibuprofen-loaded nanoparticles.

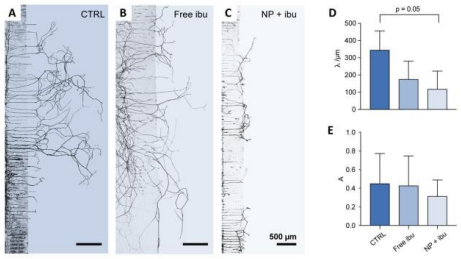

Figure 3 – Axonal growth was assessed in the inflammatory condition (A) and following treatment with free ibuprofen (B) or ibuprofen-loaded nanoparticles (C). Axonal growth was quantified: the λ value (D), turns the extension of growth, and the A value (E) gives the fraction of axons that effectively cross the microgrooves. Data represented as mean ± SD.

CONCLUSIONS

We designed, optimized and validated an advanced NeuroVascular Bone chip (NVBchip) model. We have transposed into a single chip the key cellular players and the cascade of events that are likely to occur in bone inflammatory pathologies. This is the first bone microenvironment model, including, not only the important vasculature contribution, but also the sensory innervation intimately connected to the pain perception, which will substantially impact the bone research field. Our model proved to be a valuable tool to screen the effect of compounds or drug delivery systems on bone neurovascular system, supported by robust data retrieved from the quantitative measurements as permeability and axonal growth quantification in this platform. This neurovascular bone model will be a useful platform to carry out studies such as validation and comparative therapeutic tests with the opportunity to perform the administration in the vascular compartment and assess the drug effects axonal and bone cells functions.

ACKNOWLEDGEMENTS

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Regioselectively Oxidized Hyaluronate for Enhanced Cisplatin Delivery

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INTRODUCTION

Severe side effects of platinum anticancer complexes led to the development of various drug delivery strategies, including their conjugation to polysaccharide carriers, which offer excellent biocompatibility, biodegradability, and potential for targeting malignancies. However, only several polysaccharides can directly bind anticancer drugs, and these often have a low density of suitable biding groups. For instance, hyaluronate (HA) has cisplatin (CP) loading efficacy of only ~50% and a loading capacity limited to 25 wt.%,[1] because each molecule of CP requires two units of HA to bind. The resulting crosslinking also limits the conjugate solubility. Modifications aimed to improve these issues are usually centered around the COOH group of HA,[2] which, however, impairs its ability to bind to the proteoglycan receptor CD44, thus negating one of HA's greatest advantages as a drug carrier –tumor targeting.[3]
Hence, a sequential regioselective oxidation scheme,[4] that introduces a pair of carboxylic groups at C2 and C3 of glucuronate has been developed and advantages of prepared 2,3-dicarboxy hyaluronate (DCH, *Fig.1*) over unmodified HA as a carrier for CP are demonstrated.

EXPERIMENTAL METHODS

Sodium hyaluronate (Contipro, Czech Republic) was regioselectively oxidized; -OH groups at C2 and C3 of glucuronate are initially selectively oxidized by periodate (primary oxidation), and formed aldehyde groups are converted to –COOH by chlorite (secondary oxidation). The impact of various reaction parameters (duration of the oxidation, the concentration of oxidizing agents, etc.) on the degree of oxidation (*DO*) and molecular weight (M_w) was studied. Prepared DCH derivatives were characterized (NMR, GPC, FT-IR…), loaded with CP, and compared with HA of similar molecular weight in terms of drug loading efficacy, carrier capacity, drug release rates, and cytotoxicity against malignant (A2780) and healthy (NIH/3T3) cell lines.

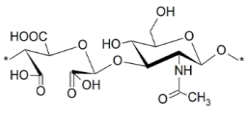

Fig. 1 Structure of DCH.

RESULTS AND DISCUSSION

Screening of reaction parameters - The duration of primary oxidation was found to impact both the *DO* and M_w of DCH. The amount of periodate was found to only minor influence M_w, but have a significant impact on *DO*. The shorter duration of SOX (below 12 h) leaves a significant amount of –CHO groups unoxidized, which negatively affects the biocompatibility of DCH. To avoid considerable degradation and non-selective oxidation, the concentration of $NaClO_2$ has to be below <0.1 M.

DCH vs. HA as cisplatin carriers - Cytotoxicity of partially oxidized DCH-P (DO=70%, M_w=139 kDa) and fully oxidized DCH-F (DO=99%, M_w= 56 kDa) towards NIH/3T3 cell line is low; cellular viability started to decrease only for very high doses >10 mg/mL. Subsequently, DCH samples and unmodified HA (M_w= 90 kDa) were loaded with CP and compared. The comparative study showed superior CP loading efficacy, up to 2x greater loading capacity, and much slower release kinetics (~2x less of CP released after 8h) of the DCH samples compared to HA (*Fig. 2*). This is a result of effective CP chelation by DCH. Moreover, the CP-loaded DCH-F sample showed significantly higher cytotoxicity towards A2780 cell lines than CP-HA.

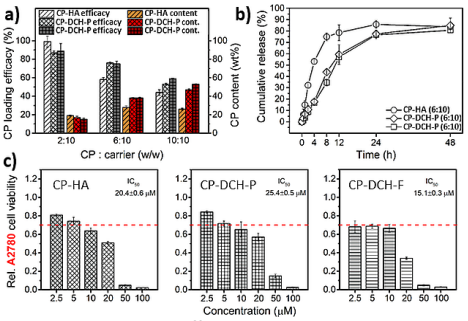

Fig.2: a) CP loading efficacy and content in conjugates; b) CP release profiles; c) Relative A2780 cell viability.

CONCLUSION

The regioselective oxidation of HA essentially triples the amount of available –COOH groups in its structure, greatly improving its capabilities as a CP carrier without modifying the N-acetyl glucosamine unit or C6 of glucuronate. DCH also possesses a large potential for further modifications.

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ACKNOWLEDGMENTS

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Drug-eluting metals by cold sintering processing: high strength and prolonged drug release

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INTRODUCTION

Biodegradable biomaterials have been extensively investigated in bone fracture fixation as they eliminate the need for a second surgical intervention for implant removal. In addition, biodegradation can be capitalized on to release drugs locally. Drug loading into the implant bulk requires low processing temperatures to preserve the cargo's chemical integrity and pharmacological activity. To unlock the potential of biodegradable iron (Fe) for local drug delivery, advanced cold sintering (CS) process for fabricating metals at ambient temperature has been developed. In the present work, we report the processing, mechanical and antibacterial properties of vancomycin hydrochloride (VH)-loaded biodegradable Fe biomaterials.

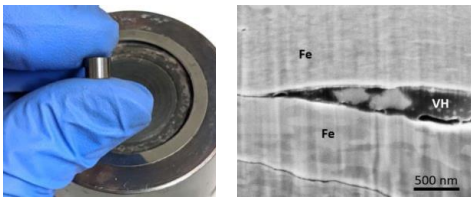
EXPERIMENTAL METHODS

To prepare a Fe-VH blend, VH powder was manually mixed with Fe powder (5-9 μm). Then, the blend was CS-ed in high-speed tool steel die at pressure 2.5 GPa (Fig. 1), room temperature to obtain dense drug-carrying samples. Samples were characterized by high-resolution scanning electron microscopy (HR-SEM), focused ion beam (FIB) slicing technique, and Avizo software for 3D-reconstruction of sample microstructure. Static degradation immersion tests were conducted on samples without drugs according to ASTM G31 standard in Modified Hank's (MH) solution [1]. Degradation was characterized by samples weight loss after 1-week intervals during 8 weeks. The mechanical properties before and after 8 weeks degradation period were tested in compression. *In vitro* antibacterial activity was analyzed by the zone of inhibition (ZOI) technique on VH-sensitive *Staphylococcus aureus* (*S. aureus*) for 2 weeks [2].

RESULTS AND DISCUSSION

The proposed processing approach allows drug loading during the fabrication of bulk metallic samples. Near dense, 97.37 ± 0.73% of theoretical density, Fe samples loaded with 1 wt.% VH (Fe-1wt%VH) were prepared by CS (Fig. 1a). High-pressure-induced plastic deformation enables metallic particles to sinter and encloses drug powder between the metallic surfaces. This is verified by cross-section micrographs of the sample (Fig. 1b) and a 3D reconstruction of its microstructure.

Pure Fe samples show high compressive strength: yield strength of 540.0±17.9 MPa and ultimate strength of 890.0±10.2 MPa. After 8 weeks of degradation in MH solution, Fe samples remain strong, with a 744.3±42.5 MPa of the ultimate compressive strength. Fe degradation rate is 1.01±0.04 mm/year. Loading with 1 wt.% of VH decreases the compressive strength, resulting in yield strength of 481.0±10.1 MPa and ultimate strength of 584.0±15.1 MPa.



(a) (b)

Fig. 1 – Fe-1wt%VH sample: (a) photo of the extracted sample next to the consolidation die; (b) micrograph of the sample cross-section

ZOI analysis shows the antibacterial activity of Fe-1wt%VH during 9 days (Fig. 2a). Cross-sectional micrographs of the sample surface and interior at a depth of ~210 μm after 2-week release are shown in Fig. 2b,c, respectively. To investigate the sample interior, it was polished down by grinding paper. Fig. 2b,c unveil that VH release during the first 9 days occurs due to drug dissolution from the sample surface and the system of open pores adjacent to outer layers. Sample interior after 2 weeks remains similar to the surface of the as-consolidated sample (Fig. 1b) and contains the enclosed drug. These results show that enclosed in the interior, VH will be released later according to the degradation of the adjacent metallic matrix.

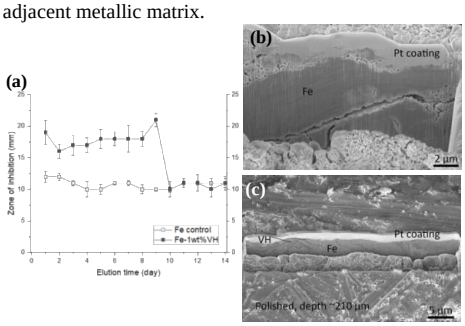


Fig. 2 – Fe-1wt%VH after incubation for 2 weeks: (a) ZOI test (against *S. aureus*); (b) cross-section micrograph at the surface; (c) at the depth ~210 μm (polished down)

CONCLUSION

Our findings demonstrate the promise of local delivery of bioactive substances at bone healing sites via degradation of temporary metallic implants. We show that fabricated drug-eluting degradable metals have high mechanical strength and enable prolonged drug release.

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Stimulation of Neovascularisation by Controlled Delivery of VEGF using an Implantable Hydrogel Loaded Soft Robotic Drug Delivery System

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INTRODUCTION

The survival and subsequent functioning of transplanted islets is largely dependent on vascularisation of the implantation site. Over 60% of macroencapsulated islets are lost immediately post transplantation due to hypoxia from inadequate early vascularisation[1]. Prevascularisation of implantation sites by delivering pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF) is a potential solution to overcome islet loss[2]. However, VEGF has a half-life of only 50 mins *in vivo* and if delivered in large quantities systemically can result in the formation of leaky blood vessels, edema, etc[3,4].

We aim to stabilise VEGF by electrostatically interacting it with our carboxymethylcellulose-sodium alginate (CMC-SA) based hydrogel and loading into the soft robotic drug delivery (SRDD) system. This SRDD system will be implanted and actuated to release a predetermined amount of VEGF in a timely manner to stimulate angiogenesis at an implant site for future islet transplantation.

EXPERIMENTAL METHODS

Animal protocols were carried out in accordance with the Italian Ministry of Health (Authorisation number 719/2020-R). Female Sprague-Dawley rats (350-450 g, N=17) were anaesthetised and subcutaneously implanted with SRDD systems loaded with CMC-SA based hydrogel without (N=8) or with (N=9) VEGF. Implanted SRDD systems were actuated once daily for 7 days with an actuation regime (10 psi, triangle wave, 10 cycles of 10 sec on 90 sec off) optimised in earlier *in vitro* release studies. On day 8, all rats were euthanised and SRDD systems and surrounding tissues were explanted *en bloc* and stained with CD31 (neovessel density) and αSMA (vessel maturity and stability) for the histological analysis of angiogenesis.

GraphPad Prism 8 was used for statistical analysis. Normality was tested using Shapiro-Wilk test. Unpaired t-tests were performed for normally distributed data. A one-way or two-way ANOVA with post-hoc Tukey's multiple comparison was used to compared two groups. Mann-Whitney U was used for not normally distributed data. Statistical significance was accepted when $P < 0.05$.

RESULTS AND DISCUSSION

Preclinical studies found VEGF released locally on a daily basis increased the abundance (p=0.0310, Figure 1a) and length density (p=0.0310) of CD31+ neovessels and decreased radial diffusion distances (p=0.0460) compared to the no VEGF controls. VEGF also significantly increased the diameter of CD31+ blood vessels (p=0.0130, Figure 1b). However, the percentage of αSMA+ blood vessels was not significantly increased (p=0.3030, Figure 1c) likely due to the short follow-up period limiting the maturation of newly formed blood vessels.

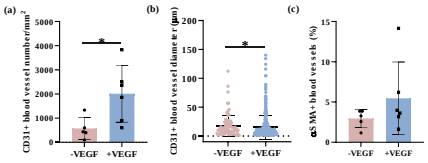

Figure 1 VEGF increases CD31+ blood vessel (a) number and (b) diameters but does not increase (c) percentage of αSMA+ blood vessels. Data represented as mean ± SD. No VEGF, N=5; VEGF, N=6; * $P < 0.05$.

CONCLUSION

Electrostatically interacting VEGF with our CMC-SA based hydrogel stabilised VEGF *in vivo* and its controlled delivery using our SRDD system stimulated neovascularisation at the implant site. Future preclinical studies will have an extended follow-up period of at least 14 days to determine whether these neovessels mature and become functional to prevascularise an implant site for future islet transplantation.

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ACKNOWLEDGMENTS

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>> 16:45 - 17:45 | PSOP-19 - POLYMER I
Chairpersons: Hélène Van den Berghe & Andreas Lendlein
Location: Room A

16:45 | O1 Polymer I - Mesenchymal Stem Cells Sense the Toughness of Interfaces
Julien GAUTROT, School of Engineering and Materials Science, Queen Mary, University of London, London, United Kingdom

17:00 | O2 Polymer I - Effect of WS2NT on PLLA Microstructure During Bioresorbable Stent Manufacturing Process
Lison ROCHER, School of Mechanical and Aerospace Engineering, Queen's University Belfast, UK

17:15 | O3 Polymer I - The Degradation and Encrustation Behaviours of Poly Glycerol Sebacate Citrate in Artificial Urine
Lu ZHANG, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

17:30 | O4 Polymer I - Role of Macromolecular Characteristics on the Morphology of Thermosensitive Chitosan Hydrogels
Pierre MARQUAILLE, Molecular, Macromolecular Chemistry and Materials, ESPCI Paris, CNRS, PSL University, France// Équipe de Recherche sur les Relations Matrice Extracellulaire-Cellule, CY Cergy Paris Université, France

Mesenchymal Stem Cells Sense the Toughness of Interfaces

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INTRODUCTION

Stem cells are known to sense and respond to a broad range of physical stimuli arising from their extra-cellular environment. In particular, the role of the mechanical properties (i.e. Youngs or shear modulus, viscoelasticity) of biomaterials has extensively been shown to have a significant impact on the adhesion, spreading, expansion and differentiation of stem cells (1). In turn, cells exert forces on their environment that can lead to striking changes in shape, size and contraction of associated tissues, and may result in mechanical disruption and functional failure. However, the impact of biomaterials toughness on stem cell phenotype has not been explored to date. In this report, we show how the design of macromolecular architecture regulates interfacial toughness and how this, in turn, controls the expansion of mesenchymal stem cells.

EXPERIMENTAL METHODS

To modulate interfacial toughness, we exploited the ability to assemble polymers and co-surfactants at liquid-liquid interfaces, forming thin (10-20 nm) protein/polymer nanosheets displaying strong interfacial mechanical properties. Such behaviour has previously been shown to enable cell adhesion, expansion and to maintain stem cell phenotype (2-4). In this work, we combine interfacial rheology and magnetic tweezer-assisted interfacial micro-rheology to characterise nanosheet mechanics at multiple length scales. Using these platforms, we develop a methodology to characterise the toughness of corresponding interfaces. In turn, we characterise the mechanism of adhesion of mesenchymal stem cells (MSCs) using microscopy and demonstrate the application of such systems for the long term expansion of MSCs on emulsions stabilised by nanosheets (PCR, flow cytometry, differentiation assays).

RESULTS AND DISCUSSION

In this report, we demonstrate that the molecular weight of the polymers used for assembly at liquid-liquid interfaces controls the toughness of the resulting nanosheets, independently of their interfacial shear mechanics. Indeed, we show that although kinetics of assembly differ as a function of molecular weight, the ultimate interfacial shear modulus of resulting nanosheets is almost unaffected by the molecular weight of the polymer. However, their toughness is modulated by the molecular weight over 2 orders of magnitude. The interfacial stress at which nanosheets are found to fracture when assembled from the highest polymer molecular weights is above the range of stress typically reported to be generated by cells on soft silicones or hydrogels. This confers sufficient toughness to resist cell-mediated deformation and fracture. We propose that this phenomenon is enabled by the bilayer structure of nanosheets (Figure 1), with extended soft segments enabling effective stress dissipation and preventing crack propagation.

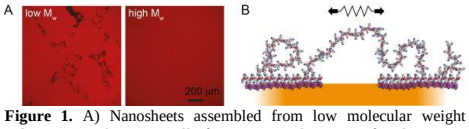

Figure 1. A) Nanosheets assembled from low molecular weight polymers (<50 kDa) typically fracture at moderate interfacial stresses, whereas high Mw polymers enable the formation of tougher nanosheets continuous at the macroscopic scale. B) Bilayer architecture of nanosheets and proposed mechanism for energy dissipation during crack propagation.

In turn, we find resulting nanosheets enable cell adhesion and the long term expansion of MSCs (over the equivalent of 8 passages) at the surface of microdroplets (emulsions). Cells cultured in such way retain comparable phenotypes (CD73/CD90/CD105 triple marker expression, THY/NES/VCAM1 expression of and ability to undergo osteo/adipo/chondrogenic differentiation) to cells grown for the same period of time on tissue culture plastic or solid microcarriers.

CONCLUSION

Overall, our data demonstrate the importance of nanoscale and interfacial toughness on the adhesion and phenotype of adherent cells. We propose a simple mechanism via which interfacial mechanics and toughness can be rationally modulated. The system presented demonstrates the ability to culture stem cells over prolonged periods of time on microdroplets, paving the way to a new generation of bioreactors based on emulsions, allowing cost reduction, scale up and the replacement of microplastics for stem cell manufacturing.

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ACKNOWLEDGMENTS

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Effect of WS_2NT on PLLA Microstructure During Bioresorbable Stent Manufacturing Process

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INTRODUCTION

For treatment of artery diseases, bioresorbable stents (BRS) offer a thrilling alternative to permanent metal stents that can fully resorb after 2-3 years[1], leaving "nothing behind" and avoiding late-stage complications[2]. A poly(L-lactide) (PLLA)/tungsten disulphide nanotube (WS_2NT) nanocomposite could respond to current unmet needs in (PLLA)-based BRS[3]: increasing strength to enable thinner devices, reducing microstructural heterogeneities, and improving radiopacity. Previous work, using *lab-scale preparation techniques*, reported a good dispersion and reinforcing potential of WS_2NTs[4]. In this study, the effect of WS_2NTs on PLLA was investigated during tube extrusion and tube expansion - two of the main steps of a BRS *industrial manufacturing process*. This work improves understanding of the effect WS_2NTs on the PLLA microstructure evolution during the process and its link with the mechanical properties of the nanocomposite scaffold.

EXPERIMENTAL METHODS

Medical grade PLLA (Purasorb PL38, Corbion) tubes reinforced with 0.5 and 3wt% of WS_2NTs were melt-processed using a twin-screw extruder. High resolution computed tomography (µ-CT) was used to evaluate WS_2NTs dispersion and radiopacity of the nanocomposite material. Scanning electron microscopy (SEM) allowed to determine WS_2NTs orientations. Thermal and morphological properties were determined using differential scanning calorimetry (DSC). Extruded preforms were expanded with 2 types of equipment. First, radial expansions were performed with a custom rig which allowed in situ collection of small- and wide-angle x-ray scatterings (SAXS/WAXS) to observe oriented-crystal formation. Second, an industrial expander was used to add an axial stretch and produce biaxially expanded tubes (hoop/axial stretch ratios of 3.6:1, 2.5:1, 3.6:1.9, 2.5:2.5) which were characterised via WAXS and tensile testing in the axial and radial directions.

RESULTS AND DISCUSSION

While well-dispersed, axially oriented nanotubes were observed on SEM images. The radiodensity of the tungsten element allowed the quantification of agglomerates (>100µm^3) in representative volumes using µ-CT (Fig.1a). Both techniques indicated good overall dispersion, which decreased with the loading (93% vs 87% of NTs considered dispersed at 0.5 and 3 wt%). DSC revealed a strong nucleation effect from the nanotubes and WAXS confirmed the strong orientation of the NTs in the extrusion direction observed on SEM. The 0.5wt% tubes were selected to be compared to neat PLLA tubes during the expansion process. Upon radial expansion, in-situ SAXS/WAXS patterns indicated that, despite the high hoop strain, WS_2NTs remained aligned in the extrusion direction and that PLLA crystals formed, perpendicular to the NTs, along the hoop direction (Fig.1b). For both expansion experiments, the nucleation ability of the nanotubes did facilitate crystallisation at lower expansion temperature and strain which led to mechanical improvements (up to 20% increase of tensile strength for "Rad" conditions, Fig.1c). However, direct reinforcement from the WS_2NTs was not identified for amorphous tubes or when comparable crystallinity was observed ("Extruded" and "Biax" conditions). During biaxial expansion, preliminary results suggest that the nanotubes, aligned in the axial direction of the tubes encourage the growth of crystals in that direction. Further SAXS studies are currently in progress to identify the nature of this phenomenon.

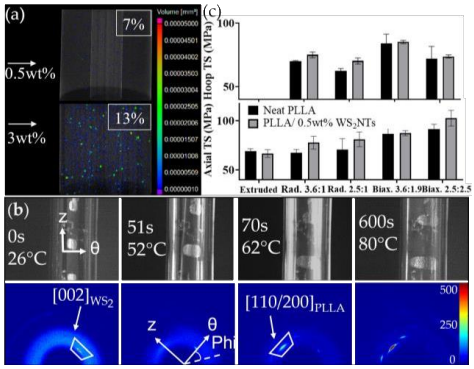

Figure 1 (a) µ-CT inclusion analysis with % of agglomerated NTs (b) Camera images and WAXS 2D patterns of a PLLA/WS_2NT (0.5 wt%) tube at different stages of the expansion process (c) Tensile strength (TS) in hoop and axial directions under various ratios of hoop and axial stretch ("Rad" for only radial expansion and "Biax" for biaxial).

While the nucleation ability, remarkable dispersion, and alignment in the extrusion direction of the nanotubes point to a good affinity with the polymer, the lack of reorientation of the nanotubes during expansion and mechanical reinforcement at a comparable degree of crystallinity point toward a lack of interaction. Additional characterisation using Raman spectroscopy and atomic force microscopy is in progress to study the interface between WS_2NTs and the PLLA matrix and try to address this counter-intuitive observation. Another hypothesis is that the production of PLLA/ WS_2NTs in an industry-relevant process might have caused significant damage to the nanotubes (qualitatively observed on SEM) and consequently dropped its reinforcing potential.

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ACKNOWLEDGMENTS

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The Degradation and Encrustation Behaviours of Poly Glycerol Sebacate Citrate in Artificial Urine

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INTRODUCTION

The urinary catheters that we use today were introduced over 80 years ago. The long-dwelling types have a tendency to develop complications, the most common being catheter encrustation and blockage caused by the formation of crystalline biofilms[1]. To tackle this problem, a coating made of biodegradable poly glycerol sebacate citrate (PGSC) is envisaged. The degradation of PGSC releases citric acid which is believed to have anti-encrustation properties[2]. However, increasing the polymer's citric acid content also leads to more rapid degradation. Hence, the degradation behaviour of PGSC samples with varying citric acid contents was investigated.

EXPERIMENTAL METHODS

Polymer sample preparation:

PGSC sheets (about 2 mm thick) with 3 different citric acid molar concentrations (0.25, 0.50, 0.75 with respect to glycerol's molar concentration) were synthesised by dissolving respective masses of citric acid and 9.70 g of PGS prepolymer (glycerol and sebacic acid molar ratio 1:1, synthesised using method specified here[3]) in 25 ml of ethanol. The resultant solutions were poured into PTFE coated moulds and degassed in a vacuum oven at 90℃. The pressure was slowly reduced to below 2 mbar over 2 hours. The temperature was then raised to 130℃ and the vacuum valve fully opened to maintain a pressure below 0.16 mbar for 48 hours. Disks (d=8 mm) were punched from the sheets using a biopsy punch. The initial mass (M_0) of all the punched PGSC disks was measured prior to degradation. The disks were then sterilised using UV radiation for 15 minutes on each side.

Degradation media preparation:

Artificial urine (AU) was prepared by dissolving the salts commonly found in human urine based on a previously developed formula[4]. The PGSC degradation behaviour, and anti-encrustation properties were examined by adding jack bean urease solution (1 unit/ml) into AU (1:5 volume ratio[5]).

Degradation specimen preparation:

2 PGSC disks and 5 ml of AU were added into a 7 ml Bijou tube to form a specimen. 3 specimens were prepared for each time point. The degradation time was chosen to be 7 days with a 1-day interval at 37℃. At each time point, the media pH was measured with a digital pH meter and the wet mass (M_w) of the PGSC disk was measured. The PGSC disks were then dried under vacuum for 21 days and their dry mass (M_d) measured. Another set of specimens was prepared with added urease solution. At each time point, their pH was also measured, and visual checks were performed for encrustation formation.

RESULTS AND DISCUSSION

The degradation of PGSC containing citric acid caused the solution pH to decrease over time. As shown in **Figure 1**, the higher the citric acid content, the lower the solution pH reached. The rate of decrease was the greatest on the first day and the solution pH reached a plateau on Day 6 and 7.

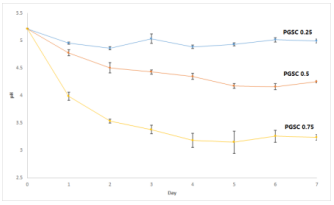

Figure 1 Degradation media pH change for PGSC with different citric acid content

Encrustation resistance measurements indicated that (**Figure 2**) there was a white precipitate present on the 0.25 samples which was absent on the 0.75 samples suggesting higher citric acid content PGSC has better encrustation resistance. Furthermore, as degradation continued, the amount of precipitation present on the 0.5 samples decreased over time which suggests the citrate ions released from polymer degradation induced the dissolution of some of the precipitates. However, high citric acid content led to surface cracks and very significant swelling. Therefore, it is evident that a balance between encrustation resistance and degradation rate needs to be struck and the optimum citric acid molar ratio lies between 0.5 and 0.75.

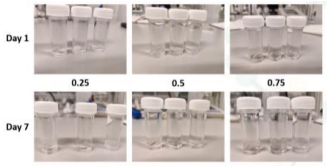

Figure 2 Photo comparison for Day 1 and 7 degradation specimens with added urease solution

CONCLUSION

The degradation experiments with PGSC samples containing different levels of citric acid demonstrated that PGSC is capable of lowering the urine pH and resisting the formation of encrustation. Hence, it has the potential for use as a catheter coating to combat the formation of crystalline biofilms. However, faster degradation that comes with higher citric acid content means an optimum concentration lies between 0.5 and 0.75 molar ratio.

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Role of Macromolecular Characteristics on the Morphology of Thermosensitive Chitosan Hydrogels

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INTRODUCTION

Thermosensitive hydrogels are widely studied matrices for cell encapsulation and delivery. Solutions of chitosan CS and phosphate salts are particularly interesting as they exhibit a thermosensitive sol/gel transition induced by phase separation.[1,2] Such systems can be held liquid at room temperature while exhibiting a sol/gel transition within minutes at 37°C, forming a macroporous hydrogel. Using mixtures of two phosphate salts, bGP and AHP, a formulation platform where pH, osmolarity and gelation kinetics of CS/bGP/AHP mixtures can be finely adjusted to be compatible with cell encapsulation, has recently been developed[1]. The delicate salt equilibrium leading to phase separation and thus gelification and macroporosity of the CS/bGP/AHP hydrogels is very sensitive to the composition of the mixture. To enlarge the range of properties of the system without losing its controlled thermogelling behaviour, we developed tools to observe and quantify evolution of gelation kinetics and microstructure upon modification of the chitosan, e.g. molecular mass M_w, degree of deacetylation DD, or biofunctionalization. The present work reports the use of this method to analyze the influence of M_w, DD and origin of the chitosan on microstructure and gelification.

EXPERIMENTAL METHODS

Materials: C-Sh-1 (M_w 188 kDa, DD 0.91, from shrimp), C-Sh-2 (M_w 205 kDa, DD 0.95, from shrimp), C-Sh-3 (M_w 564 kDa, DD 0.91, from shrimp) and C-Sq-1 (M_w 205kDa, DD 0.94, from squid) were used to create 0.8%wt gels following the formulations of CS/ βGP /AHP given by the platform[1].

Tube inversion: CS/ βGP/AHP mixtures at 4°C were immersed in a thermostatic bath at 37°C. Tube was flipped regularly until formation of the gel.

Confocal microscopy (LSCM): Fluorescently grafted CS was added to the previous formulations. Images were acquired overtime with Zeiss Axiovert 200M (laser 488 nm, oil immersion objective x40/1.3 oil DIC), at room temperature, after 2h of gelation at 37°C.

Image analysis: Images were deconvoluted and binarized to determine the porosity as the ratio of white and black pixels and were analyzed with the morphological sieve technique to estimate the pore size distribution[3].

RESULTS AND DISCUSSION

Gelation time at 37°C remained around 2 ± 0.5 min for each formulation, ensuring injectability for every CS batch. The microstructures were studied using confocal microscopy (Fig 1 (A)) and analysed with morphological sieve algorithm. It appeared that the strongest changes could be observed as M_w or CS origin were changed (Fig 1 (B)). Indeed, porosity diminishes as M_w increases, as chain length affects interchain interactions and space filling. Changes for squid-CS were also observed. Such differences can be explained by different amounts of α or β-polymorphs of chitin in shrimp or squid. These differences affect solubility, crystallinity, pore size and porosity. Finally, no significant change was observed at different DD, as, according to the platform, we adapt salts ratios to neutralize the NH_2 functions.

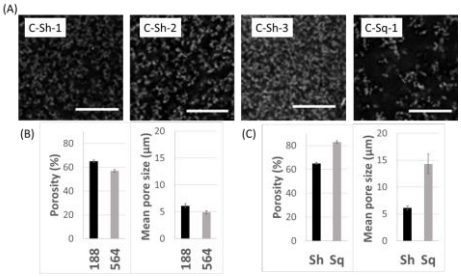

Figure 1: (A) Confocal microscopy picture for gels at 0.8%wt with different CS grades. Scale = 50 µm. Evolution of porosity and pore size depending (B) on M_w (kDa), and (C) on origin.

CONCLUSION

This work proved that, by the use of our rational platform, as long as an user knows the DD, the M_w and the origin of the CS, hydrogels based on CS, βGP and AHP can be prepared, with controlled properties, such as cytocompatibility and injectability, essential for applications in the biomedical field. We demonstrated that this formulation platform was adaptable to a large spectrum of CS batches, with different M_w or deacetylation degree DD.

The proposed method will also offer a tool to characterize the effect of CS biofunctionalization on the hydrogel macroporosity and morphology. Indeed, such modification, by peptide sequence or adhesion proteins, is especially important to improve bioactivity of CS hydrogels.

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>> 9:00 - 10:30 | PSOP-20 - BIOMIMETIC AND BIOINSPIRED MATERIALS I

Chairpersons: Rui Reis & Christèle Combes
Location: Room A

9:00 | KL Biomimetics I - Engineered spider silk hybrid proteins with transforming growth factor b3 modulates stem cell response and anticipate new prospects for tendon repair
Albina FRANCO, 3B's Research Group –I3BS Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Braga/Guimarães, Portugal

9:30 | O1 Biomimetics I - Automated fabrication of biomimetic platforms to present bone morphogenetic proteins
Elisa MIGLIORINI, Univ. Grenoble Alpes, CEA, INSERM, U1292 Biosanté, CNRS EMR 5000 BRM, Grenoble, France

9:45 | O2 Biomimetics I - Combination of Proteolytic Sequences, VEGF-mimetic Peptide and Laminin-derived Peptide for the Spatiotemporal Direction of Angiogenesis and Neurogenesis in Elastin-Like Recombinamer Scaffolds
Fernando GONZÁLEZ-PÉREZ, G.I.R. BIOFORGE, Universidad de Valladolid CIBER-BBN, Valladolid, Spain

10:00 | O3 Biomimetics I - Four-axis fabrication of a biocompatible vascular graft mimicking mechanical vessel properties
Tim TEN BRINK, Department of Complex Tissue Regeneration (CTR)/MERLN Institute for Technology Inspired regenerative Medicine, Maastricht University, Maastricht, The Netherlands

10:15 | FP01 Biomimetics I - Characterization and Modeling of Functional Gradients for Enabling Tough Biomimetic Devices
Mauricio CRUZ SALDIVAR, Department of Biomechanical Engineering, Faculty of 3mE, Delft University of Technology, Delft, The Netherlands

10:20 | FP02 Biomimetics I - Development of tough 3D-printed biomimetic calcium phosphate scaffolds for bone regeneration
Maria-Pau GINEBRA, Biomaterials, Biomechanics and Tissue Engineering Group, Department of Materials Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain; Barcelona Research Centre for Multiscale Science and Engineering, Universitat Politècnica de Catalunya, Barcelona, Spain

10:25 | FP03 Biomimetics I - Polyurethane-based Freeze-dried Systems Combined with Chondroitin Sulphate and Caseinophosphopeptides for Tendon Tissue Engineering
Eleonora BIANCHI, Department of Drug Sciences, University of Pavia, Pavia, Italy

Engineered spider silk hybrid proteins with transforming growth factor-β3 modulates stem cell response and anticipate new prospects for tendon repair

A. R. Franco[1,2], S. M. Bakht[1,2], M. T. Rodrigues[1,2], M. Gomez-Florit[1,2], R. M. A. Domingues[1,2], Isabel B. Leonor[1,2], Rui L. Reis[1,2], Manuela E. Gomes[1,2]

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INTRODUCTION

Recombinant spider silk (SS) proteins inspired a new class of tunable functional polymers[1]. We can engineer biomaterials with biological cues like the TGF-β superfamily aiming to modulate cell response providing a unique microenvironment that potentiates the cell differentiation in tendon tissues[2]. Herein, we assessed SS hybrid proteins as prospective bioinstructive materials under the hypothesis that stem cells positively respond to SS-derived biochemical signals.

EXPERIMENTAL METHODS

Hybrid protein: The transforming growth factor-β3 (TGFβ3) peptide was genetically fused with the SS protein (6mer). The new hybrid protein (6mer-TGFβ3) was expressed and purified.

Multifunctional films: Films were prepared by solvent casting combining different concentrations of SS hybrid protein (2% and 10%) with silk fibroin (8%; SF) extracted from *Bombyx mori* cocoons and water annealed for 24h. The hybrid films were characterized in terms of degradation, and secondary structure accessed by FTIR and XRD.

Cell studies: The biological potential of the hybrid films was studied, namely metabolic activity, proliferation, and extracellular matrix (ECM) production by culturing human adipose stem cells (hASCs) for up to 14 days. The expression of tendon- and chondrogenic-related markers was evaluated by Western-Blot and RT-PCR. The experiment was made in triplicates, and statistical analysis was performed ($P < 0.05$).

RESULTS AND DISCUSSION

The successful expression of the new hybrid proteins 6mer-TGFβ3 was achieved(Fig. 1A). The inclusion of TGFβ3 did not influence the ability of 6mer protein to develop β-sheet conformation (Fig. 1B and C), and slow degradation profile.

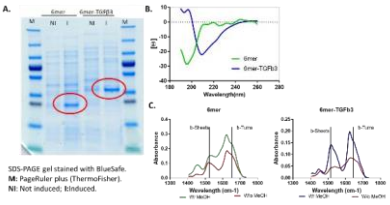

Fig. 1. A- Expression of the 6mer and 6mer-TGFβ3; B and C-protein hybrid secondary structure.

In the cell studies, the hybrid films with 10% 6mer-TGFβ3 elicit a higher transcript level of tendon-related markers tenomodulin (TNMD) and scleraxis (SCX) (Fig. 2A). The cells also deposited tendon-related ECM proteins, including collagen types 1 (Col-I) and 3 (Col-III) and tenascin-C (TNC) (Fig. 2A).

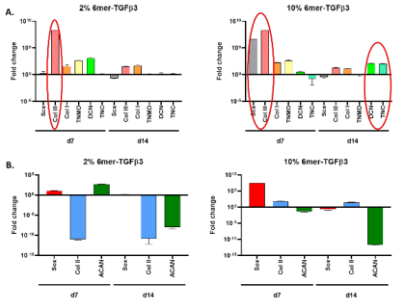

Fig. 2. A-Expression of tendon-related genes. B-Expression of chondrogenic-related genes.

As for the films with 2% 6mer-TGFβ3, they induced tendon-related markers and chondrogenic-related markers SOX9 and Aggrecan on day 7 (Fig. 2A,B).
This result strongly suggests that the concentration of 6mer-TGFβ3 modulates cell responses, affecting the commitment of hASCs into chondrogenic (2% 6mer-TGFβ3) or tenogenic (10% 6mer-TGFβ3) lineage. In the films with 6mer or SF alone, the same behavior was not observed.

CONCLUSION

The presence of the hybrid protein 6mer-TGFβ3 supported the expression of tenogenic and chondrogenic cues, evidencing cellular commitment towards tenogenic and chondrogenic lineages. These findings provide new insights into the use of recombinant spider silk proteins with tenogenic peptides to develop multifunctional living constructs aimed at tendon repair applications.

ACKNOWLEDGEMENTS

PTDC/BII-BIO/28870/2017; PTDC/BTM-MAT/2844/2021; ARF grant SFRH/BPD/100760/2014; SMB grant PD/BD/129403/2017.

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Automated fabrication of biomimetic platforms to present bone morphogenetic proteins

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INTRODUCTION

Over the last decade there has been a growing interest in the development of new materials to improve bone morphogenetic proteins (BMPs) delivery for tissue regeneration. *In vivo*, BMP2, 4, 6 and 7 are bound to extracellular matrix (ECM) components, mostly to fibronectin and heparan sulfate (HS) and with less extent to chondroitin sulfate[1]. The development of materials which are able to control BMPs molecular presentation by mimicking its *in vivo* presentation is an essential approach for a deeper understanding of BMPs functions and the modulation of its biological activity[2].
These biomaterials should present different types of ECM-derived molecules with controlled orientation and surface density. There is therefore the growing need of (i) automated deposition of molecules inside multiwell plates (ii) *in situ* characterization of the resulting biomaterials to improve the reproducibility and (iii) automated analysis of cellular responses.

EXPERIMENTAL METHODS

Biomimetic streptavidin (SAv) platforms were assembled on glass supports to study the interface between the ECM and mammalian cells by immobilization of biotinylated molecules such as adhesion peptides: cyclic RGD (cRGD) and the glycosaminoglycan HS, to which growth factors such as BMP2, 4, 6 and 7 can bind (Figure 1).
We developed a novel automated workflow, based on previous development of the group[3], to deposit different biomimetic SAv platforms inside multiwell plates for cellular studies. By applying the optical technique imaging correlation spectroscopy (ICS) we characterized the adsorption of SAv and of biotinylated components *in situ*. C2C12 myoblast were seeded on SAv platforms and thanks to an automated imaging acquisition and analysis[4] we quantified the level of pSMAD1/5/9, 1.5 h after stimulation with different BMPs.

RESULTS AND DISCUSSION

Thanks to the ICS technique – for the first time applied for biomaterials characterization – we measured a densely packed and homogeneous SAv monolayer with 336 ± 34 ng/cm^2, in agreement with standard *ex situ* spectroscopic ellipsometry measurements[5]. The quantification of the adsorption of biotin-Atto permitted to characterize the number of available biotin sites on SAv layer (1 every 9 SAv molecules). With the automated protocol we functionalized a full 96-well plate presenting BMP 2, 4, 6 and 7 in five different concentrations soluble and adsorbed to immobilized HS to study the effect of HS on different BMPs bioactivity (Figure 1). We demonstrated that BMP2 is the most efficient BMP to induce SMAD 1/5/9 phosphorylation when presented in solution or bound to HS.

CONCLUSION

For the first time we have shown that, the functionalization of biomimetic SAv platforms can be fully automated and characterized inside multiwell plates for cellular studies. The high flexibility makes our approach a powerful candidate to investigate the role of ECM components on cell signaling by developing a large variety of biomimetic surfaces.

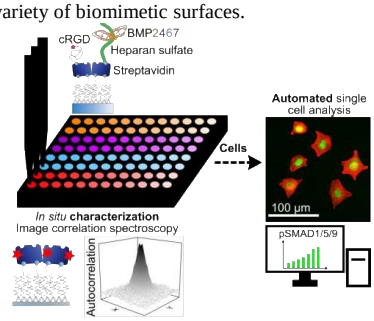

Figure 1: representation of the automated workflow to deposit the biomimetic platforms on glass bottom multiwell plates, to characterize them *in situ* with ICS and to perform automated cell analysis of the p-SMAD 1/5/9 response.

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ACKNOWLEDGMENTS

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Combination of Proteolytic Sequences, VEGF-mimetic Peptide and Laminin-derived Peptide for the Spatiotemporal Direction of Angiogenesis and Neurogenesis in Elastin-Like Recombinamer Scaffolds

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INTRODUCTION

In the area of tissue engineering and regenerative medicine (TERM) the development of three-dimensional (3D) scaffolds able to promote a spatiotemporal control of cell colonization, vascularization and innervation are of great interest. To achieve this goal, specific peptide sequences with fast and slow proteolytic rates towards the urokinase plasminogen activator uPA, namely GTAR and DRIR, the vascular endothelial growth factor mimetic peptide (QK) and the IKVAV laminin-devired peptide for neuronal adhesion and proliferation have been described in literature[1,2]. Hydrogels based on Elastin-like recombinamers (ELRs) appear as promising candidates for this purpose, as bioactive domains such as the QK or IKVAV peptide can be covalently tethered into their backbone and their recombinant nature allow the genetic encoding of proteolytic sequences in an easy and tailored way.

EXPERIMENTAL METHODS

ELRs containing fast and slow degradable proteolytic sequences (GTAR and DRIR), the RGD cell-adhesion domain, the QK pro-angiogenic peptide or the IKVAV pro-innervation peptide were prepared in this work. In addition, click catalyst-free crosslinkable domains were tethered to the backbone of the ELRs to produce the intended hydrogels under physiological conditions. Porosity of ELR hydrogels was evaluated by microscopic imaging, whereas *in vitro* studies were conducted to evaluate the effect of IKVAV peptide over C6 glial cells adhesion. To evaluate the ability of ELRs to promote angiogenesis and neurogenesis, we fabricated a 3D model containing two different cylindrical ELR hydrogels (Image 1). Specifically, the first cylinder contains the GTAR fast-proteolytic sequence and the QK peptide, whereas the second cylinder contains the GTAR fast-proteolytic sequence and the IKVAV peptide, in order to evaluate both bioactivities. In contrast, the outer part lack or display the slow-proteolytic sequence (DRIR). Subcutaneous implantation in Swiss CDR-1 mice and subsequent histology and immunohistochemistry (IHC) allow the analysis of cell infiltration, angiogenesis and neurogenesis in space and time.

RESULTS AND DISCUSSION

Scanning electron microscopy confirmed the porous structure of the ELR hydrogels suitable for soft tissue regeneration. *In vitro* analysis revealed the pro-innervation effect of the IKVAV peptide promoting C6 glial cells adhesion. *In vivo* results of the 3D model showed a faster cell colonization of the internal tubes containing the fast-proteolytic sequence, in comparison with the outer part lacking or displaying slow-proteolytic sequences. In addition, histology and immunohistochemistry confirmed an increased angiogenesis in the presence of the QK peptide and an increased neurogenesis in the presence of the IKVAV peptide following the pre-design structure (Fig. 1).

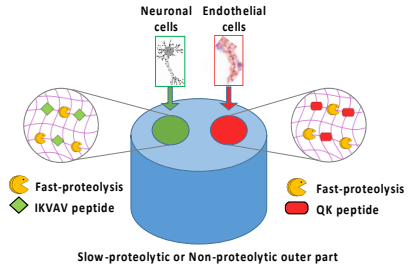

Image 1. 3D ELR construct

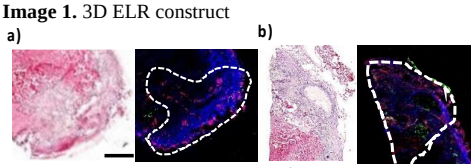

Figure 1. Representative images of the hematoxylin and eosin, DAPI (nuclei, blue), CD31 (endothelial cells, red) and β-III tubulin (neuronal cells, green) stained 3D scaffolds, where (a) correspond to the QK-containing cylinder and (b) correspond to the IKVAV-containing cylinder, after 6 weeks of implantation. Scale bar = 500 µm.

CONCLUSION

The combination of proteolytic-sensitive sequences, the QK pro-angiogenic peptide and the IKVAV pro-innervation peptide into 3D ELR systems showed the capacity to spatiotemporally control angiogenesis and innervation in vivo. Specifically, the cylinder containing the QK peptide showed a faster endothelialization, whereas the cylinder displaying the IKVAV peptide showed a faster innervation, following the pre-designed pattern. These results pave the way for the fabrication of ELR scaffolds intended for an optimal tissue repair in the area of TERM applications.

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ACKNOWLEDGMENTS

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Four-axis fabrication of a biocompatible vascular graft mimicking mechanical vessel properties

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INTRODUCTION

Cardiovascular disease is a frequent cause of mortality and often requires surgical replacement of blood vessels by autologous or synthetic grafts. Although common, these medical grafts exert limited effectiveness with failure rates up to 50% post-implantation. Recent advances in tissue engineering and biofabrication have tried to overcome implant comorbidities through a novel, self-regenerating alternative defined as Tissue Engineered Vascular Grafts (TEVG). These grafts are defined as tubular constructs mimicking vascular biomechanical properties whilst promoting vessel formation and self-assembly of cells. Although promising, no vascular graft currently exist that is capable of fully sustaining the required mechanical properties and mimic a biocompatible environment for complete vascular regeneration.[1] Here, we focus on creating TEVGs derived from biocompatible synthetic polymers replicating the biomechanical properties of native arteries, effectively optimizing grafts for physiological conditions.

EXPERIMENTAL METHODS

Tubular grafts consisting of varying biodegradable polymers (poly(ε-caprolactone), Mw. 45000g/mol^{-1}; poly(ethylene oxide terephthalate)/poly(butylene terephthalate) 1000PEOT70PBT30 were fabricated by means of four-axis fused deposition modeling (FDM), a technique combining regular FDM functionalities with a rotational fourth axis (R°). Designs were modeled after the human carotid artery, spanning a diameter of Ø4mm. Skeletal supports were designed in a waved Z-pattern to support multiple controllable parameters whilst resembling the stress/strain relation of vascular matrix protein fibrils. In this study, pattern amplitude, supporting strut fiber quantity and recurring pattern frequency have been adjusted to optimize flexibility, strain capacity and vascular strength of the scaffold. An additional microfiber (~40 µm) luminal layer was printed to support future integration of endothelial cells. Mechanical characterization of the TEVGs was performed through both longitudinal and circumferential uniaxial tensile strength and fatigue analysis with induced incremental stretch over time. All results were compared to porcine carotid arteries for biological validation.

RESULTS AND DISCUSSION

Results of our study showed that alterations in design allowed substantial control over extension capacity, graft strength and flexibility. In particular, amplitude changes along the longitudinal axis significantly alters the maximum strain capacity and Young's Modulus. Optimized grafts displayed toe regions in stress/strain curves, mimicking vascular-like elastic behavior. Compared to porcine biological samples, our grafts fit within physiological ranges up to 250% strain.

The radial stiffness found in circumferential stress analyses were roughly 4.5-fold higher than the longitudinal direction, which is in accordance with longitudinal/radial mechanical anisotropy of the natural carotid artery. Further fatigue testing displayed resistance to natural stretch rates (Δ1.1-1.5λ), where additional increase of stretch rate displayed optimized TEVGs exceeding the natural arterial strain capacity tenfold in the longitudinal direction.[2] Assessment of the microfiber luminal layer displayed no damage at physiological ranges, and is thus suitable for future tissue culturing.

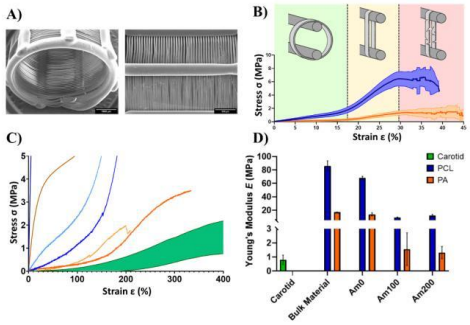

Figure 1) A) SEM images of PCL TEVG scaffolds. B) Representative curves of the circumferential stress/strain behaviour. C-D) Comparison of optimized scaffolds to porcine carotid arteries.

CONCLUSION

This study successfully established the formation of biodegradable TEVGs with control over mechanical properties regardless of material. Optimized scaffolds managed to mimic mechanical behavior of natural arteries and displayed a tenfold resistance to physiological pressure whilst maintaining natural flexibility. This model can be adjusted in diameter and length to resemble the majority of macrovasculature, hereby laying the foundation for on-demand production of cardiovascular implants.

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Characterization and Modeling of Functional Gradients for Enabling Tough Biomimetic Devices

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INTRODUCTION

Functional gradients (FGs) are among the most common structural elements that Natural materials have evolved to survive perilous environments[1]. Their presence enhances the mechanical performance of multifunctional structures by releasing interfacial stresses through the systematic transition of mechanical properties (*e.g.*, power, exponential)[2]. Today, voxel-based additive manufacturing techniques allow mimicking such FGs due to its point-wise freedom for depositing both hard (mineral-like) and soft (collagen-like) phases across space[3]. However, the non-linear transition in mechanical properties between both phases depends on the voxels' hard material fractions (ρ) and spatial distributions, where no models have been introduced to estimate their behavior. Therefore, it is fundamental to derive and validate such models, enabling the realization of fully tuned biomimetic devices with enhanced toughness.

EXPERIMENTAL METHODS

We used a Polyjet 3D printer (Stratasys, USA) for manufacturing an FG with a linear ρ transition discretized by randomly depositing the hard-soft voxels (Figure 1A). We performed nanoindentation experiments with a Hysitron TI 950 Triboindenter (Bruker, USA) to measure the elastic modulus response of the FG. Then, we used a simplified model obtained from the literature to characterize its behavior, which takes the form[4]:

$$E/E_H = \rho^b + E_S/E_H$$

where b is a fitting parameter and E, E_H, and E_S are the elastic moduli of the composite, hard, and soft material, respectively. We validated this model by designing two intervertebral discs (IVDs) and testing them under quasi-static compression. The first one was designed with a non-graded interface between its hard-soft connections. In contrast, the second IVD was graded, and its hard material distribution averaged the same elastic response as the first design. We hypothesized that the graded IVD would have an improved toughness before critical failure.

RESULTS AND DISCUSSION

The nanoindentation results denoted an elastic modulus transition that variated non-linearly (Figure 1B), similar to what has been reported in the literature[3]. Besides, the parameterized model (b = 2.06) adequately followed the non-linear behavior of the experimental data with a high correlation (R^2 > 97%) across three different orders of magnitude. These outcomes indicate that the proposed model is an excellent candidate for tunning the hard-soft composite's response since only a single parameter is necessary to describe their entire behavior.

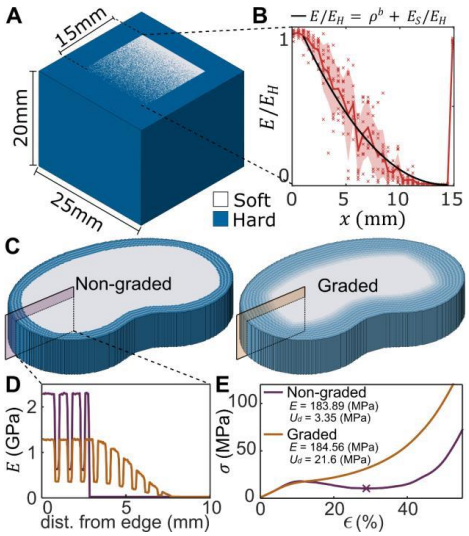

Figure 1: The nanoindentation specimen (A) and its response (B). Its modeling enabled designing fully tuned IVDs (C-D), where the graded one was tougher (E).

The proposed model enabled discretizing and manufacturing IVDs with functions of elastic modulus that resemble those of their lamellae (Figure 1D). The resulting average elastic modulus of both IVDs was nearly identical (Figure 1E), validating the accurate performance of the parameterized model. Furthermore, the graded design enabled a better distribution of stresses across the IVD, resulting in a hardening-like response with an improved toughness compared to the critical yielding of the non-graded design.

CONCLUSION

These findings prove that the introduced model enables the design of fully-tuneable biomimetic devices with only a single relation, where the application of FGs enhanced their overall non-linear performance.

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Development of tough 3D-printed biomimetic calcium phosphate scaffolds for bone regeneration

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INTRODUCTION

Direct ink Writing enables the fabrication of bone scaffolds with a precise control of both external shape and internal porosity. The development of reactive calcium phosphate (CaP) inks that are able to harden at body temperature through a setting reaction has brought numerous advantages. Besides resulting in biomimetic compositions, closer to the mineral phase of bone and avoiding shrinkage problems[1], it opens the door to the combination with organic second phases and the design of composite scaffolds. Polycaprolactone (PCL) has drawn much attention for biomedical applications due to its high toughness and slow degradation compared to other polyesters[2]. This study aims at overcoming the inherent brittleness of 3D-printed CaP ceramic scaffolds by using PCL organogels as binders in alpha-tricalcium phosphate (α-TCP) based inks. Moreover, the addition of polyethylene glycol (PEG) is explored as a plasticizer[3]. Our goal is to analyze the effects of the PCL and PEG contents as well as of the ceramic loading on the rheological properties of the ink and the mechanical, physico-chemical and biological properties of the 3D printed scaffolds.

EXPERIMENTAL METHODS

The inks were prepared by loading PCL organogels (15-20 wt.%), containing different amounts of PEG (0-10 wt.%), with increasing proportions of α-TCP powder. An α-TCP ink prepared with Pluronic F127 hydrogel as a binder was used as a control (C ink). Rheological studies were carried out in a rotational rheometer. 3D printed bar-shaped scaffolds with a nozzle diameter of 410 µm and a distance between filaments of 250 µm, were used to assess the mechanical properties by 3-point bending tests. Printability and shape fidelity, adhesion between printed filaments and microstructure of the scaffolds were assessed by optical and scanning electron microscopy (SEM). Porosity was quantified by mercury intrusion porosimetry (MIP), specific surface area (SSA) by nitrogen adsorption and the skeletal density by helium pycnometry. The in-vitro cell behavior of the PCL containing scaffolds and the C ink was evaluated using human mesenchymal stem cells. Cell adhesion, viability and proliferation were assessed at 24 h and 7 days.

RESULTS AND DISCUSSION

The rheological behavior of the PCL ink showed an elastic to plastic transition, in contrast to the typical viscoelastic behavior presented by the C ink. A minimum ceramic loading was required for optimal printability. The addition of PEG enhanced the adhesion between printed layers (Fig. 1A and B). However, a too high concentration of PEG (10 wt.%) resulted in a plastic behavior of the as-printed samples. The addition of PEG did not modify the SSA (15.2-16.5 m^2/g), the skeletal density (2.54-2.59 g/cm^3) and the microporosity. The presence of both polymers did not disturb the setting reaction and an entangled network of needle-like CDHA nanocrystals was obtained in all cases, with PCL fibers homogenously distributed (Fig. 1C and D). Regarding the mechanical properties, the use of the PCL organogels resulted in a 2-fold increase of the bending strength, and a 5-fold increase of the work of fracture compared to the C ink, therefore reducing significantly the brittleness of the ceramic scaffolds. Excellent cell adhesion and proliferation was found on the PCL containing scaffolds.

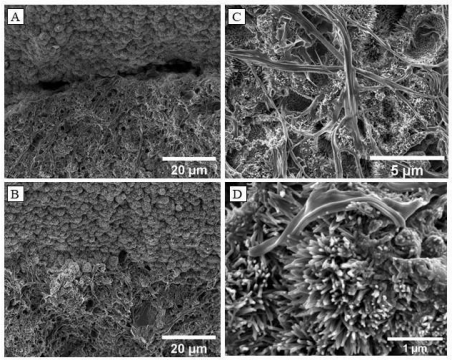

Fig. 1. Scanning electron microscopy images. A) Layer adhesion of PEG0. B) Layer adhesion of PEG5. C and D) Microstructure of the PCL containing scaffolds.

CONCLUSION

The incorporation of biocompatible and biodegradable polymers in the binder of self-setting inks is a promising method for enhancing the mechanical properties and reducing the brittleness of calcium phosphate bone scaffolds, preserving the excellent biological properties.

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ACKNOWLEDGMENTS

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Polyurethane-based Freeze-dried Systems Combined with Chondroitin Sulphate and Caseinophosphopeptides for Tendon Tissue Engineering

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INTRODUCTION

Tendon pathologies are medical conditions that include ruptures and overuse injuries, accompanied by inflammatory and degenerative alterations, such as tendinopathies. Biopolymers based scaffolds have been proposed in tissue engineering to replace and restore tendon tissue, due to their ability to mimic the structural, biomechanical, and biochemical functions of the extracellular matrix (ECM)[1]. Moreover, the combination of the scaffolds with caseinophosphopeptides (CPP), obtained from casein, provides an interesting approach to promote the reconstruction of the tendon-bone interface. In fact, they have been demonstrated to affect calcium uptake and osteoblast differentiation[2,3], and scavenge free radicals[4]. Given these premises, the aim of this work was the design and development of freeze-dried systems based on thermoplastic polyurethane (TPU), combined with chondroitin sulphate (CS), a glycosaminoglycan effective in wound healing[5], and with CPP, in order to enhance the tissue healing potential.

EXPERIMENTAL METHODS

Preparation of the freeze-dried systems.

Two blends containing respectively 8% w/w (namely T1) and 10% w/w (namely T2) of TPU in water:acetic acid (10:90) were prepared. Two more blends with the addition of 1% w/w CS were also prepared (namely T1-CS and T2-CS). The blends were refrigerated (-80°C overnight) and freeze-dryed for 24 h.

The four systems were put in contact with cell medium containing CPP to promote their adsorption into the structure. Three different concentrations of CPP (2.4, 3.2, and 6.4 mg/ml) were used to evaluate their influence on the cell adhesion and proliferation.

Characterization of the systems.

The systems morphology was assessed using SEM and the pore areas were calculated using ImageJ software.

The mechanical properties were evaluated using a Texture Analyzer, and the structure elesticity was also evaluated by means of an Ultra Small-Angle X-Ray Scattering (USAXS).

Statistical analyses were performed using Astatsa statistical calculator. One-way analysis of variance (ANOVA) was followed by Scheffè for post-hoc comparisons. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

The systems were characterized by a homogenous microporous morphology (pore area (μm^2): T1 = 442±181.4; T2 = 321.3±143.1; T1-CS = 378±170.6; T2-CS = 301.1±124.9). Moreover, the CPP adsorption did not cause morphological changes (fig. 1).

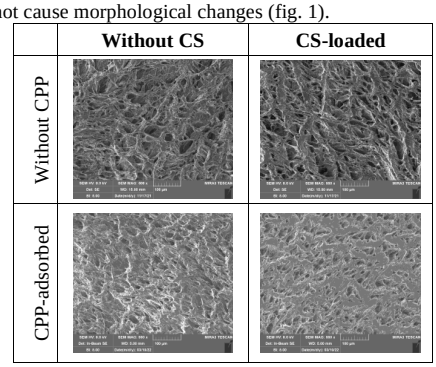

Fig. 1. SEM micrographs of the freeze-dried systems with and without CS, and before and after CPP adsorption.

The presence of CS led to an increase of the systems mechanical properties. In particular, the T2-CS scaffolds reached Fmax values of 12 MPa, which are comparable to that of the native tendon, and were characterized by remarkable values of elongation % (651.1±69.2 %).

Moreover, the USAXS analysis demonstrated that the systems were characterized by elastic behavior since they were able to stretch and return to the initial conformation, interestingly mimicking the crimps of the native tendon.

CONCLUSION

In conclusion, freeze-dried systems combined with CS and CPP were successfully prepared. The scaffolds possessed adequate morphology and mechanical properties, representing a promising structure to stand the mechanical loads during the tissue regeneration. Further analyses are ongoing to test the cell adhesion and proliferation onto the scaffolds and the influence of CPP on their ability to produce ECM. In vivo evaluation of the systems safety is also on plan.

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ORAL SESSION | PSOP-21 Biomaterials for cancer

>> 9:00 - 10:30 | PSOP-21 - BIOMATERIALS FOR CANCER
Chairpersons: Catherine Picart & Jens Puschhoff
Location: Room E

9:00 | KL Biomaterials for cancer - Applications of organoids in bioengineering
Jens PUSCHHOF, Epithelium Microenvironment Interaction Laboratory, Division Microbiome and Cancer, German Cancer Research Center, Heidelberg, Germany

9:30 | O1 Biomaterials for cancer - Designing a Peptide Hydrogel for Early Detection of Cancer
Niall MAHON, Department of Materials, Manchester Institute of Biotechnology, Manchester, UK

9:45 | O2 Biomaterials for Cancer - Patient-derived nanocarriers for precise hyperthermia against glioblastoma multiforme cells
Daniele DE PASQUALE, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), Italy

10:00 | O3 Biomaterials for cancer - Reactive Oxygen/Nitrogen Species-Scavenging Hydrogels with Therapeutic Potentials in Triple-Negative Breast Cancer
Amir ALSHARABASY, CÚRAM, SFI Research Centre for Medical Devices, National University of Ireland, Galway (NUIG), Ireland

10:15 | FP01 Biomaterials for cancer - Multifunctional biomimetic pancreatic cancer cell membrane-camouflaged vitamin E-based prodrug micelles
Miguel PEREIRA DA SILVA, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

10:20 | FP02 Biomaterials for cancer - A 3D Co-Culture Spheroid Model to Assess the Response of Bone Metastases to Anticancer Drugs
Ceri-Anne SUURMOND, Department of Dentistry – Regenerative Biomaterials, Radboud Institute for Molecular Life Sciences, Radboud University Medical Center, Nijmegen, The Netherlands

10:25 | FP03 Biomaterials for cancer - Polymeric Nanoparticles Targeting Glycans in Gastric Cancer Cells Under Live Flow Conditions
Francisca DINIZ, i3S- Institute for Research and Innovation in Health, University of Porto, Portugal; ICBAS-Institute of Biomedical Sciences of Abel Salazar, University of Porto, Portugal

Applications of organoids in bioengineering

Jens Puschhof[1,2], Yorick Post[2], Joep Beumer[2], Cayetano Pleguezuelos-Manzano[2], Axel-Rosendahl Huber[2], Hans Clevers[2*]

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In the last decade, adult stem cell-derived organoids have become versatile tools in disease modelling and bioengineering.

The possibilities to expand healthy human tissue of almost every organ and control differentiation makes these 3D tissue models a suitable resource for diverse applications.

Here, I will highlight our recent advances in several areas of organoid models.

In the first part of my talk, I will focus on the possibility to produce bioactive molecules in their original cell types using the example of snake venom gland organoids. I

n the second part, I will describe our efforts to increase the complexity of tissue culture models by combining organoids with microenvironmental components on diverse platforms.

Finally, I will elaborate on our recent efforts to engineer organoids for better representation of rare human cell types in order to study their function at single cell resolution.

Designing a Peptide Hydrogel for Early Detection of Cancer

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INTRODUCTION

Cancer early detection is pivotal to patient survival. The small non-coding nucleic acid sequences, microRNA (miRNA) are a captivating molecular target for cancer early detection. miRNA are dysregulated during the early stages of cancer[1], it is found in stable amounts in blood plasma and serum. Therefore, a minimally invasive liquid biopsy screening device would allow for point of care diagnostics. Current miRNAs detection methods are cumbersome and lack reproducibility along with poor sensitivity and low accuracy. To overcome these challenges we aim to develop a new diagnostic platform using a functional 3D peptide hydrogels for sequence specific[2, 3], PCR-free, fluorescent detection of miRNAs in a "one-pot" assay. This work will assess the suitability of the novel hydrogel-based technology for rapid, robust and reliable screening of the unique miRNA fingerprint of difficult to detect cancers.

EXPERIMENTAL METHODS

Using a split probe system with a FRET pair conjugated to an anti-parallel β-sheet peptide hydrogel, can be formulated that allow complementary strands of cancer miRNA biomarkers to be identified via fluorescence. As depicted in Figure 1.

Diffusion characteristics were evaluated via plate reader and Fluoroblok well insert. Allowing to measure the rate at which fluorescently label DNA analogue of miR-21 diffuse into peptide hydrogels.

Cell culture of Panc-1, MIA PaCa-2, LNCaP and PC-3 cells in 2D and 3D (multicellular tumour spheroids) forms was undertaken to validate the sensor. The hydrogels under investigation were also evaluated in their ability to support the four cancer cell lines spheroid structure.

RESULTS AND DISCUSSION

Four de novo designed self-assemble peptide hydrogels (SAPH) were tested to understand the diffusion characteristics of miRNA and select system that allow fast trapping and detection of miRNA. MiRNA being negatively charge it was found that positively charted hydrogels promoted miRNA trapping. The mesh size of the hydrogel used (<40nm) also allowed to filter and avoid interference from larger cell debris usually present in biological samples.
The bio-compatibility of the four peptide hydrogels provides a 3D platform for cancer cell culture, and in situ bio-sensing.

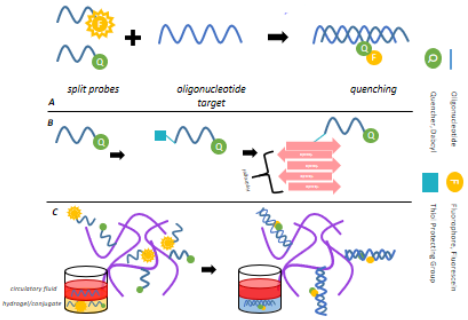

Figure 1: Diagram of peptide hydrogel sensor.

CONCLUSION

The self-assemble peptide hydrogel is an extremely versatile material. Has the potential to harbour fluorescent properties, to allow for biosensor application in the early detection of cancer.
Future work on 3D culturing of other pancreatic and prostate cancer cells for quantification of the key secreted biomarkers linked to the two cancers that require early detection.

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ACKNOWLEDGMENTS

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Patient-derived nanocarriers for precise hyperthermia against glioblastoma multiforme cells

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INTRODUCTION

Glioblastoma multiforme (GBM) is the most malignant brain cancer, with a heterogeneous histological nature, rapid progression, and low survival rates, all features that make the current treatments against GBM highly inefficient[1]. Finding a strategy that can precisely target new anticancer actions in the tumor area to support canonical chemotherapy could improve the survival of patients affected by GBM.

In recent years, localized hyperthermic treatment against GBM displayed promising results. Nanocomposite materials based on superparamagnetic iron oxide nanoparticles (SPIONs) are used for their ability to generate heat upon alternated magnetic field (AMF) stimulation[2]. Selective targeting towards GBM could solve some issues related to adverse drug reactions or to the patient's immunological response. In this view, a new tumor targeting strategy consists in coating the nanostructures with membranes extracted from the tumor cells, by exploiting the cancer cell homotypic recognition.

In this work, we propose a nanovector composed of a lipid core, where SPIONs and an anticancer drug, regorafenib, are encaspulated, and coated with membranes derived from primary GBM cells, to allow homotypic recognition of the nanovectors.

EXPERIMENTAL METHODS

Primary cells extracted from GBM surgical samples of eight different patients (GBM-derived primary cells - GDPC-), were cultured according to standard procedures, and with relevant ethical permission (CER Liguria 341/2019).

GDPC plasma membranes were extracted and used to coat the nanovectors to obtain cell-derived magnetic nanovectors loaded with regorafenib (Reg-CDMNVs). *In vitro* tests were carried out using GDPC cells. Reg-CDMNVs + AMF antitumor action was studied by WST-1, and by the expression of molecular markers such as HSP70, TRAP-1 (immunostaining), and caspase-8 activation (flow cytometry). The release of lysosomal content after Reg-CDMNVs + AMF was demonstrated by a lysosomial marker and cathepsin D location (immunostaining). CDMNV selective tumor targeting was studied with dynamic flow experiments, and compared to representative human healthy brain cell lines: astrocytes, pericytes, endothelial and neuron-like cells. These experiments were carried out for CDMNVs and CDMNVs with digested membrane proteins (CD*MNVs), the latter used as control for assessing efficient homotypic targeting.

RESULTS AND DISCUSSION

Targeting experiments in flow conditions showed that CDMNVs were able to excellently target GDPC cells (12 ± 2.9% cell area with nanoparticles), while CD*MNVs lose their targeting abilities (8 ± 1.1% cell area with nanoparticles), highlighting the importance of membrane proteins for tumor targeting. Reg-CDMNVs (300 µg/ml) induced a decrease in cell viability of 40.2 ± 2.9% with respect to control cells. In comparison, plain regorafenib, at the corresponding concentration, was less effective in reducing cell viability (68.4 ± 0.9 %). After chronic AMF stimulation cell viability of cultures pretreated with Reg-CDMNVs was significantly lower, *i.e.* 20 ± 4%. No change in viability with chronic AMF was detected for control cells and cells treated with the plain drug. The internalization fate of CDMNVs was studied at different time points (6, 24, and 96h). CDMNV internalization in the lysosomes increases with time, and, upon AMF, they induce lysosomal membrane permeabilization with consequent leakage of the lysosomal content, in particular cathepsin D, a proteolytic enzyme that can trigger apoptotic pathways through caspases activation.

After the treatment with AMF + CDMNVs or AMF + Reg-CDMNVs, a net increase in the synthesis of the mitochondrial stress marker TRAP-1 and of the heat stress marker HSP70 (Figure 1) could be detected, further validating the efficacy of magnetic hyperthermia induced by CDMNVs. Finally, the treatment with both CDMNVs and Reg-CDMNVs followed by AMF stimulus led to caspase-8 activation.

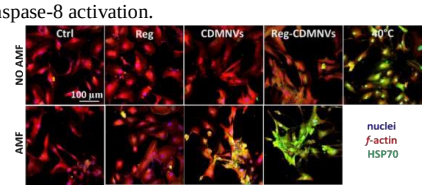

Figure 1. Immunostaining of HSP70 in GDPC without and with 2 h of AMF stimulation.

CONCLUSION

Obtained results demonstrate that the combined action Reg-CDMNVs + AMF exerts a clear antitumor action based on localized hyperthermia. In addition, the effective targeting promoted by these new systems, together with the patient-derived coating strategy, could render this strategy a valid option for personalized nanomedicine against GBM.

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ACKNOWLEDGMENTS

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Reactive Oxygen/Nitrogen Species-Scavenging Hydrogels with Therapeutic Potentials in Triple-Negative Breast Cancer

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INTRODUCTION

The excessive production of various reactive oxygen and nitrogen species (RONS), has been proved to be one of the main contributors to the progression of triple-negative breast cancer (TNBC).[1] Nitric oxide (•NO) is among these RONS, which exert different cellular effects through the nitrosation of specific molecules, controlled by its concentrations and the oxidation into other congeners.[2] Similarly, various reactive oxygen species (ROS) have different functions in tumour cell migration, with H_2O_2 and $OH^{\bullet-}$ being the most studied species in cancer.[3] Accordingly, the fabrication of a hydrogel system able to scavenge NO and other RONS represents a potential strategy for maintaining the nitrosative redox homeostasis in tumor cells. Herein, hyaluronic acid (HA) was employed as one of the main components of the tumour extracellular matrix proved to have regulatory roles for tumour cell migration and tumour growth.[4] The specific objectives of this study were: (i) to test the efficiency of HA polymer and hydrogels to scavenge NO and ROS; (ii) to evaluate the potential of hemin to scavenge NO and protect HA against the RONS-induced degradation; (iii) to investigate the efficiency of hemin-loaded hydrogels to scavenge the intra and extracellular NO and (iv) to explore the downstream effects of NO-scavenging potential on the angiogenesis and tumour cell migration.

EXPERIMENTAL METHODS

HA interactions with NO and ROS: In PBS, 1 MDa HA was incubated with NO-donors or H_2O_2 and lyophilized. The HA products were tested by ^{1}H-NMR, FTIR, gel electrophoresis and HPLC. Considering hemin an efficient NO-scavenging compound, NO's effects on HA/hemin mixture were evaluated. Fabrication of hemin-loaded Hydrogels: Hemin-loaded chemically-crosslinked HA hydrogel was fabricated. The NO-scavenging efficiency was measured electrochemically. In vitro testing: The effects of HA products from different treatments and the HA hydrogels on the intracellular NO levels were evaluated. Moreover, the downstream effects of NO-scavenging on the migration of MDA-MB-231 cells were studied via trans-well migration assays.

RESULTS AND DISCUSSION

HA interactions with NO and ROS: via the characterization of the HA products resulting from the interactions between HA and NO released from different donors with different rates and H_2O_2, it was concluded that HA is an efficient scavenger for NO and ROS. These interactions worked as inducers for fragmentation of the long polymeric chains, and the suggested mechanism is shown in **(fig. 1)**. However, these reactive species' effects were not as significant as those of the tested hyaluronidase control concentrations.

NO-scavenging by HA hydrogels and *in vitro* testing: Via the incubation of hemin-loaded and unloaded hydrogels with different concentrations of DETA-NO for two weeks, followed by seeding of MDA-MB-231 cells in the presence of fresh 300 µM DETA-NO for invasion study through the hydrogels, the effects of hemin can be identified. The hemin-loaded hydrogels caused a decrease in the migration efficiency of cells compared to the unloaded ones **(fig. 2),** referring to the essential functionality of hemin within the hydrogel matrix for prolonged scavenging NO in biological systems.

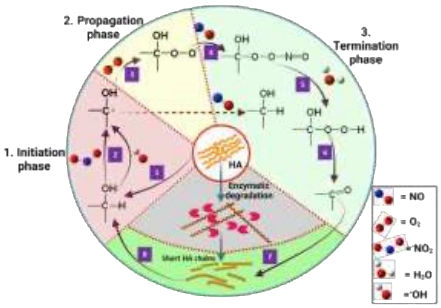

Figure 1: A summary for the main extracellular radical reactions involved in the oxidative degradation of HA.

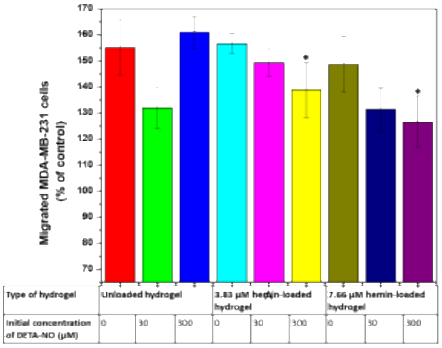

Figure 2: the effects of 300 µM DETA-NO on the invasion of MDA-MB-231 cells through hemin-loaded and unloaded HA hydrogels, pre-treated with different concentrations of DETA-NO. Data are represented as mean ± SD, n=3. $^{*,\#}$, $P < 0.05$ versus the unloaded hydrogel group, treated previously with 30 and 300 µM DETA-NO, respectively.

CONCLUSION (S)

In addition to studying how HA binds ROS, the NO-scavenging efficiency of the polymer and HA hydrogels was evaluated alongside the downstream effects of NO-elimination on TNBC migration. Moreover, the essential functionality of hemin as a NO-scavenging compound within the hydrogel matrix was proved, which paves the way for the application of these hydrogels as new therapeutics for breast cancer.

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Multifunctional biomimetic pancreatic cancer cell membrane-camouflaged vitamin E-based prodrug micelles

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INTRODUCTION

Pancreatic cancer (PC) is currently one of the deadliest and most aggressive cancers worldwide, bearing a dismal 5-year survival rate of only 11% [1]. PC is characterized by a desmoplastic and dense stroma barrier and to rapid emergence of a multidrug resistant phenotype to conventional chemotherapeutic agents. Gemzar® is a clinically-approved gemcitabine (GEM) solution widely used in PC treatment, mostly in combination regimens, but evidences poor stability, accelerated clearance and extensive systemic toxicity [2]. Nanoparticles have been receiving increasing attention as advanced drug delivery systems capable to protect and enhance stability of encapsulated drug payloads as well as to enable controlled drug release at target sites [2,3]. In recent years, attention has been paid in developing all-functional nanoparticles bearing intrinsic biofunctionalities, thus able to function as active ingredients. In this work, a strategy was explored to leverage serum stability, blood circulation half-life and PC targeting features of GEM by preparing all-functional polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer /vitamin E succinate (VES)-GEM (VES-GEM) prodrug micelles (Soluplus®/VES-GEM) camouflaged with PC cell membrane (PCCM@M). Soluplus® has multidrug resistance reversal features, and antioxidant, anticancer and p-glycoprotein inhibition properties are ascribed to VES. Further coating with pancreatic cancer cell membrane (PCCM) is expected to work as a biomimetic stealth conferring prolonged blood circulation profile, as a source of antigens to drive immune reactivation of PC microenvironment, and as a display of receptors for improved PC targeting through homotypic mechanisms [4].

EXPERIMENTAL METHODS

Soluplus®/VES-GEM micelles were firstly prepared through solvent evaporation method. BxPC3 cell line was used for PCCM extraction, through hypotonic lysis, sonication and differential centrifugation steps. Aliquots of obtained PCCM pellet suspension (200 µL, 0.5 mg/mL) were added to diluted Soluplus®/VES-GEM micelle dispersions (100 µL, 1 mg/mL of Soluplus®) attaining a 1:1 (w/w) ratio. The resulting product was subjected to ultrasonication (3 min, 3 s on /1 s off). A similar approach was carried out to prepare PCCM nanovesicles. Next, the hydrodynamic size, polydispersity index (PDI) and zeta potential (ZP) of Soluplus®/VES-GEM micelles (M), PCCM nanovesicles (PCCM) and PCCM@M were measured by dynamic light scattering (DLS) (Zetasizer Nano ZS, Malvern Instruments, UK).

RESULTS AND DISCUSSION

Results showed PCCM@M had size= 108.8 ± 3.6 nm, ZP= -13.89 ± 1.49 mV an PDI= 0.306 ± 0.034. When compare to Soluplus®/VES-GEM micelles (M), size increased ca. 15 nm which is in accordance to the average width of cell membranes, and ZP decreased from -2.52 ± 0.25 mV (M) to -13.89 ± 1.49 mV (PCCM@M) typical of cell membranes, which suggests a successful coating with the PCCM (**Figure 1**).

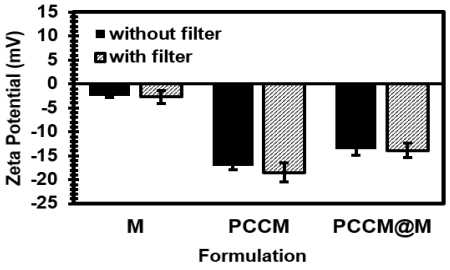

Figure 1. Zeta potential of M, PCCM and PCCM.

CONCLUSION

PCCM-camouflaged Soluplus®/VES-GEM micelles were prepared and characterized regarding size, PDI and ZP. The substantial decrease in ZP of PCCM@M accompanied by slight increase in size suggests successful coating of the micelle core with PCCM.

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ACKNOWLEDGMENTS

The authors would like to thank FCT (grant SFRH/BD/148771/2019) for providing financial support to this project.

A 3D Co-Culture Spheroid Model to Assess the Response of Bone Metastases to Anticancer Drugs

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INTRODUCTION

Prostate and breast cancer are the types of primary cancers that metastasize most frequently to bones. Similar to primary bone cancers, bone metastases are often treated using tumor curettage, which creates a bone defect that requires filling with a suitable graft material. Moreover, cancer cells may still retain in these defects since tumor margins are typically difficult to determine.[1] To prevent recurrence of bone cancer, chemotherapeutics can either be systemically administered or locally delivered from biomaterials carriers that are used to fill bone defects. To date, several bone-regenerative materials with anticancer properties have been developed, but in vitro and in vivo testing of therapeutic efficacy is still performed in relatively simple 2D cell cultures that are far from mimicking physiological bone tumor conditions.[2,3] Early 3D methods composed of cancer cells showed responses more similar to in vivo efficacy[4], and efforts now have started to explore 3D co-cultures.[5] Here, we aim to develop an easy to use, but clinically highly relevant 3D co-culture spheroid model for application as a tool to investigate anti-cancer efficacy of chemotherapeutic drugs and materials.

EXPERIMENTAL METHODS

All cells were cultured in alphaMEM (Gibco) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco). Combinations of primary human bone marrow stromal cells (hBMSC) with either prostate (PC3) or breast cancer cells (MDA-MB-231) were cultured in various platforms (hanging drop with petri dishes and ultra low attachment plates (PHCBI)) to create 3D spheroids. Various seeding densities and media supplements (30 ug/mL collagen type 1A from rat tail (Corning), 0.24 or 1.2 mg/mL methocel (MC, Sigma Aldrich), and a combination of both) were used to optimize 3D spheroid formation with tight cellular connections. After optimization of a protocol for the effective formation of 3D spheroids, different types of 3D spheroids with cell ratio variations were imaged with brightfield and laser scanning confocal microscopy (Carl Zeiss LSM880). hBSMSCs were stained with CellTrace CFSE green (Invitrogen) before mixing, and cancer cells with CellTrace Far Red (Invitrogen). Hemispheres of 3D spheroids were then further characterized with z-stack confocal imaging. Quantitative image analysis was performed to determine the numbers of hBMSC and cancer cells, morphological parameters, and spatial distribution of the cells throughout the 3D spheroids at days 1, 3, and 7.

RESULTS AND DISCUSSION

Alternatively, 3D spheroids were generated using ultra low attachment plates as opposed to the hanging drops from a petri dish, as these were more easily collected from the plates. 3D spheroids of different sizes were generated by varying the initial cell seeding numbers at 2,000, 4,000, and 6,000 cells per 3D spheroid.
With image analysis, the circularity (parameter for stable spheroids) was determined, showing optimal values for 3D spheroids with 2,000 cells per spheroid and 30 ug/mL collagen supplementation. Brightfield images and confocal z-stacks demonstrated that cancer cells quickly outgrew the co-culture 3D spheroid and this effect was most pronounced for 3D spheroids with an initial 1:1 cell ratio. The 9:1 ratio seemed to work the best, as the number of cancer cells remained balanced with the number of hBMSCs over a 7 day culture period (Figure 2).

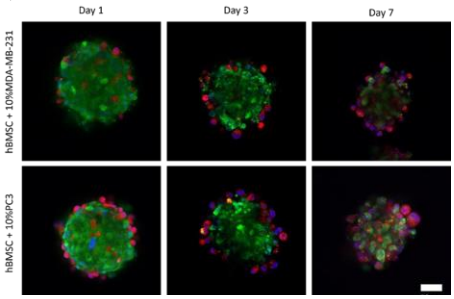

Figure 2 Laser scanning confocal images at 20x magnification of 3D spheroids (2,000 cells per spheroid) of 90% hBMSC (green) with 10% MDA-MB-231(red) or PC3 (red) cells, with stained nuclei (blue) at days 1, 3, and 7 in culture. Scale bar of 50 um.

Furthermore, 3D spheroids showed to decrease in size and form a tight core of predominantly hBMSCs and cancer cells growing peripherally.

CONCLUSION

In conclusion, a 3D spheroid co-culture model consisting out of 2,000 cells per spheroid with 30 ug/mL collagen media supplementation was established, showing generation of stable spheroids for a culture period of up to 7 days. The combination of multiple microscopy techniques demonstrated re-organization of the different cell types within the 3D spheroids. This model paves the way for testing new drugs and biomaterials.

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Polymeric Nanoparticles Targeting Glycans in Gastric Cancer Cells Under Live Flow Conditions

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INTRODUCTION

Gastric cancer (GC) represents the fifth most incident and the third most lethal cancer in the world. Thus, find an effective treatment of GC remains a therapeutic challenge[1]. Recently, nanoparticles (NPs) have been widely used as an innovative tool to specifically deliver drugs into tumor cells, minimizing cytotoxicity and drug's off-target effects in normal cells. To increase the specificity of NPs for the target site, specific ligands, such as glycans, can be attached to the surface of the NPs[2]. Glycan structures are present on the cell's surface. An altered glycan pattern can occur in cells due to different expression of specific enzymes involved in glycosylation and can be associated with the induction of malignant phenotypes in cancer. Sialylated glycans are aberrantly and exclusively expressed in several epithelial cancers, including GC, being important antigen targets for a delivery nanosystem[3]. Thus, our aim was the development of functionalized NPs with an antibody targeting a specifically sialylated glycan and validate the specificity and recognition capacity of this new nanosystem. Usually, the *in vitro* NPs tests are performed under static fluid conditions without any shear stress. However, flow exposure reveals to be an important biomechanical parameter that should be taken into account[4]. Therefore, in this work we evaluated the behavior of the novel NPs under static and flow conditions.

EXPERIMENTAL METHODS

Poly(lactic-co-glycolic)-polyethylene glycol (PLGA-PEG) COOH NPs were produced by nanoprecipitation method and loaded or not with a hydrophobic tyrosine kinase inhibitor (TKI). Then, NPs were surface functionalized by carbodiimide chemistry method with an antibody targeting a sialylated glycan (NP-Gly). As a control, we used unspecific IgG antibody (NP-IgG) The physicochemical properties of NPs were assessed by DLS, ELISA, LC-MS and TEM. To confirm the presence of the antibody on NPs surface we performed an ImunoTEM. The targeting ability and the specificity of these NPs to the cells were assessed under static fluid conditions using flow cytometry and immunofluorescence methods. For under flow experiments, live cell imaging was performed using fluorescence widefield microscopy. We used two GC cell models with different expression levels of the targeted sialylated glycan: MKN45 SIA (positive expression of the sialylated glycan) and MKN45 WT (negative expression of the sialylated glycan).

RESULTS AND DISCUSSION

We showed a successful production of NPs ranging from 140-220nm, with a polydispersity index around 0.2 and a surface charge ranging between -18 and -12mV. Functionalized NPs with an antibody targeting a specific sialylated glycan and IgG1 were developed, and the conjugation efficacy was 85% and 75%, respectively. ImunoTEM demonstrated the successful conjugation of NPs with the monoclonal antibody. Our in vitro assays showed that under static conditions NP-Gly specifically binds to MKN45 SIA and not to the MKN45 WT (Figure1 A and B). Furthermore, the targeting ability and specificity of the NPs is maintained under flow conditions since we observed aggregates of NP-Gly binding only to the MKN45 SIA cell model. The control NP-IgG did not bind to any cell model, reinforcing the specificity of our nanosystem. Finally, we demonstrated the successful encapsulation of TKI, with an association of encapsulating up to 50% of 1mg of TKI and a drug loading capacity was about 2.2%.

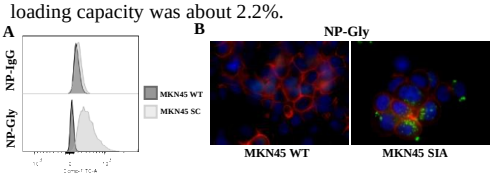

Figure 1: Targeting ability and specificity of NP-Gly under static conditions by Flow cytometry (A) and Immunofluorescence (B).

CONCLUSION

We developed a NP conjugated with an antibody against a glycan that is able to interact specifically with a sialylated glycan-expressing cancer cells under static and flow conditions. This work unveils the importance of using a potential platform for the live tracking of NPs that plays a crucial role in simulating physiological flow and better mimics the *in vivo* environment. Additionally, this developed nanosystem can be also applied in *in vitro* drug studies. The specificity of these novel NPs demonstrates a great potential to be translated to *in vivo* studies of drug delivery.

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ACKNOWLEDGMENTS

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>> 9:00 - 10:30 | ESB SISTERS SOCIETIES - SUSTAINABILITY OF BIOMATERIALS

Chairpersons: Maria Grazia Raucci & Marc Bohner
Location: Room B

9:00 | KL1 Sustainability of biomaterials - Sustainability of Additive Manufacturing Technologies to create Smart Scaffolds for Regenerative Medicine
Lorenzo MORONI, Complex Tissue Regeneration Department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, The Netherlands

9:20 | KL2 Sustainability of biomaterials - Sustainable Biomaterials of bacterial origin and their use in Biomedical Applications
Ipsita ROY, University of Sheffield, Sheffield, United Kingdom

9:40 | KL3 Sustainability of biomaterials - Are bio-based and circular solutions more sustainable? A scientific perspective to assess environmental, economic and social sustainability of bio-circular options.
Massimo PERUCCA, Project HUB-360, Turin, Italy

10:00 | O1 Sustainability of biomaterials - Sustainable highly elastic hydrogels made from naturally-derived materials for biomedical applications
Guy DECANTE, 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Barco GMR, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

10:15 | O2 Sustainability of biomaterials - Physiological polyphosphate, a smart nano-/bio-material for tissue regeneration and a prophylactic drug against SARS-CoV-2 infection
Werner MÜLLER, University Medical Center of the Johannes Gutenberg University Mainz, Germany

Sustainability of Additive Manufacturing Technologies to create Smart Scaffolds for Regenerative Medicine

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INTRODUCTION

Scaffolds for regenerative medicine applications have been historically produced by the adoption of industrial manufacturing technologies, such as textile, moulding, and foaming. With the advent of additive manufacturing (AM), it has been possible to design scaffolds geometry and structure. AM not only enabled the design of architected porous biomaterials with anatomical customization, but also a more efficient production of small-batch implants that can be tailored for patients specific requirements. This has also introduced the production of less waste, thus improving sustainability and cost-effectiveness. Whereas these advantages are common across a large palette of biomaterials, they are even more exacerbated in case of biomaterial composites used to make scaffolds. In this study, we introduce several examples of composite scaffolds fabricated by AM and aimed at skeletal tissue regeneration.

EXPERIMENTAL METHODS

Scaffolds have been fabricated by extrusion-based AM and digital light processing (DLP) technologies. In case of extrusion-based AM, composites have been created by either polymer blending or by a novel printerhead capable of compounding polymers with different inorganic fillers, at the same time changing the composition of the fillers during production, directly during extrusion. Poly(Ɛ-caprolactone), poly(lactic acid), poly(ethylene oxide terephthalate)/poly(butylene terephthalate) and copolymers thereof were used either alone or in combination with nano- and micro-hydroxyapaptite, hydrotalcite, zirconia phosphate, and graphene oxide. The fabrication process was controlled by modulating the applied pressure, temperature, and speed of translation of the printerhead onto the depositing stage. In case of DLP, either photosensible polyurethanes or poly(ethylene glycol) diacrylate were used in combination with magnetic anisotropic nanoparticles to provide scaffolds externally actuatable properties.
Scaffolds were characterized for their mechanical properties, as well as physico-chemical properties by a gamma of characterization techniques. Scaffolds were further studied for their capacity to support or induce skeletal tissue formation after culturing with mesenchymal stem cells. Cell activity was assessed by metabolic and proliferation assays, whereas cell differentiation was assessed by PCR at genetic level and by biochemical assays relevant to determine production of bone or cartilage related extracellular matrix.

RESULTS AND DISCUSSION

Scaffolds with different filler contents, varying both in chemistry and concentration, were successfully fabricated with a modulation of mechanical properties that could range values comparable to different skeletal tissues, from articular cartilage to subchondral bone. Scaffolds were also able to support stem cell differentiation, regenerate both cartilage, and bone tissues, in vitro and in vivo.

CONCLUSION

This study shows how AM technologies could be used to produce in an easy manner a library of scaffolds for regenerative medicine applications, with contained waste and increased cost-effectiveness. These features are even more enhanced in case of biomaterial composites thanks to the use of new printerheads able to compound and change filler concentration on the fly during production.

ACKNOWLEDGMENTS

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Sustainable Biomaterials of bacterial origin and their use in Biomedical Applications

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INTRODUCTION

Currently there is a huge need to find replacements for petrochemical-derived plastics which are not sustainable, degradable and lead to high concentrations of recalcitrant plastics in the soil and in the sea. In the medical arena, currently there is a lot of use of plastics for packaging, implants, tissue engineering and drug delivery. However, there is hardly any attention paid to their sustainability and environmentally friendly aspects. This group of plastics also lead to a huge environmental impact.

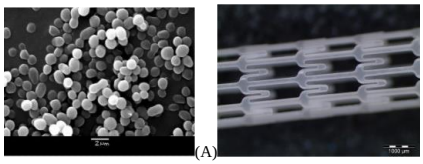
In this work we have focused on the production and use of bacteria-derived sustainable biomaterials for use in biomedical and environmental applications. Two main types of biomaterials have been focused on, including polyhydroxyalkanoates (PHAs)[1] and bacterial cellulose (BC)[2]. PHAs are polyesters produced by a range of bacteria including *Ralstonia eutropha*, *Psuedomonas putida* and *Bacillus subtilis*. These polymers are biodegradable in the soil and in the sea. In addition, they are also resorbable in the human body and are highly biocompatible. BC can also be produced by a range of bacteria including *Gluconobacter xylinus and Sarcinia ventriculi.* BC is also a green polymer, is sustainable and degradable in the soil. It is also highly biocompatible and can be used in biomedical applications.

EXPERIMENTAL METHODS

PHAs were produced using fed batch fermentation of *Pseudomonas putida* and *Bacillus subtilis* to produce medium chain length PHAs (mcl-PHAs) and Poly(3-hydroxybutyrate) respectively. The polymers were purified using Soxhlet extraction and characterized with respect to chemical properties using GC, GC-MS, FTIR and NMR; mechanical properties using tensile testing; thermal properties using DSC and TGA and molecular weight using GPC. The polymers have been used to produce solvent cast, electrospun, 3D moulded and 3D-printed scaffolds which have been used for hard and soft tissue engineering and to produce medical devices. In addition, oil in water emulsion technology was used for microsphere production to be used for controlled drug delivery.

RESULTS AND DISCUSSION

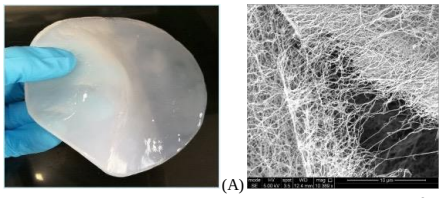
P(3HB), a short chain length PHA (scl-PHA) and a mcl-PHA was produced with high yield, 48.25g/L and 44.8g/L respectively. P(3HB) was used for bone tissue engineering, drug delivery, medical device development such as coronary artery stents, and the mcl-PHA for cardiac, nerve, pancreas, kidney and skin regeneration (Figure 1). For bone tissue engineering P(3HB) was used along with composites of P(3HB) with Bioglass®, hydroxyapatitite and carbon nanotubes. The mcl-PHAs have been used for development of cardiac patches, nerve guidance conduits, wound healing patches, bioartificial pancreas and bioartificial kidney filtration unit.



(A) (B)

Figure 1: (A)P(3HB) based microspheres (B) PHA-based coronary artery stent

Bacterial cellulose was produced under static culture conditions using *Gluconobacter xylinus*. This is a highly nano-fibrillated structure (Figure 2). Bacterial cellulose has been surface modified to create antibacterial bacterial cellulose. We have also used BC as a filler for P(3HB) based composites since BC is one of the stiffest known materials.



(A) (B)

Figure 2: (A) Bacterial cellulose pellet (B) SEM of BC

CONCLUSION

This work confirms the use of sustainable and green polymers, PHAs and Bacterial Cellulose for a range of biomedical applications, a great step forward.

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Are bio-based and circular solutions more sustainable? A scientific perspective to assess environmental, economic and social sustainability of bio-circular options.

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INTRODUCTION

Polyurethanes (PU) foam products have multi-role applications in different industrial sectors like building & construction, automotive, as well as in the biomedical sector such as in blood bags, closures, fittings, oxygenation tubing, cardiac assist pump bladders, catheters, dental cavity liners, reconstructive surgery materials, skin dressing, just to mention few[1]. The broad, intensive and ever-growing diffusion of PU represents a quite relevant market, whose size is expected to grow from $67.27 billion in 2021 to $136.65 billion in 2026 at a CAGR of 15.0%[2]. As the overwhelming majority of commercialised PU are derived from fossil origin, this poses serious concerns on the associated environmental burden. The bio-based solutions along with the circular economy design principles provide interesting options for more sustainable application of PU. But are circular models always more sustainable than linear ones? The ISO standardized Life Cycle Assessment (LCA)[3] is the quantitative methodology needed to provide fair comparisons on environmental impacts associated to linear and to circular functional systems (FS) as proposed in the following specific case study. An alternative strategy to bio-circular model for polymers relevant to biomedical application is to consider bio-degradable polymers which offer a "natural" way to implement circularity by directly interconnecting techno-sphere to ecosphere at both starting and ending points of the technological exploitation. The sustainability of Polylactic Acid (PLA)[4] or cellulose option is also being considered in this work by LCA in a comparative perspective.

EXPERIMENTAL METHODS

The comparative sustainability assessment of circular bio-PU vs. linear fossil-based PU functional systems (FS) through their life cycle has studied by applying LCA methodology according to ISO 14040-44:2006 standards[3]. Bio-PU rigid foams circular model was

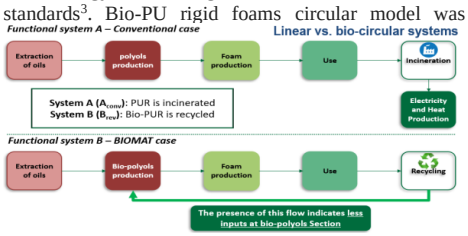

Figure 1: Conventional linear vs. bio-circular FS

assessed according the 'cradle to cradle' analysis, whereas the conventional linear model for fossil-based PU was studied according to the 'cradle to grave' analysis. Recycled polyols from bio-PU source are reintroduced in the circular FS and blended with virgin polyols, whereas incineration was considered for the end-of-life fossil PU foams of the linear FS (see Fig 1), whose energy credits have been taken into account through a FS extension. The functional unit (FU) for both systems considered in the comparative case study is represented by 1kg PU output having comparable thermal conductivity and durability (15 years lifetime). Primary data of the whole circular system from bio-based oil extraction, polyols production, bio-PU foaming and bio-polyol recycling are referred to the BIOMAT project data provided partners. Secondary data were obtained by literature as for the fossil-PU incineration process considering the energy credits as the best-known end-of-life treatment route. The reference DB used was Ecoinvent DB v.3.7, and the impact method CML 2001.

RESULTS AND DISCUSSION

The LCA results were obtained through the mass-energy balance by accounting inputs and outputs through each FS, separately, referred to the FU in terms of the eleven environmental impact categories (see Fig.2). The circular bio-PU offers reduced impacts, this is particular evident for the following categories: climate change (GWP), human and eco-toxicity potentials (HTTP, FAETP, MAETP, TETP) and for the photochemical ozone (POCP). Only ADP-f and POCP performance is lower.

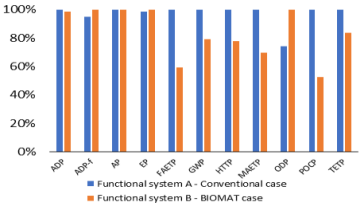

Figure 2 results of the comparative life cycle impact assessment between the linear (conventional) and circular bio-PU systems

CONCLUSION

Sustainability of circularity options has to be assessed on a single case basis by applying quantitative methods. The LCA analysis results of the specific case study shown that, for several impact categories, the benefits of the circular FS deriving from the use of a bio-based raw material source and from the reintroduction of recycled polyols in the loop for a raw material second life largely exceed the ones of the linear system deriving by the exploitation of the material feedstock energy at end-of-life, indicating a good sustainability potential of the BIOMAT proposed circular model.

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ACKNOWLEDGMENTS

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Sustainable highly elastic hydrogels made from naturally-derived materials for biomedical applications

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INTRODUCTION

Lignin is second most abundant biopolymer on Earth, accounting for around 30% of the organic carbon in the biosphere[1]. It is widely used in the papermaking industry which generates 42 to 60 million tons of alkali lignin every year[2]. This byproduct displays biocompatibility, biodegradability, antioxidant, and antimicrobial[3]. Yet, it remains greatly underemployed as around 98% of industrial lignin is currently discarded or burnt[4]. Here we describe the study of an innovative class of sustainable alkali lignin – gelatin hydrogels which revalorizes this resource. These hydrogels display great elasticity, tunable mechanical properties and stability. Their low-cost, ease of production and biocompatibility are rarely seen for highly elastic hydrogels, with great potential for biomedical applications.

EXPERIMENTAL METHODS

Preparation of the gels: alkali lignin (5-10% wt/v) and gelatin (10-20% wt/v, twice the content of alkali lignin) are mixed in water at 70°C and stirred until homogeneous, before being cooled down. Secondary processes such as crosslinking in an epichlorohydrin solution (5% (v/v)) and freeze-drying allows to tune their properties. Degradation study: gel samples were immersed in PBS at 37°C. The evolution of their weight was recorded until complete dissolution. Rheological study: The shear-thinning behavior of the gels was assessed by measuring the evolution of their shear viscosity as a function of the shear rate at controlled temperatures. Compressive mechanical properties study: the Young's modulus of the gels were measured on a uniaxial testing equipment at 20°C. Colloidal titration: the zeta potential of solutions of 0.01% (wt/v) alkali lignin and various gelatin contents (0 to 1% (wt/v)) was measured. Quartz crystal microbalance with energy dissipation (QCM-D): the deposition of material over the sensor during alternating circulation of solutions of gelatin (0.4% (wt/v)) and alkali lignin (0.2% (wt/v)) was measured over time.

RESULTS AND DISCUSSION

Crosslinking and freeze-drying the gels can increase their stability in physiological conditions from a few hours up to being practically unchanged after 90 days. Crosslinking had little impact on the compressive mechanical and rheological properties of the gels, unlike their contents of gelatin. All formulations present shear-thinning behaviors, but higher contents of gelatin increase the temperatures at which the gels exhibit these behaviors: from 32°C to 37°C between gels made with 10% and 20% (wt/v) gelatin. Higher contents of gelatin also increase the stiffness of the gels: doubling the content of gelatin of the gels can multiply their Young's modulus by up to 4.5. The elasticity of the gels was hypothesized to be due to electronic interactions, and were assessed by colloidal titration. The zeta potential of a solution of 0.1% (wt/v) alkali lignin is negative, and adding gelatin shifts this value towards the positive, and 0.08% (wt/v) gelatin is the concentration at which the zeta potential switch to positive values. Additionally, the QCM-D demonstrated that lignin and gelatin would readily bind through weak interactions, but deposited material would be washed away if deionized water was circulated in the system, similarly to what was observed with scaffolds.

CONCLUSION

Altogether, we present here an inexpensive, sustainable and straightforward way to produce an innovative class of highly elastic hydrogels with properties and stability which could easily be modified to fulfil the requirements of the desired application. These gels can be 3D printed or mold-casted to create scaffolds with various shapes, properties, and possible applications. Such hydrogels would allow to revalorize readily available and underutilized materials and have great potential applications for biomedical applications

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ACKNOWLEDGMENTS

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Physiological polyphosphate, a smart nano-/bio-material for tissue regeneration and a prophylactic drug against SARS-CoV-2 infection

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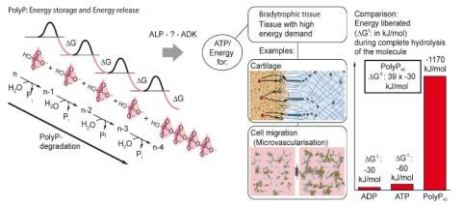
INTRODUCTION

Inorganic polyphosphate (polyP) is one of the oldest chemical energy-providing molecules in biological systems. This polymer, containing a much longer sequence of high-energy phos-phate units than the universal energy donor adenosine triphosphate (ATP), has attracted in-creasing attention for potential biomedical applications because of its diverse metabolic and regulatory functions and its ability to form biologically active nano/microparticles. Very recently, we investigated the effect of polyP in innate immunity on the binding of the recep-tor-binding domain (RBD) of the SARS-CoV-2 spike protein to the cellular ACE2 receptor and disclosed a potential therapeutic benefit of polyP against SARS-CoV-2 infection.



EXPERIMENTAL METHODS

To stabilize polyP against the polyP-degrading alkaline phosphatase, the soluble polymer was encapsulated in silica/polyP nanoparticles.

Together with suitable hydrogel-forming polymers and divalent cations, polyP is used for the fabrication of hybrid biomaterials with defined porosity and mechanical properties; they display in vitro and in vivo morphogenic activity (promoting cell growth, differentiation and migration). A distinct property of polyP is to generate metabolic energy, which is utilized by the extracellular macromolecules to organize the complex extracellular matrix.

RESULTS AND DISCUSSION

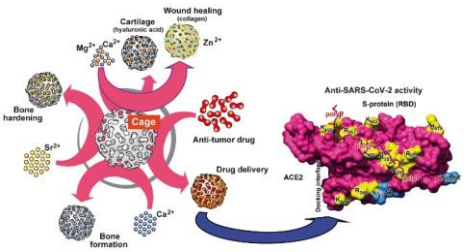
Generator of extracellular metabolic energy: PolyP consists of linear chains of orthophosphate residues, linked by high-energy phosphoanhydride bonds. This energy which is released during the enzymatic cleavage of the phosphoanhydride bonds by the alkaline phosphatase is stored in ADP. Subsequently, ADP is up-phosphorylated to ATP via the ade-nylate kinase. This extracellular formed ATP feeds the kinase and chaperon reactions reac-tions and provides the metabolic energy for the organization of the extracellular fibrillar network.

Anti-SARS-CoV-2 activity: By modeling approaches it became overt that on the surface of the receptor-binding domain at the tip of the viral S-protein a pronounced cationic groove exists that matches with anionic segments, especially present in polyphosphate. Bind-ing studies verified that Na-polyP as well as silica-nanoparticle-associated polyP significant-ly inhibited the interaction of the S-protein with ACE2 at a concentration of 1 µg/mL, close to the level present in blood. PolyP retains its anti-viral activity in a flushing solution, allow-ing an application in both prophylaxis and treatment of SARS-CoV-2 infection in the oro-pharyngeal cavity.

CONCLUSION

With the discovery of polyP and the characterization of the multiple functions of this energy-rich biopolymer, a new physiological molecule has been introduced into the growing group of biomaterials of biomedical interest, which adds a novel principle: metabolic energy-delivery in addition to morphogenetic/regenerative activity. There is no other biomaterial that is provided with this property combination.

In addition, polyP is an anti-SARS-CoV-2 prophylactic which contributes to a strengthening of the human innate immunity system in COVID-19 patients.



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>> 9:00 - 10:30 | SYMP-14 - PUSHING FORWARD BIOPRINTING TECHNOLOGIES FOR IN VITRO MODELS AND TISSUE ENGINEERING APPLICATIONS

Chairpersons: Alessandro Polini & Franscesca Gervaso
Location: Room C

9:00 | KL Bioprinting technologies - Advances in Light-based Bioprinting: Layerwise and Layerless Volumetric Technologies for Organoid Culture and Tissue Engineering
Riccardo LEVATO, Living Matter Engineering and Biofabrication group, Regenerative Medicine Center Utrecht; Department of Clinical Sciences, Faculty of Veterinary Medicine; Department of Orthopaedics, University Medical Center Utrecht, Utrecht, The Netherlands

9:30 | O1 Bioprinting technologies - Development of Advanced Binary Cell-laden Hydrogels Consisting of Guanosine and Guanosine 5-Monophosphate for the 3D bioprinting of scaffolds for soft tissue defects
Maria MERINO-GÓMEZ, Bioengineering Institute of Technology, International University of Catalonia, Sant Cugat del Vallès, Spain

9:45 | O2 Bioprinting technologies - Fabrication of vascularized 3D mammary gland model using hybrid volumetric bioprinting and photoablation
Dominic RUETSCHE, Tissue Engineering + Biofabrication Laboratory, Department of Health Sciences and Technology, ETH Zürich, Zürich, Switzerland

10:00 | O3 Bioprinting technologies - Bone Mimetic Composite Biomaterial-Ink combining Hyaluronan, Collagen and Calcium Phosphate Particles for the Delivery of Chemically Modified RNA for Treatment of Bone Defects
Daphne VAN DER HEIDE, AO Research Institute Davos, Davos, Switzerland; Department of Health Science and Technology, ETH Zürich, Zürich, Switzerland

10:15 | FP01 Bioprinting technologies - Multi-Material Approach For The Replacement Of The Temporomandibular Joint
Joanna BABILOTTE, Complex Tissue Regeneration department, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, the Netherlands

10:20 | FP02 Bioprinting technologies - ECM mimicking hydrogel scaffolds for liver tissue engineering
Nathan CARPENTIER, Polymer Chemistry and Biomaterials group, Ghent University, Ghent, Belgium

10:25 | FP03 Bioprinting technologies - 3D printed, anisotropic, and porous dense collagen hydrogels to model cardiac extracellular matrix
Marie CAMMAN, Sorbonne Université, CNRS, UMR 7574, Laboratoire de Chimie de la Matière Condensée de Paris, Paris, France/Sorbonne Université, Institut de Biologie Paris-Seine (IBPS), CNRS UMR 8256, Inserm ERL U1164, Biological Adaptation and Ageing, Paris, France

Advances in Light-based Bioprinting: Layerwise and Layerless Volumetric Technologies for Organoid Culture and Tissue Engineering

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INTRODUCTION

The function of living tissues is intimately linked to their complex architectures. Advances in biofabrication technologies offer unprecedented opportunities to capture salient features of tissue composition and thus guide the maturation of engineered constructs into mimicking functionalities of native organs. Conventional bioprinting relies on the layer-by-layer deposition of basic building blocks, following the principle of additive manufacturing. Albeit powerful and versatile, this poses relevant limitations on the production of constructs having clinically relevant size, as well as on the generation of free-form and support free overhanging, porous structures, typical of native anatomy. In this lecture, the design of novel layerless, volumetric biofabrication strategies and printable biomaterials to enable the reconstitution of complex 3D structures with precise composition is discussed. Architectures designed to stimulate the native interaction between multiple (stem) cell types and to steer the functionality of self-assembled organoids are introduced, with a particular focus on applications in musculoskeletal regeneration and liver tissue engineering.

EXPERIMENTAL METHODS

Volumetric Bioprinting was performed via a computed tomography-inspired approach. Briefly, visible light laser light was modulated into tomographic projection of the object to be printed with a digital micromirror device. The projections are sequentially casted onto a volume of cell-laden photoresponsive gelatin hydrogels, causing the intended volume of the cell-lade hydrogels to crosslink. For bone and cartilage tissue engineering, bone marrow derived mesenchymal stromal cells and cartilage-derived progenitor cells were printed. For liver tissue engineering, intrahepatic bile duct cells were isolated from human liver biopsies, expanded in a spinner flask bioreactor to grow them as organoids forming cystic structures with a clearly identified inner lumen. Upon harvesting, organoids were suspended in gelatin-based hydrogels, volumetrically printed into different mathematically-defined gyroidal structures, and differentiated into hepatocytic structures. Liver function markers were assessed under static culture and dynamic perfusion.

RESULTS AND DISCUSSION

Large (>4 cm3) cell-laden structures could be printed in <30 seconds. Volumetrically bioprinted cells showed high viability (>80%), comparable to conventional printing techniques. Anatomically-correct, bioprinted trabecular bone models allowed for rapid neo-capillary formation from embedded endothelial cells, while printed meniscus-like constructs embedding chondroprogenitor cells showed the ability to mature over 28 days of culture to acquire compressive properties comparable to native fibrocartilage (compressive modulus ≈ 300 kPa). Moreover volumetric bioprinting allowed the mild processing of delicate liver organoids. While these are partially disrupted by shear stresses in extrusion printing, they preserved high viability and undisturbed organoid size (300 µm) upon tomographic printing. Bioprinted organoids underwent hepatocytic differentiation showing albumin synthesis, liver-specific enzyme activity, and remarkably acquired native-like polarization. Organoids embedded in soft gelatins (<2 kPa compressive modulus) were bioprinted into several mathematically-defined Schwarz lattices, and cultured under perfusion. The architectural profile of these constructs affected their function as metabolic biofactories, with more convoluted designs enhancing the liver-specific ammonia detoxification by organoids.

CONCLUSION

Light-based bioprinting technologies offer a unique potential to generate complex and convoluted geometries in engineered tissues. Overall, the combination of the different strengths of advanced bioprinting technologies opens new opportunities for steering organoid fate and for the biofabrication of large constructs for tissue engineering.

ACKNOWLEDGMENTS

The author would like to acknowledge financial support from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No. 949806, VOLUME-BIO) and from the European's Union's Horizon 2020 research and innovation programme under grant agreement No 964497 (ENLIGHT).

Development of Advanced Binary Cell-laden Hydrogels Consisting of Guanosine and Guanosine 5-Monophosphate for the 3D bioprinting of scaffolds for soft tissue defects

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INTRODUCTION

Patients requiring soft tissue reconstruction often require biomaterials that provide void volumes for subsequent vascularization and new tissue formation. Importantly, due to their 3D structure similar to the native extracellular matrix and their capacity to entrap and sustain living cells, supramolecular hydrogels represent promising candidates. In guanosine (Guo)-based hydrogels, the nucleoside self-assembles into ordered G4-quadruplex structures, ultimately forming nanofibrillar networks by the π-π stacking of G-quartets and the coordination of central K^+ ions.[1] While such hydrogels typically show a short lifetime, the use of boronic acid (BA) significantly enhances their stability.[2,3] With this study, we aimed to produce binary cell-laden hydrogels consisting of Guo and guanosine 5-monophosphate (GMP) stabilized by BA and K^+, and to optimize their bioprintability and the survival of the entrapped cells. Such hydrogels would allow the 3D bioprinting of scaffolds tailored to the respective soft tissue defect, thus greatly improving the outcome of tissue reconstruction.

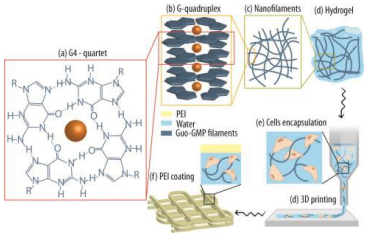

Figure 1. Schematic representation of advanced Guo/GMP cell-laden hydrogels for 3D bioprinting.

EXPERIMENTAL METHODS

Guo (10-120 mM), GMP (10-120 mM), BA (10-60 mM) and KOH (10-60 mM) were mixed at 80 °C, slowly cooled down, and assessed for gelation by tube inversion test. Printability was evaluated by a semi-quantitative filament collapse and fusion test. Best hydrogel formulations were then immersed in a hyperbranched poly(ethylenimine) (PEI) solution and characterized by scanning electron microscopy (SEM) and rheological studies (strain sweep, dynamic step-strain sweep and peak-hold assay). Subsequently, nutrient permeability (FITC-Dextran) and hydrogel stability (immersion in complete medium at 37 °C) were determined. Finally, rat mesenchymal cells (rMCs) were entrapped and studied for 21 days after 3D bioprinting, including cell viability and morphology assessment by confocal laser scanning microscopy (CLSM), and monitoring adipogenic differentiation using Oil-red O solution.

RESULTS AND DISCUSSION

From 49 hydrogel compositions that passed the inversion test, 15 exhibited suitable 3D printing properties. Long-term stability was improved by subsequent treatment with PEI, and the best composition was analyzed by SEM, showing nanofibrillar structures evident of G4-quadruplex formation. Rheological analysis revealed favorable printing and thixotropic properties, while passive diffusion of FITC-dextran molecules (70, 500 and 2000 kDa) into the hydrogel confirmed that the scaffolds may allow for nutrient transport throughout the printed hydrogel. Finally, a cell viability of 85% after 21 days was observed, but with exclusively rounded morphology. However, the presence of lipid droplets after 7 days indicates cell functioning and successful differentiation in adipogenic conditions.

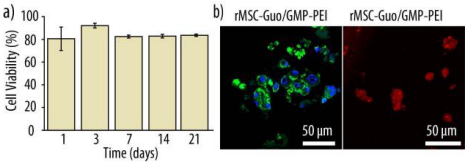

Figure 2. (a) Cell viability, (b) morphology (left), and lipid droplet formation (right) of embedded rMSCs

CONCLUSION

Our 3D bioprinted binary Guo/GMP hydrogels exhibited extensive nanofibrillar networks, showed good printability and favorable thixotropic properties. PEI coating enabled hydrogel stability for ≥21 days in medium, and the embedded rMSCs showed good cell survival (>80%) despite rounded morphology. However, formation of lipid droplets under adipogenic conditions confirmed successful differentiation and functionality of the entrapped cells. Finally, due to their demonstrated 3D bioprintability, our advanced Guo/GMP hydrogels hold great potential for the reconstruction of soft tissue defects.

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ACKNOWLEDGMENTS

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Fabrication of vascularized 3D mammary gland model using hybrid volumetric bioprinting and photoablation

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INTRODUCTION

Volumetric bioprinting (VBP) is a novel biofabrication method that promises the generation of complex living tissue constructs in a fraction of the time required using conventional methods, while at the same time allowing for higher resolution.[1,2] VBP achieves negative feature sizes ranging from several mm down to almost 100 µm for biocompatible gelatin-based photoresponsive polymers.[3] Smaller-sized features in cell-laden constructs have proven to be impossible to print in a reproducible manner due to light scattering. To overcome this limitation, we developed a hybrid approach that aims at combining VBP with 2-photon ablation (2PA) to generate a vascularized and perfusable mammary gland model, mimicking the complex alveolar architecture found in the human breast. Our multivascular model comprises both, an interconnected arterial and venous circulatory system, as well as mammary glands, which are connected to a mammary duct system and enveloped by microcapillaries (Fig. 1A).

EXPERIMENTAL METHODS

Model Design: 3D models with hollow channels with different lumen diameters and of varying complexity were generated using Solidworks and converted into .stl files. *VBP-printing*: A fully biocompatible resin (gelatin-norbornene, gelatin-thiol) was used in the fabrication of the models. In particular, models (Fig 1B) were printed in the following dimensions: 20mm x 8mm x 5mm (L x W x H) using the tomographic mode of the Tomolite (Readily 3D). An average light dose of 140mJ/cm^2 was used to induce polymerization (0.05% (w/v) LAP as photoinitiator). Models were post-cured using 405nm excitation wavelength. *2PA photoablation:* A 3D model comprising both a mammary gland and enveloping microvasculature was ablated and connected to arterial, venous and mammary gland ducts using 2PA (SP8 multiphoton microscope, Leica). *Cell experiments:* Empty capillary channels were seeded with endothelial cells (HUVEC, HUAEC) and perfused using a custom microfluidics setup.[4] Similarly, the mammary gland and duct were seeded with epithelial cells and perfused (peristaltic 4-channel pump; 5 µl/min).

RESULTS AND DISCUSSION

We exploited the rapid thiol-ene crosslinking chemistry of gelatin-norbornene and gelatin-thiol to produce complex hollow channel architectures based on VBP. This allowed the fabrication of branched and entangled multivascular networks with excellent resolution and features size (Fig. 1B).[4] Printing is rapid (7-10s) and independent from the complexity of the model[2]. This process allows fabrication of mesoscale vasculature models exhibiting lumen diameters in the range of 200-800 µm which were fully endothelialized within 2-3 days of cell seeding (Fig. 1C). Alveoli with a continuous surrounding microvasculature were ablated with 2PA such that microcapillaries could connect to both arterial and venous ducts. Recent research showed that ablated void spaces are readily invaded by HUVECs.[5]

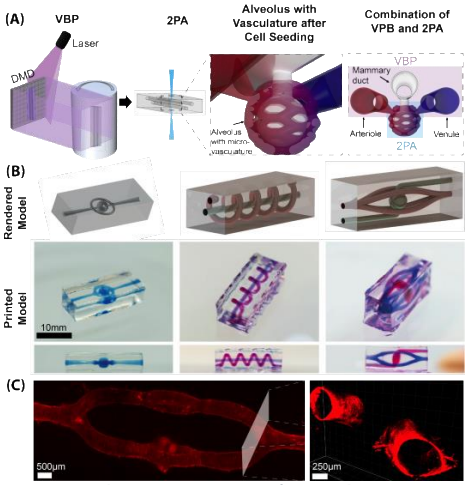

*Figure 1:**A.** Schematic representation of VBP and 2PA process to fabricate a mammary gland supported by hierarchical vasculature **B-C.** Printed models are perfusable and support endothelialization.*

CONCLUSION

This work demonstrated the combination of VBP with 2PA to generate multiscale vascular models containing meso- to microscale vessels. The capillary system was fabricated in parallel with a mammary gland model and the two systems successfully integrated. This *in vitro* model system could be used to study mammary gland pathologies like breast cancer and mastitis or bacterial and viral infections.

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ACKNOWLEDGMENTS

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Bone Mimetic Composite Biomaterial-Ink combining Hyaluronan, Collagen and Calcium Phosphate Particles for the Delivery of Chemically Modified RNA for Treatment of Bone Defects

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INTRODUCTION

In cases of large bone defects due to trauma, tumor resection, skeletal abnormalities and infections, the self-healing capacity of bone is insufficient, representing an enormous clinical challenge. This study aims to develop a 3D-printable composite biomaterial-ink to fabricate patient-specific bone graft substitutes for bone regeneration, that provide control over shape, architecture, and composition. Inspired by the natural composition of bone, the biomaterial-ink combines osteoinductive calcium phosphate particles (CaP) in a tyramine modified hyaluronic acid-Collagen type I (THA-Col) matrix for the delivery of chemically modified RNAs (cmRNAs) to induce neurogenesis, angiogenesis, and osteogenesis for bone regeneration (**Fig. 1**).

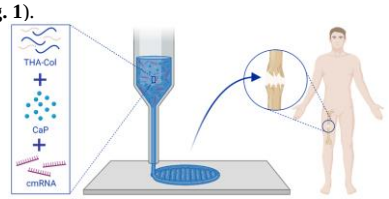

Fig. 1: Composite biomaterial-ink composed of THA-Col organic matrix, CaP, and cmRNA for bone regeneration.

EXPERIMENTAL METHODS

A range of 0-30% w/v CaP with size 45-63 or 45-106 µm were incorporated in 35 mg/mL THA or 35 mg/mL THA and 5 mg/mL rat tail Col mixed 1:1, with 0.5 U/mL horseradish peroxidase (HRP) and 0.01% w/v Eosin Y. For pre-crosslinking, 0.085 mM H_2O_2 and 6 mM NaOH was added to form a viscoelastic gel with shear thinning properties for 3D-printing. After the extrusion of the desired structure, scaffolds were cured by light crosslinking for 30 minutes (505 nm). THA was heat sterilized at 121°C for 20 minutes, and influence on gelation was investigated. Different vectors for the delivery of cmRNAs (1% v/v) were mixed into the pre-polymer solution, where impact on gelation and distribution of vectors within the ink was then assessed. Composite formulations were further characterized for printability (continuous strut, line with spacing, lattice, and an overhanging strut on a pillar structure), cohesion, swelling, degradability (in 100 U/mL hyaluronidase), and compression modulus. Additionally, formulations were evaluated *in vitro* using a metabolic activity assay after 1, 3, 7, and 14 days and are being assessed for *in vitro* osteogenic potential (viability, mineralization, alkaline phosphatase (ALP) production, gene expression, and protein production) using human mesenchymal stem cells (hMSCs).

RESULTS AND DISCUSSION

THA and THA-Col enzymatic gelation showed a 3.5-fold and 3.4-fold increase in G' and light crosslinking resulted in a 24-fold and 19-fold increase in G', respectively (**Fig. 2A**). All formulations showed viscoelastic and shear-thinning properties, with the formation of a continuous strut, good shape retention and without waviness (**Fig. 2B**). Sterilization of THA and addition of cmRNA vectors did not influence the gelation mechanism and the viscoelastic behaviour was preserved. All formulations were fully swollen after 24 hours. Compressive modulus was increasing while degradation rate was decreasing with increasing CaP content. Additionally, formulations of THA-Col showed both higher metabolic activity and ALP content after 14 days of osteogenic differentiation, compared to THA alone (**Fig. 2C and D**).

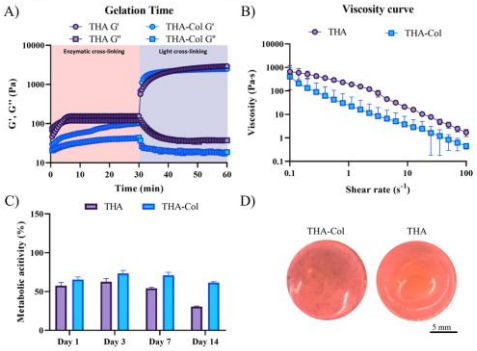

Fig. 2: A) Gelation time of THA and THA-Col showing enzymatic crosslinking (0-30 min) and light crosslinking (30-60 min), n=3. B) Viscosity carve of THA and THA-Col, n=3. C) Metabolic activity hMSCs on THA and THA-Col, n=2. D) ALP staining (blue) after 14 days of hMSCs osteogenic differentiation, n=1.

CONCLUSION

To summarize, a 3D-printable composite biomaterial-ink of THA-Col with CaP was biofabricated, suitable for the delivery of cmRNAs/vectors, holding significant potential as bone graft substitute for bone regeneration.

ACKNOWLEDGMENTS

Prof. Marcy Zenobi-Wong for valuable input. For financial support: EU Commission H2020 grant No. 874790 and 857287 and the AO Foundation.

Multi-Material Approach For The Replacement Of The Temporomandibular Joint

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[+]Joanna Babilotte and Monize Caiado Decarli contributed equally to this work

INTRODUCTION

Temporomandibular joint (TMJ) disorders impair masticatory function and speaking, reducing patients' quality of life. Even though synthetic TMJ implants are commercially available, only a limited range of sizes can be found, thus not often meeting patient needs. The use of a patient-specific TMJ implant would overcome this limitation[1]. 3D printing technologies are the most appropriate fabrication techniques to provide a high-precision scaffold with a patient-specific design, in a controlled and reproducible way. Due to the complex anatomical structure of TMJ, it is clear that multi-material approaches, that combine different manufacturing techniques, can be very promising.
Here we aimed at developing an innovative scaffold that will meet the biological and mechanical criteria to replace TMJ. The objective of this work was to evaluate one specific combination of a Xantham-gum (XG) hydrogel reinforced with fibrous scaffolds made with polycaprolactone (PCL) or copolymers of polycaprolactone and polylactic acid (PCL-LA).

EXPERIMENTAL METHODS

Fibrous scaffolds were produced by melt-electrowriting (MEW). Polycaprolactone (PCL) and a copolymer of polycaprolactone-polylactic acid (PCL-LA, 30:70 wt%) were used to fabricate ten layers of fibrous scaffolds. PCL scaffolds were manufactured at 105˚C, varying speeds from 10-50 mm/s, 0.3 bar, 5 kV and 4mm height. PCL-LA scaffolds were manufactured at 170˚C, varying speeds from 10-50 mm/s, 0.8 bar, 6 kV and 4 mm height. Multi-layered constructs of XG hydrogel were 3D printed, varying speed from 40-60 mm/s and pressure from 50-70 KPa using smooth flow tapered tip. XG hydrogels were studied with and without stabilization using ionic crosslinking.

The morphology of both produced scaffolds was observed through optical and scanning electron microscopes regarding fibers/filaments size and porosity. The different scaffolds were manually assembled before the crosslinking step. Shear stress tests evaluated the improvement of mechanical properties. The stability of the constructs in culture media for 28 days was also analyzed. Finally, the cell survival and proliferation on both scaffolds were evaluated with human mesenchymal stem cells (hMSCs).

RESULTS AND DISCUSSION

Given the complexity of the TMJ, there is not just one material that could be considered an ideal to replace and restore the function. PCL and PLA are both biocompatible and bioresorbable polymers with great interest in tissue engineering[2].
Regular and well-defined PCL and PCL-LA meshes were obtained using MEW. The range of fibers size obtained was 30-50 µm and the interfiber space was 0.5-1 mm, depending of the parameters used. Increasing the printing speed generally led to reducing the size of the fibers. The interfiber spacing (IFS) also influenced pattern fidelity, reducing the IFS resulted in the misalignment of the fibers.
Multi-layered XG constructs with high shape fidelity were also obtained through 3D printing. The pore size was 3±0.5mm and the line width was 1±0.2mm. The ionically crosslinked XG constructs were stable in cell culture media over 28 days, and porosity was maintained. Both materials were functionalized by photocurable methacrylated (MA) groups to ensure a strong binding, avoid delamination and improve mechanical properties.
Alone, usually hydrogel shows too weak mechanical properties for our application. The XG hydrogel will be reinforced with a fibrous scaffold to improve the mechanical properties. It is expected to observe changes depending on the fibrous scaffold structure (pattern and number of layers).
Finally, hMSCs showed good cell viability and survival overtime on the hydrogel and fibrous scaffolds independently. Further investigation on cell differentiation will be performed to explore the benefits of our multimaterial.

CONCLUSION

Overall, based on the hybridization between both processing techniques, employing a multi-material approach, as well as including a double crosslinking strategy, we show a promising approach for interfacial tissue regeneration with improved mechanical properties.

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ACKNOWLEDGMENTS

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ECM mimicking hydrogel scaffolds for liver tissue engineering

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INTRODUCTION

Annually, millions of people die because of liver failure, while the waiting duration for a donor liver is around 12 months.[1] Herein, we target hybrid 3D-printed scaffolds to serve liver tissue engineering applications.
As starting materials gelatin in combination with a polysaccharide was used to develop printable hydrogels. As polysaccharides dextran (Dex) and chondroitin sulphate (CS) were selected as mimics for the liver extracellular matrix (ECM) to explore their effect on the cell response. Methacrylated gelatin (GelMA) served as benchmark. The hydrogel materials were characterized on 2D-as well as on 3D-level.

EXPERIMENTAL METHODS

Development of Methacrylated gelatin

Methacrylated gelatin (GelMA) was developed by a protocol described before by Van Den Bulcke *et al.*[2] GelMA was subsequent crosslinked using UV-A light in the presence of Li-TPOL as a photo-initiator.

Development of DexNB-GelSH

Thiolated gelatin (GelSH) was developed by a protocol described before by Van Vlierberghe *et al.*[3]
Norbornene-modified (NB) dextran was developed by coupling the hydroxyl groups of dextran with the carboxylic acid groups of 5-Norbornene-2-carboxylic acid using DMAP and DCC. GelSH and DexNB were subsequent crosslinked under the same conditions as GelMA.

Development of CSNB-GelSH

NB modified CS was developed by coupling of the hydroxyl groups of CS with NB carboxylic acid using DMAP and Boc_2O as coupling reagents.
GelSH and CSNB were subsequent crosslinked in the same way as the other two materials.

3D-scaffold development

The different hydrogel materials were processed into hydrogel scaffolds using an indirect printing technique using PLA scaffolds as a sacrificial mold for the hydrogels.

Characterization

The compressive modulus of the whole scaffold was assessed using compression tests, the microscale stiffness was assessed using atomic force microscopy (AFM). The biocompatibility was assessed using an MTS proliferation assay, live-dead staining and a albumin quantification assay.

RESULTS AND DISCUSSION

On a 2D-level, DexNB-GelSH and CSNB-GelSH were superior over GelMA as they mimicked natural liver tissue (NLT) to a greater extent with respect to swelling and mechanical properties. The swelling ratio of GelMA, DexNB-GelSH and CSNB-GelSH were respectively 9.1±0.5 and 9.6±0.5 and 8.7±0.2 which is in line with the swelling of NLT (i.e.10).[4]

AFM measurements revealed superior microscale mechanical properties of the DexNB-GelSH hydrogel compared to the other materials. DexNB-GelSH exhibited a stiffness of (196±24)kPa, CSNB-GelSH of (106±2)kPa and GelMA of (291±11)kPa. NLT exhibits a stiffness of (183±48)kPa. The higher the stiffness, the more the material mimics the ECM of a cirrhotic liver ((411± 63)kPa)[5].

On a 3D-level, DexNB-GelSH scaffolds exhibited a compressive modulus of (4.8±1.6)kPa which is in excellent agreement with that of NLT (i.e. 1–5kPa)[4] as compared to GelMA which resulted in a modulus of (8.5±1.9)kPa and CSNB-GelSH (12.6±1.9)kPa.
So far, the biocompatibility of DexNB-GelSH was assessed and comparable to GelMA. The live-dead staining showed that the cells grew more into clusters on the DexNB-GelSH scaffolds compared to the more spread morphology which the cells exhibited on the GelMA material.

CONCLUSION

DexNB-GelSH and CSNB-GelSH scaffolds are promising hybrid materials to support LTE as they exhibit similar physico-chemical properties compared to NLT, while cell viability and proliferation of the hepatocytes were preserved.
In future research, different cell types such as primary hepatocytes and organoids will be included in the biological evaluation. Furthermore decellularized liver ECM will be incorporated into the hydrogel material in order to improve the cell interactivity and proliferation.

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3D printed anisotropic and porous dense collagen hydrogels to model cardiac extracellular matrix

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INTRODUCTION

Despite the crucial role of the cardiac extracellular matrix in the organotypic organization and conduction of contraction, most 3D heart models[1] do not mimic its specific characteristics, namely its biochemical composition, stiffness, anisotropy and porosity[2]. Recent approaches of 3D heart models used non porous hydrogels fabricated from low concentrated collagen to encapsulate cardiac cells. Hence, the *in vivo* properties are not reproduced. Here, we developed a 3D printed collagen hydrogel that mimic the cardiac extracellular matrix, *i.e* collagen anisotropy, adequate stiffness and two ranges of porosity (one to ensure nutrients and oxygen diffusion and the other for cell cultivation).

EXPERIMENTAL METHODS

Dense collagen solutions (30 $mg.ml^{-1}$) were printed through the 23G flat bottom needle inside a buffer bath. The extrusion process aligned the collagen molecules along the axis of extrusion. The buffer bath played two major roles: it "froze" the collagen alignment and triggered collagen gelling. The printing process was performed unidirectionally for each layer to create an intrinsic porosity between the different collagen filaments. After a rapid period of collagen gelling, needles were introduced within the hydrogel to generate large pores. An additional gelling period was performed to tune the mechanical properties. Cardiac spheroids, made with 85% of cardiomyocytes derived from induced pluripotent stem cells and 15% of ventricular fibroblasts, were then seeded within the printed hydrogels to evaluate spheroid fusion, contraction rate and assess their maturation.

RESULTS AND DISCUSSION

By tuning the extrusion speed and the gelling process, a 3D printed hydrogel with aligned collagen fibers was obtained. A combination of two gelling strategies (24h PBS 5X + 24h NH_3) was optimal to obtain both anisotropy and adequate mechanical properties (E=10 kPa). Scaffold anisotropy was obtained at two different scales: all filaments were printed in the same direction (macroscopic) and collagen fibers were aligned inside printed filaments (microscopic). Concerning the porosity, changing the height between two successive layers allowed to create an intrinsic porosity of 100 µm while preserving the scaffold cohesiveness. This porosity is suitable for nutrients and oxygen diffusion in the whole scaffold, thereby favoring cell viability. Larger pores created by needles molding generated straight channels of 600 µm in diameter. These were colonized by cardiac spheroids mixed with Matrigel® to create a suitable 3D environment. After 15 days in culture, cells changed in shape and contraction revealed an adaptation to this new environment

CONCLUSION

In this study, we developed a 3D printing technique to create a biomimetic cardiac extracellular matrix suitable for spheroids cultivation. Our approach focused on the extracellular matrix and its key parameters since it is deeply involved in cardiac functions and in several diseases.

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ACKNOWLEDGMENTS

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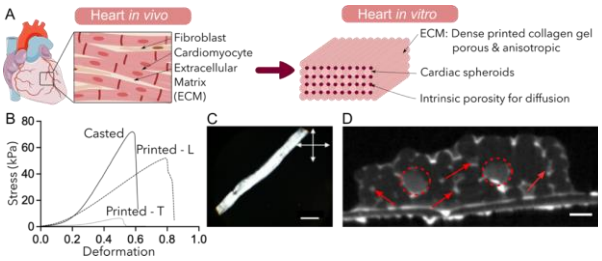

Figure 1: A- Heart in vivo and its model in vitro. B- Fiber alignment and its impact on mechanical properties (comparison between casted and printed gels either stretched in the fiber orientation (L) or perpendicular to the fiber orientation (T). C- Printed collagen observed with Polarized Light Microscopy (Scale bar 500 µm). D- MicroCT of a collagen gel with the two ranges of porosity(circles : large channels,arrows: intrinsic porosity -Scale bar 500 µm)

>> 9:00 - 10:30 | SYMP-15 - DYNAMIC MATERIALS FOR BIOENGINEERING AND DIGITAL SURGERY

Chairpersons: Matthew B. Baker & Sandra Van Vlierberghe
Location: Room H

9:00 | KL Dynamic materials - Dynamic covalent hydrogels for advanced biomaterials design
Mark W. TIBBITT, Macromolecular Engineering Laboratory, Department of Mechanical and Process Engineering, ETH Zurich, Zurich, Switzerland

9:30 | O1 Dynamic materials - Patient-derived lipid-based magnetic nanovectors: a step forward towards personalized nanomedicine
Carlotta PUCCI, Smart Bio-Interfaces, Istituto Italiano di Tecnologia, Pontedera (Pisa), Italy

9:45 | O2 Dynamic materials - Molecular constants of reversible Schiff base formation: How to design dynamic hydrogels from the bottom up
Francis MORGAN, Department of Complex Tissue Regeneration, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

10:00 | O3 Dynamic materials - Design of thermo-responsive polypeptide bioconjugates for the treatment of glioblastoma by PDT/RDT
Leslie E. DUBRANA, Laboratoire de Chimie des Polymères Organique, (LCPO), UMR 5629, Université de Bordeaux, CNRS, Bordeaux INP, Pessac, France

10:15 | O4 Dynamic materials - Mixed Reality for Surgery: A New Tool For Medical Devices
Marc PIUZZI, Lynx Medical, Paris, France

Dynamic covalent hydrogels for advanced biomaterials design

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INTRODUCTION

Dynamic covalent chemistry has emerged as an elegant molecular design strategy to engineer functional polymer networks that combine advantageous properties of both chemically and physically cross-linked materials. Dynamic covalent hydrogels are increasingly used in advanced materials design with applications ranging from recyclable thermosets to self-healing hydrogels. Despite the significant progress in the fabrication and characterization of these materials, the relationship between the underlying chemistry at the junctions of dynamic covalent hydrogels and their macroscopic properties is still not fully understood.[1] In this work we will elaborate a framework to understand how emergent properties of these materials relate to the dynamic chemistry at the junction.[2] One application of reversible hydrogels is for the encapsulation and release of complex biomolecules. We extend our structure–function–dynamics understanding in these materials to predict release behavior from dynamic covalent hydrogels.[3] Finally, we demonstrate how these materials can be applied for the thermal stabilization of biologics, including protein-based and viral vaccines (*Fig. 1A*).[4]

EXPERIMENTAL METHODS

The reversible boronic-ester-based hydrogels were formed from 4-arm poly(ethylene glycol) (PEG) macromers ($M_n \approx 10{,}000$ g mol^{-1}) end-functionalized with a phenylboronic acid derivative or a *cis*-1,2-diol containing moiety.[2] Biologic release from boronic-ester-based dynamic covalent hydrogels was modulated by the addition of sugars, as competitive displacement triggers network dissolution and cargo release (*Fig. 1B*).[3]

RESULTS AND DISCUSSION

We synthesized a range of functional biomaterials based on dynamic covalent boronic ester cross-links. We characterized the small molecular binding properties using spectroscopic and computational approaches and related these to the macroscale rheology.[2] This allowed us to develop a predictive model to understand biomolecule release from this class of materials.[3] We then applied this understanding to develop materials for the thermal stabilization of temperature sensitive biologics, including vaccines.[4]

We thermally stabilized a wide range of biologics in reversible hydrogels, starting with model enzymes (β-galactosidase, leucine aminopeptidase, and alkaline phosphatase). We attributed protection to the formation of polymer networks around the biologics, which restricted their mobility and physically restrained them from interacting with each other, reducing aggregation and activity loss after heat exposure. In addition, we showed that the gels protected extremely heat sensitive therapeutic enzymes, such as *E. coli* DNA gyrase and human topoisomerase I. Both enzymes could be stored within the gels for months at room temperature without significant activity loss, vastly outperforming the unencapsulated (free) enzymes (*Fig. 1C*). Finally, we stabilized vaccines from thermal stress, including a protein-based vaccine, recombinant influenza A H5N1 hemagglutinin, and a live virus, adenovirus Type 5 (Ad 5; *Fig. 1D*). Gel-encapsulated Ad5 outperformed free Ad5 at all tested temperatures (4 to 65 °C) and retained close to 100 % activity after 4 weeks at 27 °C.

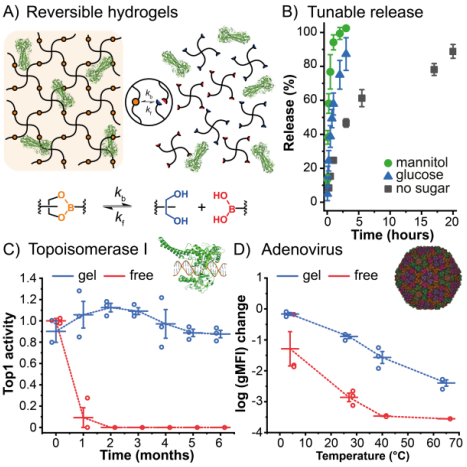

Fig. 1: (A) Thermal stabilization of biologics using reversible boronic ester hydrogels. (B) Tunable release of stabilized cargo via triggered dissolution using sugars. Protection against thermal stress was demonstrated for (C) human topoisomerase I and (D) adenovirus type 5.

CONCLUSION

Structure–function–dynamics relationships enable rational engineering of biomaterials assembled from reversible bonding mechanisms, including boronic esters. This includes the development of materials for the encapsulation and on-demand release of biologics. Our dynamic hydrogel platform offers a simple material solution to mitigate the costs and risks associated with a continuous cold chain for biologic transport and storage.

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Patient-derived lipid-based magnetic nanovectors: a step forward towards personalized nanomedicine

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INTRODUCTION

Glioblastoma multiforme (GBM) is the most lethal brain tumor, with a median survival rate of 12-15 months and a 5-year survival of ≈ 5%. Due to its invasive nature and genetic heterogeneity, GBM is very difficult to treat. Moreover, most of the drugs cannot cross the blood-brain barrier, and are not selective towards cancer cells.[1,2]
In this work, we propose new patient-derived lipid-based magnetic nanovectors that combine the action of a chemotherapeutic drug and magnetic hyperthermia. The nanovectors are made of a lipid core loaded with the drug regorafenib and superparamagnetic iron oxide nanoparticles (SPIONs), and coated with plasma membrane extracts from GBM patients' cells, derived from GBM surgical samples, to obtain cell-specific lipid magnetic nanovectors (CDMNVs). This guarantees targeting towards GBM cells thanks to homotypic adhesions, an inherent feature that cancer cells exploit to recognize each other.[3]

EXPERIMENTAL METHODS

The lipid-based magnetic core was synthetized by hot ultrasonication, and then coated with GBM cell membrane extracts (CM). The nanovectors were studied by dynamic light scattering (DLS), transmission electron microscopy (TEM), Fourier-transform infra-red (FTIR), X-ray photoelectron spectroscopy (XPS), and bicinchoninic acid (BCA) assay. Drug loading and release in different conditions was measured by high performance liquid chromatography (HPLC). The SAR of CDMNVs was measured during alternated magnetic field stimulation, AMF, (frequency = 97.55 kHz; field strength = 20 mT), and compared to bare SPIONs and uncoated lipid-based magnetic core (MNVs).

RESULTS AND DISCUSSION

CDMNVs displayed a homogeneous size distribution, with an average hydrodynamic diameter and polydispersity index (PdI) of 288 ± 1 nm and 0.17 ± 0.01, respectively, and a negative ζ-potential (-23.6 ± 0.4 mV) that guarantees electrostatic stability. MNVs showed similar size, PdI and ζ-potential, suggesting that the coating does not affect their morphology and stability. TEM images of CDMNVs highlighted their spherical, blackberry-like structure, with SPIONs encapsulated in the lipid matrix (Figure 1). SPIONs account for the 11 wt % of the total weight of CDMNVs. The presence of proteins on the cell membrane extract after CDMNVs coating was evaluated to be the 2.7 wt % of the total nanoparticles mass. The presence of the cell membrane coating was characterized by FTIR spectroscopy. In particular, the occurrence of the typical contributions of amide bonds at ≈ 1650 cm^{-1} (C=O stretching vibrations) and ≈ 1540 cm^{-1} (N-H bending and C-N stretching) in CDMNVs confirm the successful coating. CDMNVs coating was further investigated with XPS. The C signal showed a main component at 285 eV due to C-C- and C-H bonds, a component at ≈ 286.5 eV assigned to C-OH or C-O-C bonds, and a peak at ≈ 288 eV that due to COO- groups. The N signal presented two main contributions: a peak at 402.8 eV due to the lipid core, and an intense contribution at 400.1 eV, typical for amines and amides, confirming the presence of a thin coating of ≈ 10 nm or less. Upon AMF stimulation, both MNVs and CDMNVs were able to increase the temperature of the solution of ≈ 7°C in 30 minutes. MNVs showed a specific absorption rate (SAR) of 16.9 W/g, while CDMNVs showed a SAR of 14 W/g. These values are in good agreement with those found for bare SPIONs (SAR = 18.1 W/g, measured in hexane). Therefore, the coating procedure did not significantly affect the magnetic properties of the SPIONs. CMNVs were then formulated to encapsulate the drug regorafenib, with a drug loading of 1.1 wt %. The drug release was measured in different conditions, with or without the AMF application, and it seems to follow a "burst release + diffusion profile".

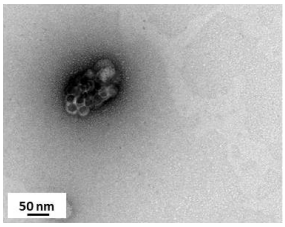

Figure 1. TEM image of CDMNVs

CONCLUSION

CDMNV formulation method led to the formation of homogeneous and well-dispersed nanoparticles, successfully coated with GBM patient-derived cell membrane extracts. The retention of important proteins involved in homotypic adhesion is important to guarantee patient-specific antitumor effects. CDMNVs were able to increase the temperature of the medium of ≈ 7°C, with a SAR of 14 W/g, comparable with that of free SPIONs. These results suggest that CDMNVs can be used as a patient-personalized tool to exploit a synergic action between drugs and magnetic hyperthermia, leading to a more efficient GBM eradication.

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Molecular constants of reversible Schiff base formation: How to design dynamic hydrogels from the bottom up

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INTRODUCTION

The native extracellular matrix (ECM) possesses complex viscoelastic mechanical properties. Changes in these dynamic mechanical properties can control cell fate, yet few synthetic strategies exist to mimic and control these complex properties.[1] There is a clear need in tissue engineering for hydrogel systems with robust control over static (stiffness), dynamic (relaxation), and process-related (printability) mechanical properties. Dynamic covalent chemistry (DCvC) is a promising tool to meet these needs, providing the time-dependent viscoelasticity that covalent crosslinks lack.

We have previously shown how leveraging the molecular constants (K_{eq}, k_1, k_{-1}) of two reversible crosslinking reactions (hydrazone and oxime) allows tuning of the mechanical properties of hydrogels based on Schiff base crosslinkers with oxidized alginate.[2]

Building upon this work, we next set out to investigate the effect of how different polymer backbones present the aldehyde group, as well as to expand the variety of Schiff base crosslinkers studied. We aim to elucidate two key relationships: 1) How does the nature of the aldehyde (natural vs synthetic polymer & presence/absence of an aliphatic spacer) effect the molecular constants for the Schiff base formation? 2) How does slight chemical variation in the amine partner change the molecular constants for Schiff base formation? We can then use these relationships to control the mechanical properties of resulting hydrogels, leading to a robust strategy for the bottom-up design of dynamic hydrogels to mimic native biomechanical niches.

EXPERIMENTAL METHODS

Oxidized alginate (**1**) was prepared by the periodate oxidation of sodium alginate. Alginate containing pendant aldehyde groups (**2**) was prepared using DMTMM and 3,3-diethoxypropan-1-amine, followed by deprotection under acidic conditions. Poly(3-sulfopropyl methacrylate *-co-* N-(3,3-diethoxypropyl)methacrylamide) (**3**) was synthesized via RAFT polymerization of the corresponding monomers followed by deprotection under acidic conditions. Polymer backbones were characterized by NMR and GPC. Various amines able to form Schiff bases were ordered or synthesized as necessary. Molecular constants were extracted from measured UV visible and NMR data by fitting to a kinetic model. Hydrogels were characterized with rheometry and tested for processability on a CELLINK BioX printer. Cytocompatibility was assessed by culturing fibroblasts on each material-Schiff base combination.

RESULTS AND DISCUSSION

In this study, we compare the equilibria and rate constants for the Schiff base formation between various amines and an aliphatic aldehyde in one of three forms (**1-3**). The aldehydes in oxidized alginate exist primarily in hydrated/(hemi-)acetal forms, which significantly reduces their reactivity. Unsurprisingly, associated K_{eq}s are up to three orders of magnitude smaller than corresponding reactions with a pendant aldehyde. This increase is attributed to the C-3 spacer present in both the covalently modified alginate and the copolymer that leads to an increase in free aldehyde concentration as shown by NMR.

Similarly, slight chemical variation of the amine partner leads to K_{eq}s spanning up to three orders or magnitude. Combined, different combinations of amine and aldehyde provide coverage of at least 6 orders of magnitude. We are now exploring how these large molecular differences influence macroscopic hydrogel properties. Indeed, hydrazone and oxime crosslinkers paired with oxidized alginate possess both shear moduli and stress relaxation times spanning 2 decades each, are cytocompatible and can target relevant biomechanical regimes to instruct cellular morphology and spreading.

CONCLUSION

Here we show that molecular variations in aldehyde/imine bonds results in K_{eq}s spanning up to six orders of magnitude. NMR indicates that an aliphatic spacer enables a higher concentration of free aldehydes (and increased reactivity) compared to oxidized alginate. Differences in molecular constants including K_{eq}s enable specific targeting of mechanical regimes. These results show a versatile structure-reactivity-property approach to bottom-up hydrogel engineering. This facile approach highlights reversible Schiff bases as a platform for the design of novel hydrogels using molecular constants, is of interest for targeting viscoelastic properties of cellular niches, and is an accessible platform for designing dynamic networks with high processability.

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Design of thermo-responsive polypeptide bioconjugates for the treatment of glioblastoma by PDT/RDT

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INTRODUCTION

Glioblastoma IDH1 WT, a grade IV malignant glioma, is one of the most aggressive and lethal cancer, with less than 7.2 % survival rates after 5 years. The current FDA-approved therapeutic approach is Gliadel® wafers, a post-operative surgical implant. This approach presents many advantages: a minimally invasive surgery, avoiding BBB crossing, and providing a reservoir of active molecules (*i.e.*, carmustine) close to malignant cells. Nevertheless, Gliadel® wafers present some limitations and side effects, including the stiffness of the wafers and their lack of specificity.[1] In our NanoGlio project, we aim at developing a post-surgical deposit, softer and more flexible than Gliadel® and with high specificity to tumor cells. We therefore exploit elastin-like polypeptides (ELPs) as biocompatible, biodegradable, non-immunogenic and thermo-responsive polymer backbones.[2] The polypeptide chain ends are conjugated on one side to verteporfin (VP, a FDA approved photosensitizer), and on the other side to glucose. VP is used for Photo- or Radio- Dynamic Therapy (PDT/RDT), *i.e.*, photochemical damage *via* ROS accumulation when activated by an IR laser or X-rays, leading to tumor cell apoptosis.[3] Glucose confers cell specificity to the resulting nanocarriers by targeting GLUT-1 transporters overexpressed in blood brain barrier (BBB) and glioma.[4]

EXPERIMENTAL METHODS

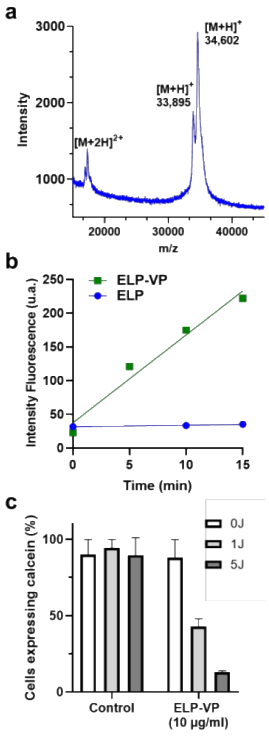
Synthesis of ELP-VP. The ELP used, MW[(VPGVG)(VPGMG)- $(VPGVG)_2]_{20}$, was produced recombinantly in *Escherichia coli* bacteria. VP was coupled to the *N*-terminal end of ELP by peptide coupling activated by HCTU. Compounds were characterized by SEC, MALDI, UV, DLS and NMR.

Photoirradiation Experiment. ELP-VP or ELP (0.125 mg/mL) were dissolved in PBS in the presence of SOSG (0.4 µM), a $^1O_2$ sensor. Irradiation was performed with a 690 nm laser, under stirring. $^1O_2$ generation was followed by SOSG fluorescence (λ_{ex} 504 nm/ λ_{em} 525 nm).

In vitro assay. P3 patient-derived cells of glioblastoma were incubated for 4 hrs with ELP-VP (10 µg/mL). Then, irradiation was performed. Cytotoxicity was studied by FACS, 48 hrs after irradiation. Calcein was used as a marker of living cells (n=2, mean+/-SD).

RESULTS AND DISCUSSION

The ELP-VP synthesis was optimized, with 90% overall yield and 80% conversion rate as evidenced by MALDI ($\Delta[M+H]^+$ = 707, M_{VP} = 718 g/mol) (Fig.1a) and using a VP UV-calibration curve. Under irradiation, only ELP-VP and not the control ELP induced an increase of SOSG fluorescence over irradiation time, meaning that the presence of VP on the polypeptide lead to $^1O_2$ generation (Fig.1b).



Moreover, preliminary *in vitro* results (n=2) showed that without irradiation, ELP-VP did not affect cell viability. However, when laser irradiation was applied, the percentage of living cells decreased with a dose-response effect. Thus, we can suggest that irradiation leads to ROS generation inducing an apoptosis of tumor cells (Fig.1c).

CONCLUSION

The synthesis of ELP-VP was optimized and the one of VP-ELP-Glu is ongoing. The generation $^1O_2$ by ELP-VP was confirmed using a fluorescent sensor. This property seemed to be confirmed in preliminary *in vitro* assay as the ELP-VP conjugate induced cell death under irradiation in a dose-response manner. Altogether these results demonstrate the potential of ELP-VP as efficient PDT agent. *In vitro* experiments are in progress on 2D cell cultures and on 3D spheroids, while VP-ELP-Glu will be investigated to assess targeting properties of the bioconjugate.

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ACKNOWLEDGMENTS

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Mixed Reality for Surgery : A New Tool For Medical Devices

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INTRODUCTION

Health is a very dynamic research field, in full expansion, and which is constantly nourished by hybridization with multiple emerging scientific fields. Among these, digital health puts the resources of artificial intelligence at the service of public health and the organization, management and evaluation of the health system. For nearly 30 years now, surgeons have been appropriating these technologies for the benefit of patients.

Adding mixed reality (the combination of virtual reality and augmented reality, usually designated by XR) is expected to be a valuable tool for surgeons.

Today, digital technology can assist the surgeon in his actions and imaging can help the surgeon to see the organs and their functioning before, during and after the operation. The first challenge to come is to assist the surgeon in his own vision of the operation by allowing him to observe simultaneously in real time the patient and his digital twin. By digitizing all the interactions between the surgeon and the patient, it becomes possible to model the entire operation and to develop artificial intelligence tools to assist the surgeon in the conduct of the entire operation.

RESULTS AND DISCUSSION

The company Lynx Mixed Reality has developed a completely new mixed reality headset with performances compatible with the requirements imposed for surgical practice. We would like to set up a joint research laboratory with Inserm. Indeed, the Lynx company and members of the INSERM U1148 unit have been in contact since the creation of the prototype of the augmented reality headset in 2017.

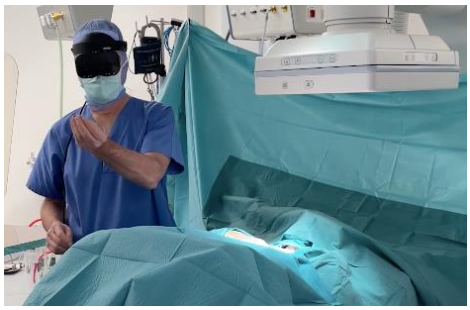
The results of these first attempts have shown that both the headset used and the operating room will need to evolve in the following years.

The operating room should also undergo some changes, such as guaranteeing a high enough internet speed would ease online expertise or courses for tutees.

The need of having virtual objects that fit the exact patient being treated within the XR system will influence the preliminary exams prescribed by their doctor.

The scientific and medical project aims to bring innovative health products to the operating room and developing a French and European industrial sector of digital surgery.

The ambition of the project partners, Inserm and Lynx Mixed Reality is a breakthrough in surgical practice, but also a breakthrough for the training of surgeons. This could be a main achievement for the implantation of medical devices in preclinical and clinical uses.



ACKNOWLEDGMENTS

The authors would like to thank the French ANR (Agence Nationale de la Recherche – Labcom 2021- LCV2-0002-01) for providing financial support to this project.

Adhesive Membranes from Marine Origin Raw Materials for Soft Tissue Engineering Applications

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INTRODUCTION

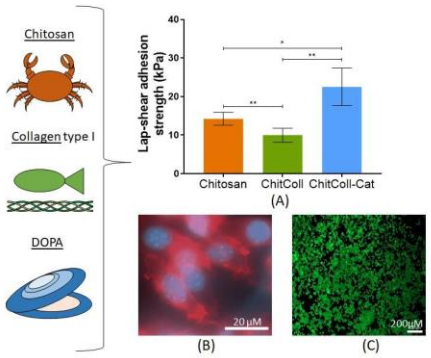
Tissue engineering (TE) has emerged as a versatile solution for re-establishment of normal biological functions by using bioactive materials to fill the injury site and thus, provide mechanical support and cell adhesive platform. Chitosan - a polysaccharide obtained by the deacetylation of chitin that is a structural element of the crustaceans exoskeleton[1], have been proposed for different TE applications. Chitosan is often combined with other polymers, e.g. collagen, to improve its mechanical properties and cytocompatibility. Collagen type I (Coll I) can be obtained from fish by-products with a reduced risk of diseases transmissions. Moreover, its production represents a sustainable strategy for marine by-products valorization with economic and ecological advantages [2]. However, the adhesive properties of Coll I and chitosan in a wet environment is limited. The marine adhesive proteins (MAPs) expressed by marine mussels contain the aminoacid 3,4-dihydroxy-L-phenylalanine (DOPA) and are outstanding moisture-resistant adhesive materials[3]. The adhesion capacity of MAPs is due to the catechol groups of DOPA. In this work, we obtained new tissue adhesives by functionalization of Coll I with the catechol derivative 3,4-dihydroxybenzaldehyde (3,4-DB). The obtained conjugate (Coll-Cat) was blended with chitosan (ChitColl-Cat) and evaluated as a material for regeneration of soft tissues.

EXPERIMENTAL METHODS

Coll I was extracted from salt-cured Atlantic cod (*Gadus morhua*) skins by supercritical fluid technology using CO_2 acidified water. The structure was confirmed by SDS-page and circular dichroism analysis. Schiff's base reaction was performed by stirring 46 mg/mL of 3,4-DB dissolved in water at 60 °C and 5 mg/mL of Coll I dissolved in acetic acid (AcOH) for 2 h at RT. The structure of the obtained conjugate Coll-Cat was confirmed by UV and ^{1}H-NMR spectroscopies. The degree of substitution was around 2%. Membranes prepared from 2.5% w/v of deacetylated chitosan in AcOH (1.5% v/v) and glycerol (5% v/v in 0.15 M NaCl) were used as controls and 1.5% w/v of chitosan solution to which was added either Coll I (1% w/v) or Coll-Cat (1% w/v) were used to obtain a ChitColl or a ChitColl-Cat blend, respectively. The membranes were processed by spin-coating (200 rpm, 3 min) in a Petri dish (Ø90 mm) and then dried at 37 °C.

RESULTS AND DISCUSSION

The obtained membranes had different macroscopic appearance: the ChitColl-Cat membranes were dark brown due to the presence of catechol groups, while the chitosan membranes were transparent and ChitColl ones were opaque. Morphological studies revealed a homogeneous and compact structure for the ChiColl-Cat membranes. The functionalization with catechol groups enhanced the water uptake and mechanical strength compared to the other two membranes. The introduction of these groups also improved the adhesion strength (22.5 ± 4.9 kPa, Fig. 1A), which is similar to the natural adhesives used in the clinical context and higher than some synthetic adhesives in wet environments. Preliminary biological tests were performed by seeding L929 fibroblastic cells on the surface of the membranes, which showed an improved cell attachment (Fig. 1B) and viability (Fig. 1C) for the obtained ChiColl-Cat membranes, proving their potential for different biomedical applications.



CONCLUSIONS

The sustainable use of biomimetic approaches and the under-exploited marine-derived polymers, such as chitosan and Coll I from codfish, demonstrated to be highly promising to develop a new class of adhesive and biodegradable membranes to adhere closely to the tissue and provide mechanical and structural support for an effective soft tissue repair.

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ACKNOWLEDGMENTS

The authors thank the financial support of: i) FCT and the European program FEDER/FEEI through PTDC/BTM-MAT/28123/2017, PTDC/NAN-MAT/31036/2017 and PhD scholarship SFRH/BD/143209/2019, ii) REMIX Project, funded by the European Union's Horizon 2020 Research and Innovation programme under the Maria Sklodowska-Curie grant agreement n. 778078.

>> 9:00 - 10:30 | SYMP-16 - ADVANCES IN THE DESIGN OF MULTIFUNCTIONAL AND CELL-INSTRUCTIVE HYDROGELS FOR TISSUE ENGINEERING

Chairpersons: Catarina Custódio & Julieta Paez
Location: Room F

9:00 | KL Multifunctional & Cell-instructive hydrogels - Design of hydrogels using natural macromolecules with improved structural and functional properties
João F. MANO, University of Aveiro, Aveiro, Portugal

9:30 | O1 Multifunctional & Cell-instructive hydrogels - Instructing Engineered Living Tissues from Within using Biophysically and Biochemically Tunable Microbuilding Blocks
Niels WILLEMEN, Dept. of Developmental BioEngineering, Faculty of Science and Technology, Technical Medical Centre, University Twente, Enschede, The Netherlands

9:45 | O2 Multifunctional & Cell-instructive hydrogel - A composite elastin derivative-based matrix for bone tissue engineering.
Nadia MAHMOUDI, BioIngenierie Tissulaire (BioTis), Inserm U1026, University of Bordeaux, Bordeaux, France

10:00 | O3 Multifunctional & Cell-instructive hydrogels - Optimized allyl-modified gelatin hydrogel for 3D cell culture of primary human umbilical vein endothelial cells and dermal fibroblasts
Alessandro CIANCIOSI, Department of Functional Materials in Medicine and Dentistry at the Institute of Functional Materials and Biofabrication (IFB), University of Würzburg and KeyLab Polymers for Medicine of the Bavarian Polymer Institute (BPI), Würzburg, Germany

10:15 | FP01 Multifunctional & Cell-instructive hydrogels - Development of a 3D polysaccharide porous membrane for the modelling of the outer blood retina barrier
Chloé DUJARDIN, Université Paris Cité, INSERM U1148, Paris, France

10:20 | FP02 Multifunctional & Cell-instructive hydrogels - Tuning the Physical Properties of Collagen/Hyaluronan Hydrogels to favor Mesenchymal Stem Cells Differentiation into NP Cells: A Step forwards Intervertebral Disc Regeneration
Christophe HELARY, Sorbonne University, CNRS, UMR 7574 – Chemistry of Condensed Matter Laboratory, Paris, France

10:25 | FP03 Multifunctional & Cell-instructive hydrogels - Critical aspects of Ti-based bulk metallic glasses for dental implant manufacturing
Laurabelle GAUTIER, 1 MATEIS UMR CNRS 5510, INSA Lyon, Université Claude Bernard Lyon 1, Villeurbanne, France

Design of hydrogels using natural macromolecules with improved structural and functional properties

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INTRODUCTION

Sophisticated strategies have been employed to engineering the microenvironment of cells in 3D matrices using distinct biomaterials, to be explored in cell and tissue engineering. Hydrogels are adequate matrices for culture of cells – examples of natural-based hydrogels with adequate mechanical and biological properties are shown.

Using bioinspired strategies, basic units of hydrogels or macro-structures with controlled geometry can be processed in mild conditions, permitting the encapsulation of living cells and other biological cargo with high efficiency. Natural macromolecules such as polysaccharides and proteins are very interesting materials to be used to produce hydrogels due to their high-water affinity and their natural engagement to interact favorably with living organisms, thus being usually biocompatible.

The proper macromolecular design may provide structural properties that are not usually characteristic of conventional hydrogels, including high toughness, adhesiveness, or self-healing capability. Examples of such natural-based hydrogels will be shown, based on marine-derived polysaccharides.

Bioactive domains existing in proteins improve cellular functions, which are important to develop constructs for tissue engineering applications. In our group we have been using human-derived proteins to produce hydrogels with such interesting functional properties.

Examples will be given on photocrosslinkable systems based on platelet lysates and proteins from the amnionic membrane.

The hydrogels have the potential to be applied in therapies based on regenerative medicine, as well as in the development of pathological tissues in vitro to be used in disease modelling and drug screening.

Instructing Engineered Living Tissues from Within using Biophysically and Biochemically Tunable Microbuilding Blocks

T. Kamperman[1*], N.G.A. Willemen[1*], C. Kelder[1], M. Koerselman[1], M. Becker[1], M. Karperien[1], and J. Leijten[1]

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INTRODUCTION

Modular tissue engineering exploits the 3D self-assembly of cells and micromaterials to create larger tissue constructs with higher complexity and resolution.[1] However, current modular tissue engineering strategies have near-exclusively relied on static non-responsive, micromaterials,[2] whereas functionality of native tissues is dictated by their inherently dynamic nature.[3] Here, we report on *in situ* tunable microbuilding blocks of which the biochemical and biophysical properties can be altered via orthogonal crosslinking strategies.

EXPERIMENTAL METHODS

Microgel production: Microgel precursor droplets composed of 5% dextran-tyramine-biotin (Dex-TAB; ~1 mM biotin) and 22 U/mL horseradish peroxidase in phosphate-buffered saline were emulsified in Novec 7500 Engineered Fluid with 2% Pico-Surf 1 using a microfluidic droplet generator, and subsequently crosslinked via controlled microfluidic supplementation with H_2O_2.[4] Dex-TAB microgels were consecutively incubated with 1 µM neutravidin, washed, incubated with 1 µM biotinylated or desthiobiotinylated molecule-of-interest (e.g., biotinylated c(RGDfK)), and washed again.

Tunable modular tissue engineering: Microgels were homogeneously co-seeded with cells into non-adherent 3% (w/v) agarose microwell chips at a density of ~50 units per microwell. The mechanical properties of microgels could be modified post-synthesis by exploiting visible-light-induced crosslinking of tyramine moieties using 1 mM ruthenium and 2.5 mM sodium persulfate as initiators. Chemical post-modification of the microgels was achieved via competitive supramolecular complexation of avidin and biotin analogs.[5] Adipogenic and osteogenic differentiation of mesenchymal stem cells (MSCs) was analyzed microscopically following Oil-Red-O and Alizarin Red staining, respectively.

RESULTS AND DISCUSSION

Reactive tyramine and biotin moieties in Dex-TAB microgels could be enzymatically post-crosslinked and/or functionalized with avidin/biotin analogues, respectively, in an orthogonal manner. This enabled virtually independent *in situ* tuning of the microgels' mechanical and chemical properties. Coating Dex-TAB microgels with biotinylated c(RGDfK) enabled integrin-mediated self-assembly with cells, even without serum.

In contrast to conventional bulk encapsulation of cell spheroids in hydrogel, incorporating cell-sized microbuilding blocks within engineered tissue uniquely supported cell-material mechanotransduction, which was essential for osteogenic differentiation of 3D stem cell cultures (**Figure 1a**). Material stiffness-induced lineage programming of modular 3D stem cell/microgel spheroids was dependent on the size, stiffness, and number ratio of microbuilding blocks, as well as the timing of material stiffness-induced mechanical cues.

In situ biochemical control over cell-building block aggregates was shown by temporally endowing the building blocks with (desthio)biotinylated fluorophores (**Figure 1b**), as well as bone morphogenetic protein (BMP)7 neutralizing antibodies, which showed temporal control over the cellular response to BMP7 using a BMP-reporter cell line.

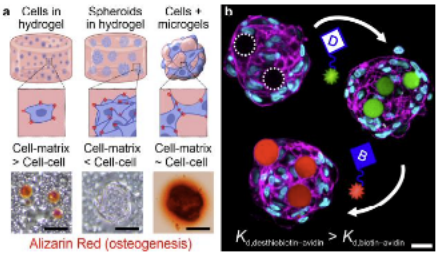

Figure 1. (a) *Providing biomechanical cues from within using stiff cell-binding microgels is key to achieve osteogenic differentiation of aggregated MSC.* ***(b)*** *In situ biochemical modification of a modular tissue via competitive complexation of neutravidin with desthiobiotinylated and biotinylated fluorophores. Scalebars indicate 50 µm (black) and 20 µm (white).*

CONCLUSION

We developed the first biophysically and biochemically tunable microbuilding blocks that control cell behavior from within engineered tissues. This *in situ* tunable instructive microbuilding blocks concept is expected to prompt the next generation of adaptive 3D tissue models that facilitate several applications including disease modeling, drug screenings, and regenerative therapies.

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A composite elastin derivative-based matrix for bone tissue engineering.

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2 Univ. Bordeaux, CNRS, Bordeaux INP, LCPO, UMR 5629, Pessac, France

INTRODUCTION

Despite continuous progress in the field of bone tissue engineering, the treatment of critical bone lesions caused by trauma, degenerative diseases or tumor resections remains an ongoing challenge. Extracellular matrix-like hydrogels are increasingly used as scaffolds for tissue engineering. Elastin-like polypeptides (ELPs) are a class of recombinant polymers of interest for hydrogel manufacture because they are monodispersed, cross-linkable, biocompatible, biodegradable, and non-immunogenic. The project focuses on the development of an innovative bioactive ELP-based composite hydrogel for bone tissue engineering. This work aims to evaluate the ability of these matrices to stimulate angiogenesis, innervation, and osteoinductivity in a subcutaneous model in mice.

EXPERIMENTAL METHODS

The recombinant ELP used in this study, noted ELP[M_1V_3-*80*], has the following sequence: MW[(VPGVG)(VPGMG)(VPGVG)$_2$]$_{20}$. It contains a total of 21 methionine residues that were chemoselectively modified by a thioalkylation reaction to introduce an alkene function for subsequent cross-linking[1]. The hydrogels were produced by cross-linking the ELP-alkene chains with two different bioactive peptides presenting two cysteine residues flanked at the C- and N-terminal ends[2,3]. The first one is derived from laminin (IKVAV/YIGSR) and is able to increase the recruitment of vascular and neuronal cells. The second, SNA15, is derived from salivary statherine and has been demonstrated to play a role in the retention of calcium phosphate. The last compound corresponds to hydroxyapatite (HA) particles, a mineral component known for its osteo-(in)(con)ductive properties.

The matrix porosity was observed by Cryo Scanning Electron Microscopy (SEM) and the pore interconnectivity was evaluated using confocal imaging of the matrices following FITC-Dextran (70kDa) incubation. To assess mineralization potential, the hydrogels with the best characteristics were implanted subcutaneously in mice and followed longitudinally after 2 and 4 weeks of implantation by X-ray computed tomography (micro-CT) using a Quantum FX caliper (Life Sciences, Perkin Elmer, Waltham, MA). To evaluate inflammation, vessel and osteoid tissue formation histological analysis were employed after 4 weeks of implantation. For this CD31 immunohistochemistry and Masson's trichrome staining were used respectively. The quantification of pores and the formation of osteoid tissue was done using the software ImageJ®. The Mineral Volume/Total Volume (MV/TV) was determined by eXploreMicroView® software (General Electric Healthcare, Milwaukee, WI).

All tests were performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, California USA), p-value<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

The average pore size varied between 235+/-107 µm for the hydrogel without HA, and 79+/-6 µm for hydrogels containing 2.5% (w/v) HA, indicating that the introduction of HA impacts on matrix microporosity. Nonetheless, the obtained porosity could be sufficient for cell colonization. Our results show a uniform diffusion of FITC-Dextran within the matrices, indicating efficient pore interconnectivity.

Then, the matrices were implanted subcutaneously with 3% (w/v) matrices without HA, or containing 1%, 2% or 2.5% (w/v) HA. The micro-CT analysis confirmed the formation of ectopic mineralized tissue within the implants (Fig.1A). The volume of mineralized tissue formed in matrices containing HA increased over time (Fig.1B). The matrices containing 2% and 2.5% (w/v) HA did not differ in the induction of mineralized tissue formed after 2 or 4 weeks of implantation, and these two matrices produced the greatest amount of mineralized tissue.

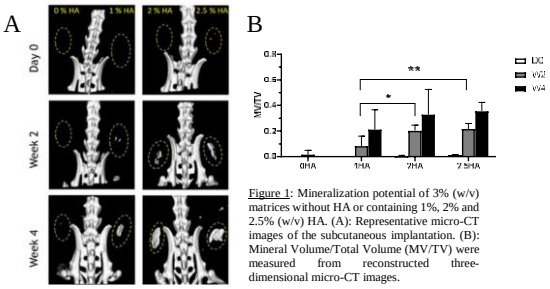

Figure 1: Mineralization potential of 3% (w/v) matrices without HA or containing 1%, 2% and 2.5% (w/v) HA. (A): Representative micro-CT images of the subcutaneous implantation. (B): Mineral Volume/Total Volume (MV/TV) were measured from reconstructed three-dimensional micro-CT images.

Gels were explanted and histological sections of the materials associated with the surrounding tissues showed no significant fibrosis. This indicates that ELP hydrogels are not rejected. Angiogenesis was evaluated secondly: in the case of the 0% (w/v) HA gels, capillaries could be observed mainly at the periphery. Conversely, for the 2% (w/v) HA formulation, larger vessels (up to 200 µm) could be observed in the periphery but also inside the hydrogels. After quantification, 28+/-12 vessels/mm² of gel were observed for the 0% HA gels. For HA-containing gels, 88+/-38 vessels/mm² in 1% HA gels, 122+/-44 vessels/mm² in 2% HA gels and 127+/-46 vessels/mm² in 2.5% (w/v) HA gels were observed. These results suggest that HA-containing hydrogels have a positive effect on vascularization. The capacity of the developed matrices to sustain innervation *in vivo*, in the scope of bone regeneration, is underway.

CONCLUSION

We obtained a cell-free and growth factor-free hydrogel capable of inducing ectopic mineralized tissue formation and angiogenesis in mice. Functionalized matrices combined with 1 to 2.5% (w/v) HA show osteoinductive properties. Further *in vivo* testing in small animals is required for pre-clinical evaluation in bone lesions.

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Optimized allyl-modifier gelatin hydrogel for 3D cell culture of primary human umbilical vein endothelial cells and dermal fib oblasts

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INTRODUCTION

Biomaterial-based hydrogels are ideal candidates to recapitulate the extra cellular matrix (ECM), providing a water inclusive network for cells to grow within a favourable mesh size, with tailorable stiffness and degradation properties.[1] Gelatin is a well-established biomaterial which is often modified with functional groups, covalently bonded into a three-dimensional polymeric network.[2] Thiol-ene click chemistry is a crosslinking strategy, which offers advantages like insensitivity to oxygen inhibition, low polymer shrinkage and homogeneous network formation.[3,4] In this study we present an optimized hydrogel precursor formulation based on allyl-modified gelatine (gelAGE) and a multi-arm thiolated polyethylene glycol (PEG-4-SH) as crosslinker, in the presence of a photo-initiator such as Lithium-phenyl-2,4,6-trimethylbenzoyl-phosphinate (LAP).

EXPERIMENTAL METHODS

GelAGE synthesis was optimized based on previous published protocols.[4] The nomenclature of the allyl-gelatin products was based on the reaction time (1-2h) and the molar concentration of sodium hydroxide (NaOH) and allyl glycidyl ether (AGE): low (L), medium (M) and high (H).The degree of modification (DoM) of two different GelAGE types (G_{1MM} and G_{2LH}) was quantified with ^{1}H-NMR and their molecular weight (Mw) with water GPC. The physico-chemical properties of the materials were analyzed by swelling, compression tests and photo-rheological measurements. Primary human umbilical vein endothelial cells (ECs) and normal human dermal fibroblasts were casted within the hydrogel precursor solution individually or as co-culture and crosslinked with UV-A light (405 nm).

Cell viability and proliferation was determined by live/dead staining. Cell morphology was visualized with Höchst and Phalloidin staining. Soluble fibronectin and soluble collagen from all samples were measured by fluorometric methods with respective assay kits. Expression of CD31 and fibronectin were confired by immunofluorescent staining.

RESULTS AND DISCUSSION

The reaction conditions cut down the native gelatin chains (type A). G_{1MM} showed a Mw substantially lower than G_{2LH} which interestingly carried fewer substituents groups (Fig. 1a, 1b). All the hydrogel precursors had equimolar amounts of allyl and thiol moieties. The compression tests conducted on cylindrical specimens (4,5 x 8 mm) displayed that both gelAGE matices were soft, even though G_{2LH} was substantially softer due to the limited DoM and required a smaller amount of crosslinker (Fig. 1c). Over 21 days, both gelAGE types showed a relatively low sol fraction and swelling ratio (Figure 1d, e). Cell viability and morphology of ECs and fibroblasts were compared between G_{1MM} and G_{2LH}. Both hydrogel types supported cell viability, but cells embedded in G_{1MM} remained rounded and did not show any morphological alterations until day 28.

In contrast, fibroblasts embedded in G_{2LH} begun to elongate on the first incubation day, and ECs on day 3. Further biological analyses were therefore performed only with G_{2LH}-embedded cells. Biological activity of individual cell types was supported in G_{2LH} hydrogels. Time-dependent secretion of soluble collagen and fibronectin was detected in both cell types, but was, higher in fibroblasts than in ECs. Immunocytochemical analysis of co-cultures indicated that endothelial cells expressed CD31 and formed cell-cell contacts. Furthermore, strong fibronectin expression in all cells, as well as vessel-like structural organization at day 14, were observed (Figure 2).

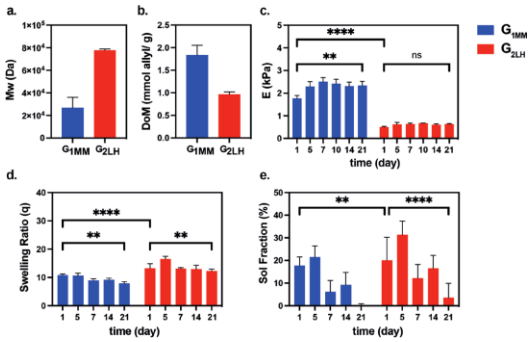

Figure 1: a. Average MW of two different gelAGE types G_{1MM} and G_{2LH}; b. Degree of modification quantified trough ^{1}H-NMR in mmol of allyl moieties per gram of gelatin of gelAGE G_{1MM} and G_{2LH}; c. Young's modulus of gelAGE G_{1MM} and G_{2LH} over 21 days; d. swelling and e. sol fraction of G_{1MM} and G_{2LH} over 21 days. (n=6)

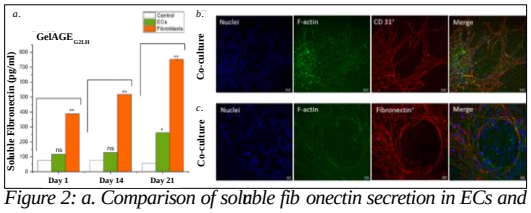

Figure 2: a. Comparison of soluble fibronectin secretion in ECs and fibroblasts embedded in GelAGE G2LH according to hydrogel control (w/o cells) b. Co-culture in hydrogels and immunofluorescent staining of endothelial marker CD31 after 14 days of incubation (20x magnification, scale bar 50 µm), c. Co-culture in GelAGE G2LH and immunofluorescent staining of fibronectin after 14 days of incubation (40x magnification, scale bar 20 µm).

CONCLUSIONS

This study demonstrated the possibility to tailor the stiffness of the matrix of gelAGE products by varing the reaction conditions. Furthermore, it was showed that G_{2LH} hydrogels support cell viability, biological function, motility, and may thus fulfil the demands of ECs and fibroblast.

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Tuning the Physical Properties of Collagen/Hyaluronan Hydrogels to favor Mesenchymal Stem Cells Differentiation into NP Cells: A Step forwards Intervertebral Disc Regeneration

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INTRODUCTION

Back pain is a major burden of the 21st century as 90% of the world population will be affected during their life time. In half of cases, this disease is associated with intervertebral disc (IVD) degeneration. The regular treatment is based on antipain treatment and physiotherapy. When the pain is too great, surgery is required (discectomy, spinal fusion). Novel strategies relying on stem cell injection have been tested. Unfortunately, the outcomes are disappointing because of cell leakage and incomplete differentiation. Nowadays, a consensus exists on the necessity to encapsulate stem cells within a hydrogel to maintain them in situ and favor their differentiation. As cell behavior depends on biochemical and physical environment, a biomimetic hydrogel would promote IVD regeneration. Nucleus Pulposus is a highly hydrated tissue working as hydraulic shock absorber. Glycosaminoglycans give a high degree of hydration whereas collagen II gives resistance and allows for cell adhesion. With the aim of developing novel biomimetic hydrogels, collagen/hyaluronic acid composites were developed to mimic the structure and the mechanical properties of Nucleus Pulposus. For this purpose, we first studied the impact of the HA content on the hydrogel physical properties. Then, the potential of the different formulations to differentiate mesenchymal stem cells (MSCs) into NP cells was analyzed in detail.

EXPERIMENTAL METHODS

HA functionalized with tyramine groups (HA-Tyr) was mixed with collagen and gelled using Horse Radish Peroxidase and H_2O_2 at pH 7.4. With a constant collagen concentration (0.4%), the HA-Tyr content was increased up to 2 % to create a platform of Collagen/HA-Tyr hydrogels with different properties. The hydrogel structure, the mechanical properties and the degree of hydration were analyzed. Mesenchymal stem cells were encapsulated within the different hydrogel types and cultivated over 28 days. The impact of MSCs on hydrogel stability, metabolic activity and cell morphology were analyzed. Last, the gene expression of Aggrecan, Collagen I and II was quantified by real time PCR.

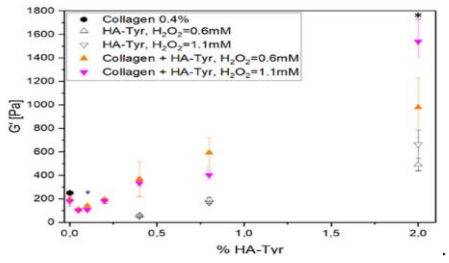

Figure 1: Mechanical properties of Collagen/HA-Tyr hydrogels evaluated by rheology.

RESULTS AND DISCUSSION

The physico-chemical study showed the impact of the HA-Tyr content on the hydrogel physical properties. At low HA-Tyr content (less than 0.4 %), the composite behaviour was driven by collagen. Hydrogels exhibited a fibrillary network and were characterized by low mechanical properties (Figure 1). From 0.8% HA-Tyr, the mechanical properties and the hydration degree increased to reach those of NP (G'= 1.5kPa) when 2% HA-Tyr and 1.1 mM H_2O_2 were added. Below 0.4% HA-Tyr, encapsulated cells contracted hydrogels after one week in culture (Figure 2). From 0.8%, hydrogels, MSCs did not contract hydrogels and their mechanical properties were stable over the time course of the experiment. With a high HA-Tyr content, cells did not proliferate, suggesting their commitment towards differentiation. At low content, MSCs spread and adopt a fibroblast like morphology (Figure 2). On the opposite, cells encapsulated within hydrogels at high HA-Tyr content were more rounded and resemble NP cells. The gene expression quantification showed that MSCs orientated towards a NP cell phenotype. When 2% HA-Tyr was used, cells highly expressed NP cells markers such as Aggrecan and Collagen II, and weakly expressed Collagen I. In contrast, cells encapsulated in hydrogels with a low HA-Tyr content weakly expressed these NP cell markers.

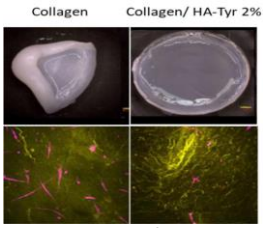

Figure2: Macroscopic view of hydrogels (top) and cell morphology (bottom) after 28 days in culture.

CONCLUSION

Taken together, these results show that Collagen/Hyaluronan Composite Hydrogels with a high HA-Tyr content (2%) mimic the physical properties of the Nucleus Pulposus and promote the differentiation of MSCs into NP cells. Hence, these hydrogels could be useful for IVD regeneration.

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ACKNOWLEDGMENT: This research has been supported by Agence Nationale de la Recherche (ANR) - grant number: ANR-19-CE06-0028.

Critical aspects of Ti-based bulk metallic glasses for dental implant manufacturing

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INTRODUCTION

Since two decades, Bulk Metallic Glasses (BMG) are often described as very promising materials for biomedical applications. They may indeed exhibit astonishing mechanical and corrosion properties, especially when they are processed in the form of very small samples. The $Ti_{40}Cu_{36}Zr_{10}Pd_{14}$ grade for example has already been proved to be biocompatible and presents a yield strength up to 2GPa[1]. This, in addition to a theoretical improvement of corrosion resistance related to its amorphous nature, would open the door to minimally invasive implants and small dental products.

However, we have recently shown that crystalized casting defects (often referred as spherulites) in this specific composition have a detrimental influence on the mechanical and electrochemical properties[2]. They are particularly numerous and large in several milimeters bulk samples, which are relevant to produce dental implants or dental products. While the exact origin of the spherulites has not yet been determined, their morphology, chemistry, crystallography based on Cu-Ti binary diagram and a growth mechanism have been studied in details[2]. It is hypothesized that if the microstructure (content and size of such spherulites) of this BMG can be better controlled through processing it can lead to a significant improvement in the mechanical properties and corrosion resistance. It was therefore the aim of this work to explore the effect of processing on the presence and the features of spherulites and their effect on mechanical and corrosion properties.

EXPERIMENTAL METHODS

The $Ti_{40}Cu_{36}Zr_{10}Pd_{14}$ bulk amorphous rods used for the experiments were obtained by classic suction copper mold casting from high purity raw metals (Copper, Titanium, Zirconium and Palladium) under high purity Argon atmosphere. Different casting parameters were investigated such as melts number before casting, arc intensity and hydrogen enriched atmosphere. The BMG rods have then been characterized by XRD, DSC, SEM and TEM. The mechanical properties were studied by compressive, tensile and four points bending tests. The electrochemical measurements were conducted in NaCl 0.9% solution at 37°C with Ag/AgCl as reference electrode and Graphite as counter electrode.

RESULTS AND DISCUSSION

In general, mechanical characterisation showed very poor reproducibility except for compressive tests which are less sensitive to casting defects (and not relevant for the real use). Failure was often correlated with the presence of a big spherulite near the sample surface. Nevertheless, the Yield Strength of this BMG is really high around 2GPa (but without any plasticity in mode I). It is believed that the spherulites could be associated to un-melted areas in the alloy during the casting process. Even with an increase of the arc intensity or a large number of fusions, the statistical analysis did not show a significant difference in distribution in spherulite's number and size. The electrochemistry experiments showed that the spherulite/amorphous matrix interface appears to be particularly prone to dissolution. The complex spherulite's microstructure showed several phases with specific orientation relationships which helps to propose a growth scenario.

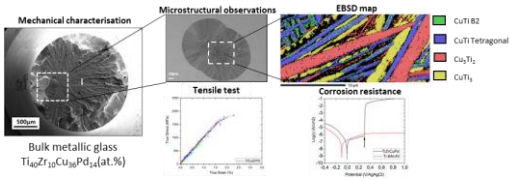

Figure 1 : On the left, SEM picture of a representative fracture surface which illustrates the presence of casting defects in $Ti_{40}Cu_{36}Zr_{10}Pd_{14}$ BMG under tensile test. On the right, EBSD map with the identified phases. On the bottom, stress-strain curves of tensile test and potentiodynamic curves in NaCl 0.9%(compared to TA6V as reference material).

CONCLUSION

The TiCuZrPd grade that has been portrayed as most promising for biomedical applications and particularly for dental implants exhibits casting defects. To date, and despite numerous attempts, we have not been able to avoid them or adjust their size in order to increase ductility and avoid pitting corrosion. This constitutes a key point to further explore for this material to become a robust candidate in biomedical applications in the near future.

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ACKNOWLEDGMENTS

The authors would like to thank the 'Agence National de la Recherche' (ANR) and its funding within the framework of the ANR-TNT-18-CE91-0005.

>> 11:00 - 12:00 | PSOP-22 - BIOMIMETIC AND BIOINSPIRED MATERIALS II

Chairpersons: Rui Reis & Christèle Combes
Location: Room A

11:00 | O1 Biomimetics II - Development of a biomimetic stem cell scaffold system for spinal cord applications

Cian O'CONNOR, Tissue Engineering Research Group, Dept of Anatomy & Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland & Advanced Materials & Bioengineering Research Centre, Trinity College Dublin, Dublin, Ireland

11:15 | O2 Biomimetics II - Microfabrication of Collagen Particles as Biomaterial Building Blocks for Bottom-Up Bone Tissue Engineering

Esra GÜBEN KAÇMAZ, Department of Instructive Biomaterials Engineering, MERLN Institute for Technology-Inspired Regenerative Medicine, Maastricht University, Maastricht, Netherlands

11:30 | O3 Biomimetics II - Beyond RGD; nanoclusters of syndecan- and integrin-binding ligands synergistically enhances cell/material interactions

Daniel HEATH, Department of Biomedical Engineering, University of Melbourne, Parkville, Australia

11:45 | O4 Biomimetics II - Designing injectable and functional graphene oxide (GO) – self-assembling peptide hybrid hydrogels for biomedical applications

Alberto SAIANI, Department of Materials, University of Manchester, Manchester, UK; Manchester Institute of Biotechnology, University of Manchester, Manchester, UK

Development of a biomimetic stem cell scaffold system for spinal cord applications

Cian O'Connor[1,2], Ian Woods[1,2], Sarah F. McComish[3], Sean Kerr[1], Maeve A. Caldwell[3], Adrian Dervan[1,2], Fergal J. O'Brien[1,2]
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INTRODUCTION

Following spinal cord injury, a complex scar forms around the resultant lesion cavity, preventing axonal regrowth. Despite ongoing development of stem cell treatments for spinal cord injury, effective repair remains a challenge in part due to the lack of a supportive environment and cell death. Therapeutics that physically bridge the cavity with a neurotrophic environment while simultaneously delivering stem cells to restore lost tissue may have potential. Building off success in our lab in peripheral nerve repair[1], we aimed to identify neurotrophic extracellular matrix proteins to develop novel, biomimetic functionalized hyaluronic acid scaffold implants and to determine their trophic capacity for spinal cord applications using multiple cell models[2]. By optimizing scaffold stiffness and matrix composition for stem cell delivery, it was hypothesized that the pro-regenerative signaling properties of trophic induced pluripotent stem cell (iPSC) derived astrocyte progenitors could be enhanced by tuning scaffold physiochemical properties to promote cord repair.

EXPERIMENTAL METHODS

Spinal cord astrocytes and neuronal cells were cultured on a range of matrix proteins to identify a novel neurotrophic substrate combination. Following the incorporation of the neurotrophic substrate mix into 3D hyaluronic acid scaffolds of varying stiffnesses, scaffold physicochemical properties were fully characterized. Spinal cord astrocytes, neurons, dorsal root ganglia (DRG) and iPSC-derived astrocyte progenitors were cultured in the scaffolds for up to 21 days and the effect of scaffold physicochemical properties was assessed using immunostaining, confocal microscopy, ELISA qPCR and analysis of metabolic activity. In addition, the impact of scaffold properties on the trophic capacity of iPSC-derived progenitors was assessed *in vitro* and *ex vivo* using neurons, DRG and spinal cord explants.

RESULTS

Screening of central nervous system matrix components revealed that a combination of collagen-IV (Coll-IV) and fibronectin (FN), synergistically enhanced neuronal outgrowth while also promoting astrocyte process extension and reducing reactive behaviour. Subsequently, hyaluronic acid scaffolds functionalized with Coll-IV/FN were manufactured using different concentrations of hyaluronic acid to produce scaffolds of varied stiffnesses ranging from soft/biomimetic to stiff/supraphysiological (0.8-3kPa). Spinal cord astrocytes cultured in soft, Coll-IV/FN functionalized scaffolds matching cord stiffness, increased metabolic activity, secretion of anti-inflammatory cytokine IL-10 and promoted resting cell phenotypes. Furthermore, these scaffolds significantly enhanced neurite outgrowth from neuronal cells and adult mice DRG explant cultures (a mature model of axonal growth) compared to stiffer scaffolds. These biomimetic soft, Coll-IV/FN scaffolds also promoted iPSC viability, outgrowth/infiltration, differentiation and functional uptake of glutamate while encouraging the growth of iPSC-derived spheroids that subsequently formed extensive neuronal/astrocytic networks within the scaffold. Conditioned media taken from iPSC-loaded scaffolds and applied to growing neurons, indicated that media from soft, Coll-IV/FN scaffolds enhanced neurite outgrowth 2.8 fold whereas media from stiffer scaffolds or scaffolds without Coll-IV/FN did not. Finally, growth of ex vivo mouse spinal cord and DRG explants showed that soft, Coll-IV/FN iPSC scaffolds promote increased cellular infiltration, resting astrocyte morphologies and axonal extension between DRG and iPSC spheres within scaffolds.

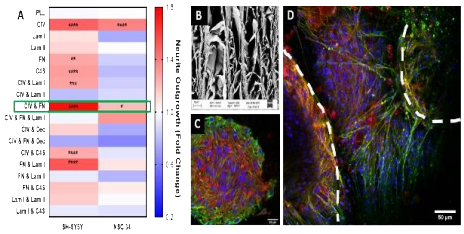

Figure 1 A) Screening of matrix proteins shows Coll-IV/FN as the most neurotrophic substrate (Green box). B) Longitudinally aligned scaffolds comprised of Coll-IV/FN were manufactured, mimicking cord tissue. C) IPSC neurospheres comprised of astrocytes (red) and neurons (Green) form in soft, Coll-IV/FN scaffolds. D) Soft, Coll-IV/FN scaffolds promote axonal extension between iPSC neurospheres (top right) and DRGs (bottom left).

DISCUSSION

These data indicate that by appropriately tuning the physicochemical properties of scaffolds to mimic that of the uninjured spinal cord, significantly enhances astrocyte immunomodulatory responses while promoting neurite extension. Furthermore, biomimetic scaffolds promote the paracrine activity of patient-derived progenitor cells that enhances axonal outgrowth. Overall, this work has significant implications for spinal cord applications while highlighting the impact of scaffold design and properties on the therapeutic effectiveness of stem cells for delivery.

ACKNOWLEDGEMENTS & REFERENCES

This work is funded by the IRFU Charitable Trust, the Anatomical Society and the SFI-AMBER centre.
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Microfabrication of Collagen Particles as Biomaterial Building Blocks for Bottom-Up Bone Tissue Engineering

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INTRODUCTION

Various bone tissue engineering (BTE) approaches have been utilized to provide effective treatments as alternatives to autologous transplants for critical-sized bone defects, for which the inherent renewal capacity of bone is often inadequate[1]. Biomaterials-based bottom-up BTE offers a promising solution to generate implantable bone microtissues to regenerate the defected bone. In this approach, cells and micron-scale biomaterial building blocks are assembled to obtain modular 3D constructs[2-3]. We suggest the use of collagen microparticles (COL-MPs), alone or combined with calcium phosphate mineralization, as building blocks for generating 3D modular bone microtissues in order to provide the cells with proper biological and mechanical cues. This research shows the first optimization steps for fabrication of shape-specific engineered COL-MPs in low-attachment micromolds.

EXPERIMENTAL METHODS

A 6.44 mg/ml solution of collagen in 10x phosphate-buffered saline (PBS), supplemented with fluorescently-labelled dextran to visualize the formation of microparticles, was casted onto a plasma treated polydimethylsiloxane (PDMS) template featuring particle micromolds. The COL-MPs were formed in the mold by incubation at 37 °C for 2 hours and crosslinked with 50 mM 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 25 mM sodium N-hydroxysulfosuccinimide sodium (NHS) overnight at room temperature. To release the COL-MPs, either a poly (vinyl alcohol) (PVA) film was casted over the micromolds or a commercial adhesive tape from PVA was laminated over them. The PVA layer was later peeled off and dissolved in water, resulting in free-standing COL-MPs. The COL-MPs were analyzed with fluorescent microscopy, scanning electron microscopy (SEM) and attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR).

RESULTS AND DISCUSSION

The collagen solution supplemented with dextran could successfully fill the micromolds in the PDMS template (Figure 1a) and form insoluble COL-MPs after crosslinking (Figure 1b). COL-MPs could be removed from the micromold with both PVA cast film and adhesive tape (Figure 1c–d). In both cases, damages to the COL-MPs were observed which might have occurred during the peeling or the drying step before SEM imaging. Therefore, we are currently optimizing the COL-MPs' releasing and drying steps. Typical collagen bands were found in the FTIR spectrum of COL-MPs. For example, amide I, II and III bands were detected in the FTIR spectra of the COL-MPs around 1700, 1550, and 1250 cm^{-1}, respectively (Figure 2)[4-5].

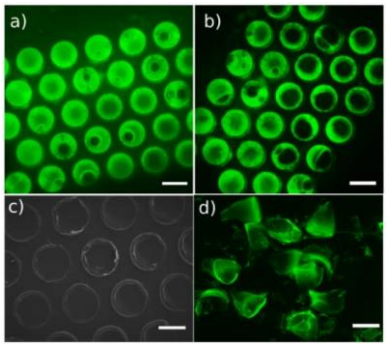

Figure 1. PDMS templates with micromolds containing a) collagen solution and b) COL-MPs after crosslinking, c) SEM image of COL-MPs removed from the micromolds using PVA adhesive tape and d) free-standing COL-MPs in water. Scale bars: 200 µm.

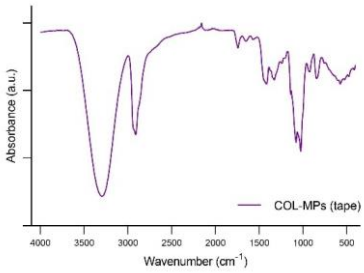

Figure 2. FTIR spectrum of COL-MPs (tape).

CONCLUSION

The present study shows the first optimization steps for the fabrication of free-standing COL-MPs. We aim to use these microparticles as matrix-mimicking building blocks for generating hybrid 3D bone-like microtissues.

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ACKNOWLEDGEMENTS

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Beyond RGD; nanoclusters of syndecan- and integrin-binding ligands synergistically enhances cell/material interactions

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INTRODUCTION

Biomaterials are commonly functionalized with peptide ligands to provide the material with bioactive properties. For instance, many materials have been functionalized with the RGD peptide. This peptide binds to multiple integrin receptors present in the membranes of adherent cells, and the functionalized materials often elicit adhesion of cells that express the appropriate integrins. Over the last 30 years, materials have been functionalized with numerous integrin-binding ligands, the relationship between ligand concentration and cell response has been investigated, and the impact of ligand patterning on cell response has been researched.[1]

Despite the advances made by this line of inquiry, most researchers have myopically focused on functionalizing surfaces with integrin-binding ligands. However, biological research has illustrated that integrin engagement alone does not elicit a full cell adhesion response. For instance, the sydencan-4 ligand must be engaged with integrins to form focal adhesion and drive cellular behavior.[2] In this work, we test the hypothesis that biomaterials functionalized with mixed populations of integrin- and syndecan-binding ligands will lead to improve cell material interactions.

EXPERIMENTAL METHODS

Comb polymers were synthesized through RAFT co-polymerization of methyl methacrylate and a PEG methacrylate. The distal end of some of the PEG side chains was functionalized with norbornene groups to allow the grafting of cysteine-terminated peptides.

Some polymers were functionalized with multiple integrin-binding peptides (RGD), syndecan-4-binding peptides (AG73), or a mixture of the two. These highly functionalized chains were blended with non-functionalized chains to enable control over the global ligand density and the local ligand density due to the random coil nature of the functionalized polymer.

Polymer surfaces with a range of global and local ligand densities were produced, and the impact of functionalization on endothelial cell behavior was assessed. Specifically, we assessed the ability of endothelial cells to adhere to the surfaces, spread, form focal adhesion, and proliferate under static conditions. We also used a laminar flow chamber to assess the impact of functionalization on the ability of cells to adhere under flow conditions and attach from flow.

RESULTS AND DISCUSSION

Surfaces functionalised with nanoclusters of integrin- and syndecan-4-binding ligands exhibited superior cell material interactions compared to surfaces functionalised with integrin-binding ligands alone at the same surface density. Specifically, surfaces with a 50/50 mix of integrin- and syndecan-binding ligands resulted in a synergistic increase in endothelial cell adhesion, and those surfaces were able to reach endothelialisation faster. The 50/50 mixed surfaces also resulted in a more spread cellular morphology and more apparent focal adhesions under static conditions.[3]

When laminar shear flow was applied, we found that endothelial cells on the 50/50 surfaces exhibited improved mechanosensing. Specifically, they were the only cells that aligned in the direction of flow, as is seen *in vivo*; they exhibited significantly greater adhesion strength to the surface as assessed through detachment assays; and significantly more cells were scavenged by these surfaces from flow.[4]

Integrins and syndecan-4 are used by all adherent cell types, so we believe we have identified a new class of biomaterials that can be used to improve a range of biomedical device and tissue engineering applications.

CONCLUSION

Materials that engage both integrin- and syndecan-4 ligands improves cell adhesion, results in faster surface coverage, improves mechanotransduction to applied stresses, increases adhesion strength of the cells, and improves scavenging of cells from flow. These novel materials have significant application in the biomaterial and tissue engineering fields.

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ACKNOWLEDGMENTS

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Designing injectable and functional graphene oxide (GO) – self-assembling peptide hybrid hydrogels for biomedical applications

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INTRODUCTION

Due to their unique combination of versatile synthesis, tunable physiochemical properties and biocompatibility, hydrogels have been the biomaterial of choice over the past two decades for many applications in tissue engineering, drug delivery and regenerative medicine. In this context, we recently developed injectable graphene oxide (GO) - self-assembling peptide hybrid hydrogels (Fig 1.) as a potential delivery platform for cells and/or drugs.[1-2]

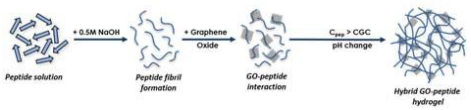

Fig 1.: Schematic representation of the gelation pathway of GO-peptide hybrid hydrogels

Intervertebral disc (IVD) degeneration, a cell-driven process that starts in the gelatinous nucleus pulposus (NP), is a major cause of low back pain[1], is a particularly relevant example where a minimally invasive cellular therapy could bring significant benefits in particular at the early stages of the disease.
In this current study, we explored the possibility of using the GO-self-assembling hybrid peptide hydrogels present as a vehicle for the sequestration and controlled delivery of transforming growth factor beta-3 (TGF- β3), an anabolic growth factor (GF) known to direct NP cell fate and function.

EXPERIMENTAL METHODS

We investigated the adsorption and sequestration of TGF- β3 on GO flakes using a range of different techniques including atomic force microscopy (AFM) and fluorescent spectroscopy. The TGF- β3-coated GO flakes were then incorporated into F8 hydrogels to form GF-loaded hybrid hydrogels that were used for the 3D culture of bovine NP cells over a 3-week period. The effect of the method used to include TGF- β3 on NP-marker gene and protein expressions was investigated by real-time quantitative polymerase chain reaction (RT-qPCR) and, histology and immuno- histochemistry (IHC), respectively. Finally, the interactions between NP cells and TGF- β3-loaded GO flakes were visualized using trans- mission electron microscopy (TEM).

RESULTS AND DISCUSSION

Our results clearly showed that GO flakes can sequestrate TGF- β3 through strong binding interactions resulting in a slow and prolonged release, with the GF remaining active even when bound to the GO flakes. The adsorption of the GF on the GO flakes to create TGF- β3-loaded GO flakes and their subsequent incorporation in the hydrogels through mixing, [(GO/TGF-β3 Ads)-F8] hydrogel, led to the upregulation of NP-specific genes, accompanied by the production and deposition of an NP-like ECM, rich in aggrecan and collagen II. NP cells actively interacted with TGF- β3-loaded GO flakes and remodelled the scaffolds through endocytosis.

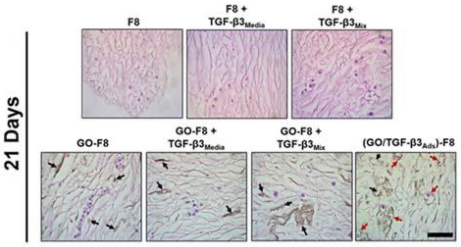

Fig 1.: Representative H&E histological staining images of hydrogels after 21 days of bovine NP 3D cell culture.

CONCLUSION

This work highlights the potential of using GO as a growth factor nanocarrier for the design of functional hybrid peptide-based hydrogels.

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ACKNOWLEDGMENTS

The authors acknowledge financial support from the Engineering and Physical Sciences Research Council (EPSRC) and the Medical Research Council (MRC): Early Career Research Fellowship (EP/K016210/1), Centre for Doctoral Training (CDT) in Regenerative Medicine (EP/L014904/1).

>> 11:00 - 12:00 | PSOP-23 - CELL AND BACTERIA SURFACE INTERACTIONS
Chairpersons: Emmanuel Pauthe & Maria Asplund
Location: Room H

11:00 | O1 Cell & Bacteria interactions - From laboratory scale to commercialization – Conception and evaluation for a virucidal surgical mask during the COVID-19 pandemic (Cidaltex®)
Nicolas BLANCHEMAIN, Univ. Lille, INSERM, CHU Lille, U1008 – Advanced Drug Delivery Systems, Lille, France

11:15 | O2 Cell & Bacteria interactions - Metallic Glass systems for biomedical applications
Ziba NAJMI, Center for Translational Research on Autoimmune and Allergic Diseases CAAD, Università del Piemonte Orientale UPO

11:30 | O3 Cell & Bacteria interactions - Controlling blood-material interaction and osseointegration potential by tuning the composition of amorphous metals
Markus ROTTMAR, Laboratory for Biointerfaces, Empa, Swiss Federal Laboratories for Materials Science and Technology, St Galen, Switzerland

11:45 | O4 Cell & Bacteria interactions - Poly-L-Lysine and Human Plasmatic Fibronectin Films as Bifunctional Coatings to Reduce Bacterial Adhesion and Enhance Tissue Integration
Anamar MIRANDA, Equipe de Recherche sur les Relations Matrice Extracellulaire Cellules, Institut des Matériaux, CY Cergy-Paris Université, Cergy-Pontoise, France

From laboratory scale to commercialization – Conception and evaluation for a virucidal surgical mask during the COVID-19 pandemic (Cidaltex®)

Nicolas Blanchemain[1*], Mickael Maton[1], Gaétan Gerber[2], Pascal Odou[3], Michèle Vialette[4], Camille Sacareau[4], Antony Pinon[4], Christel Neut[5], Bernard Martel[6]

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INTRODUCTION

The World Health Organization (WHO) advises the use of masks as part of a comprehensive package of prevention and control measures to limit among other the spread of SARS-CoV-2, the virus that causes COVID-19[1]. Respiratory and surgical masks ensure the individual and collective protection by playing a barrier toward the virus. However, viruses are trapped but not deactivated, which remains a potential source of contamination. To prevent such drawbacks and enhance their protective effect, an antiviral functionalization of these conventional mask can be done [2]. In 2011, during the H5N1 pandemic, we developed and patented a process of biocide functionalization of filtering media through the immobilization of quaternary ammonium on functionalized polypropylene fiber with polymer of cyclodextrins [3]. In March 2020, we have valorized these results to produce in record time virucidal respiratory and surgical masks on an industrial scale with the Bioserenity (Troyes, France). After the conception, the evaluations on breathability as well as comfort, biocompatibility, antibacterial and antiviral efficacy were performed before the CE marking in February 2021 and the marketing (Cidaltex®).

EXPERIMENTAL METHODS

The non-woven polypropylene (PP, 50g/m², Lydall, France) was rolled padding in aqueous solutions containing a polycarboxylic acid, a catalyst and cyclodextrin (HPβCD) and a surfactant. A curing step was then applied in the oven to provoke the crosslinking reaction. The functionalized nonwoven PP (PPCD) was then roll padded in ammonium dimethyl benzalkonium chloride (ADBAC) and dried in the oven at 100°C (PP-CD-ADBAC). The morphological architectures of nonwoven were observed by SEM (Hitachi SS 4700 SEM field emission GU). The ADBAC loading was evaluated after water and NaOH 0.1N rinsing using UPLC-DAD (Nexera i, Shimadzu, Japan) with a reverse-phase column (C18 Diphenyl SpeedCore 2,6 µm, 100×2.1mm, Fortis, England). The filtration efficiency and air permeability were analyzed with airborne particles counter (AeroTrak® model 9550). Kill-time test was performed on PP-CD-ADBAC to evaluate the kinetics of the bacterial reduction of *S. aureus* and *E. coli* according to JIS 2801/2000. The kinetics of the virus reduction of HCoV-229E was performed according to ISO 18184. The full masks were produced including the virucidal layer inside the mask in order to conduct a usability study on 12 volunteers. Statistic evaluation was performed for each test.

RESULTS AND DISCUSSION

The functionalization parameter optimization allowed to carry out textile production on an industrial line adapted to the manufacture of masks. The optimal ADBAC loading was determined for the final production. The SEM observation showed the homogeneity of the functionalization without clogging the porosity of the nonwoven. This observation was confirmed by measuring the breathability and filtration efficiency of the Cidaltex® layer, which are maintained compared to a traditional mask. The efficacy of the antibacterial layer was proven against *E. coli* and *S. aureus* with a bacterial reduction of 99.99% in less than 1 and 2 hours of contact respectively. Similarly, antiviral efficacy was demonstrated on HCoV-229E with a reduction of 99.96% in 20 minutes. 12 volunteers wore 10 masks for 4 hours each (5 conventional masks 5 virucidal masks). No significant difference was observed in terms of humidity, breathability and comfort between virucidal masks and conventional masks. Then, the different layers of the mask were analyzed before and after wearing. Extraction studies have not shown the presence of virucidal agent on the outer layers of the mask (neither in contact with the face skin nor the outer side). This study shows the usability but also the safety of using the Cidaltex®.

CONCLUSION

We proved the safety, performance and effectiveness of a new single-use surgical mask developed, validated (CE marking) and marketed during the COVID-19 pandemic. The technological transfer from laboratory to an industrial scale was achieved and opens up new prospects for the antiviral filtration of buildings and transport...

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Metallic Glass systems for biomedical applications

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INTRODUCTION

Metals and alloys contribute to about 70% of medical implants. However, the loss of metallic implants is often due to the formation of bacterial biofilm on their surface. Many have focused on metal surface functionalization and/or antibacterial coatings. The most famous antibacterial agents used in antibacterial coatings include Ag, Cu, and Zn. **Metallic glasses** are a revolutionary class of materials that their crystallization upon solidification is suppressed. Absence of crystalline structure is the reason for their extraordinary properties over traditional crystalline alloys such as wear resistance. They are more compatible with bone because of their reduced Young modulus. Moreover, it has been recently found that if efficient number of antibacterial elements such as Ag or Cu are present in their chemical composition, they can show intrinsic antibacterial properties. This is very different from when Ag or Cu is used as an antibacterial agent in the form of nanoparticles because Ag and Cu in metallic glasses are in the form of randomly distributed atoms. No insightful explanation on the mechanism of antibacterial activity in metallic glasses is provided. In this work, we have investigated series of different compositions based on Zr-Cu-Ag metallic glasses for their antibacterial properties. Our findings suggests that for metallic glasses, it is the surface energy which is responsible for antifouling properties and individual Cu and/or Ag released ions from the surface are not responsible for the antibacterial properties. The resulting investigation has opened a new door for generation of other metallic glass systems as implantable devices such as Ti-based metallic glasses for dental implants[1].

EXPERIMENTAL METHODS

Zr-based metallic glass coatings: A range of different chemical compositions were prepared via Physical Vapor Deposition magnetron by co-sputtering Zr, Cu, and Ag onto a Polybutylene terephthalate (PBT) substrate. The coated samples were investigated for their surface wettability by static and dynamic contact angle measurements. Samples were further investigated for their structural properties by XRD, and SEM. Wear release was measured by ICP-MS. Antifouling properties were evaluated by Gram-positive *Staphylococcus aureus* and the Gram-negative *Escherichia coli* strain visually confirmed by FESEM and fluorescent live/dead staining. Human mesenchymal stem cells (hMSC) were used for direct cytocompatibility evaluation and their metabolic activity was evaluated via relative fluorescence unit. The results were further visualized by FESEM, and fluorescent-staining. Finally, hMSC' cytoplasm was stained by May Grunwald and Giemsa to detect and visualize the released ions which have diffused through the cells' membrane. **Ti-based bulk metallic glass implants**: In this work, $Ti_{40}Zr_{10}Cu_{36}Pd_{14}$ bulk metallic glass was fabricated by suction casting. Series of structural analysis was conducted on the sample including XRD, DSC, TEM, SEM, DMA, TE, and wear resistance analysis. The antibacterial properties were conducted by *Aggregatibacter actinomycetemcomitans* and real human dental plaque. All tests were conducted like Zr-based metallic glass. Furthermore, high throughput proteomics analysis was also conducted here.

RESULTS AND DISCUSSION

XRD has confirmed the formation of amorphous structure of the coating. Static and dynamic water contact angle measurement shown that the hydrophobic surface of PBT($124.5^{\circ}\pm0.3$) turn into superhydrophobic surface ($150.8^{\circ}\pm0.1$,hysteresis=3.5°). Ions released from coated samples has shown that mainly copper is released into the solutions (≈50–120 ppb), and even in that case, its concentration is extremely below the toxic level for humans. These results indicate that the non-wetting surface of the coating is responsible for antibacterial properties as ion-release from samples is extremely low. Specimens' surfaces infected with the Gram+ *S.aureus* and the Gram- *E.coli* strain demonstrated a strong preventive antifouling property as ≈95% of the inoculated bacteria did not adhere into the surface determined by the count of the colony forming unit (CFU). Results were visually confirmed by FESEM images and fluorescent live/dead staining where only few viable single colonies were observed, thus excluding the formation of biofilm. Regarding direct cytocompatibility evaluation, hMSC were able to adhere and spread along coated fibers showing a metabolic activity comparable (≈90%) to those cultivated onto non-coated PBT. Similar trend was observed for bulk Ti-based metallic glass.

CONCLUSION

The combination of intrinsic antifouling properties, cytocompatibility, lower elastic modulus, and greater biocorrosion resistance makes metallic glasses as promising implantable devices to substitute the current state of the art.

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ACKNOWLEDGMENTS

E.S would like to thank European Commission for providing funding for this project under the Horizon 2020 for Marie Skłodowska-Curie Individual Fellowship, with the acronym "MAGIC" and grant agreement N. 892050.

Controlling blood-material interaction and osseointegration potential by tuning the composition of amorphous metals

William A. Lackington[1], Romy Wiestner[1], Elena Pradervand[2], Martina Cihova[2], Eike Müller[1], Yashoda Chandorkar[1], Mihai Stoica[2], Katharina Maniura[1], Jörg F. Löffler[2], Markus Rottmar[1*]

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INTRODUCTION

Metals have become a standard material class for implants as well as for blood-contacting devices. While their high thrombogenic potential can be favorable in steering the tissue response for implants, they usually require complex surface coatings and systemic anticoagulation therapy when used in blood-contacting devices. Metallic glasses are gaining increasing attention as promising materials for such applications due to their increased strength and elasticity, as well as their superior corrosion and wear resistance compared to their crystalline counterparts. This study aims to understand the impact of material surface physicochemical properties and material bulk atomic order on the blood response towards metallic glasses, in comparison to medical grade crystalline Ti64, as well as on subsequent cell responses that govern a material's osseointegration.

EXPERIMENTAL METHODS

Pd-, Pt-, Ti-, and Zr-based metallic glasses with different elemental compositions were prepared and characterized via XRD, XPS and WCA measurements. Ti64 was used as a reference material. Samples were incubated for up to 48 min in partially heparinized human whole blood, washed with PBS and fixed for subsequent analysis. Supernatants were collected for analysis via ELISA. Platelet adhesion and activation, fibrin formation as well as expression of biomarkers specific for the blood coagulation cascade (PF4, TAT, F1/F2) were analyzed by SEM, CLSM, and ELISA. Attachment, spreading and growth as well as in vitro mineralization of human primary bone progenitor cells on blood pre-incubated surfaces was assessed by CLSM and by a quantitative calcium assay after up to 28 days of culture.

RESULTS AND DISCUSSION

Upon incubation with blood, a reduced thrombogenic potential of Pd-glass in comparison to Ti64 was observed, while no significant differences between Ti64 and Ti- or Zr-based metallic glasses were found. Interestingly, enhanced platelet adhesion but reduced fibrin network formation and lower expression of further blood coagulation cascade biomarkers was found on the surface of Pd-based metallic glass in comparison to Ti64 and Ti- or Zr-based metallic glasses. Evaluating the influence of the metal atomic order on the blood response, no differences were observed when comparing the same Pd-based alloy in its amorphous and crystalline state.

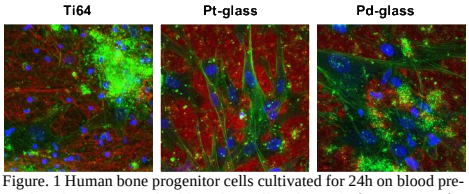

Figure. 1 Human bone progenitor cells cultivated for 24h on blood pre-incubated Ti64, Pt- and Pd-based metallic glasses before staining for fibrin (red), actin cytoskeleton (green) and cell nuclei (blue).

An enhanced blood coagulation compared to Ti64 was observed when modifying the composition of the Pd-glass and also exploring a similar Pt-glass. Both glasses exhibited a significantly higher level of mineralization of human bone progenitor cells, while cell attachment and spreading was similar on the metallic glasses and Ti64 (Figure 1).

CONCLUSION

The blood response is dependent on the chemical composition of metallic glasses and can thus be exploited to either favor or prevent blood coagulation. Notably, not the bulk metal atomic order, but the surface chemistry is decisive for controlling the blood–material interaction and subsequent osteogenic differentiation.

ACKNOWLEDGMENTS

Funding from Uniscientia Foundation is gratefully acknowledged. This study was conducted as part of the Zurich Heart project of Hochschulmedizin Zurich.

Poly-L-Lysine and IIuman Plasmatic Fibronectin Films as Bifunctional Coatings to Reduce Bacterial Adhesion and Enhance Tissue Integration

Anamar Miranda[1], Damien Seyer[1], Carla Palomino-Durand[1], Houda Morakchi-Goudjil[1], Mathilde Massonie[1], Rémy Agniel[1], Hassan Rammal[1,2], Emmanuel Pauthe[1] and Adeline Gand[1]

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[2] EFOR Healthcare Paris, Biocompatibility Platform, Levallois-Perret, France
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INTRODUCTION

Long-term dental implants success is usually compromised by a poor soft tissue fixation, leading to a breach open to bacteria colonization and infection. Strategies for implant surface functionalization are thus being explored. The Layer-by-Layer (LbL) technique, based on the alternate deposition of oppositely charged polyelectrolytes[1], allows the construction of thin films which can serve as reservoir of bioactive molecules to confer antibacterial properties and control cell behavior[2]. A bifunctional biomimetic LbL coating is here proposed, obtained by the association of fibronectin (Fn) and a 30 residues poly-L-lysine (PLL_{30}). Fn, an extracellular matrix protein is used as polyanion for its pro-adhesive properties[3] and PLL_{30} as polycation due to its intrinsic antimicrobial properties[4]. PLL_{30}-Fn film construction and characterization was studied as well as its effect on human gingival fibroblasts (HGF) and 3 bacterial strains involved in implant-related infections, initiation of dental caries and peri-implantitis lesions -*Staphylococcus aureus, Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans*-. Cells and bacteria behavior was observed both separately and in co-culture.

EXPERIMENTAL METHODS

$(PLL_{30}\text{-Fn})_{10}$ films were built on 14-mm-diameter glass slides by the LbL technique using PLL_{30} and Fn. Film construction and topography was assessed by ATR-FTIR, SEM and AFM. HGF behavior and ability to remodel Fn, was evaluated by fluorescent labelling, immunostaining and Confocal Microscopy observation. Bacterial adhesion on the coating was checked after 2 h of incubation. Co-culture systems included HGF inoculation prior to bacteria, bacteria before HGF and both simultaneously. ANOVA and independent samples t-test were used for statistical analysis.

RESULTS AND DISCUSSION

PLL_{30}-Fn LbL assembly resulted in a sub-linear, saturating growth, reaching a plateau after 10 bilayers. $(PLL_{30}\text{-Fn})_{10}$ film morphology appeared heterogeneous, with the presence of aggregates and film thickness of the ranged from 200-300 nm to 700 nm-1 µm in zones containing large aggregates. A higher rigidity was found around the aggregates (up to 46 MPa), whereas the latter showed lower rigidity values (5–30 MPa). The films were cytocompatibles and showed enhanced cell adhesion. HGF presented an increase in vinculin beams on the coating compared to bare glass or Fn monolayers. The cells were also able to reorganize the Fn within the film into fibrils, supporting the presence of focal adhesions and the good cell-matrix interaction.

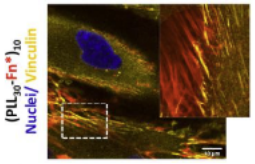

***Fig.1**. Focal adhesions of HGF on $(PLL_{30}\text{-Fn})_{10}$ films after 2 days of incubation. Fn was labelled with Alexa Fluor 568. Vinculin (Star Red-yellow), nuclei (DAPI-blue). Scale bar = 10µm*

The attachment of *S. mutans* at 2h onto the film diminished by up to 70% compared to bare glass while *S. aureus* adhesion was totally inhibited, even to a higher extent than observed for PLL_{30} monolayers. Thus, the modified surface could constitute a reservoir for the delivery of PLL_{30}, which could diffuse to the surface and interact with bacteria. Limiting the initial bacterial adhesion could compromise the biofilm formation responsible of most implant-related infections.

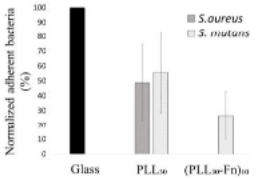

***Fig.2**. Percentage of adherent S. aureus and S. mutans after 2 h of incubation on PLL_{30} monolayers and $(PLL_{30}\text{-Fn})_{10}$ films, normalized to bacteria adhered on glass. No S. aureus are found to attach to $(PLL_{30}\text{-Fn})_{10}$ films.*

The bifunctional effect of the films was also tested in co-culture of HGF and three bacteria strains.

CONCLUSION

Here we developed a bifunctional coating that favors cell adhesion and minimizes bacterial attachment by the combination of Fn and PLL_{30} in an LbL assembly. These findings shed light on the potential of thin films constructed with Fn and PLL_{30}, to favor material-tissue integration while avoiding bacterial attachment.

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ACKNOWLEDGMENTS

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>> 11:00 - 12:00 | PSOP-24 - HYDROGELS III
Chairpersons: Elisabeth Engel & Francis Max Yavitt
Location: Room B

11:00 | O1 Hydrogels III - Enzyme-controlled, nutritive hydrogel for mesenchymal stromal cell survival and paracrine functions
Pauline WOSINSKI, Université de Paris, B3OA CNRS 7052 INSERM U1271, Paris, France & Ecole Nationale Vétérinaire d'Alfort, B3OA, Maisons-Alfort, France

11:15 | O2 Hydrogels III - Self Assembly Peptide Based Double Network Hydrogel
Zixuan LIU, Manchester Institute of Biotechnology, The University of Manchester, Manchester, UK; Department of Materials, The University of Manchester, Manchester, UK

11:30 | O3 Hydrogels III - Tuning viscoelasticity in colloidal nanoparticle hydrogels towards enhanced cell activity
Pascal BERTSCH, Radboud University Medical Center, Regenerative Biomaterials, Nijmegen, The Netherlands

11:45 | O4 Hydrogels III - A multifunctional and programmable micro-platform for localized tumor invasion studies
Pouyan BOUKANY, Department of Chemical Engineering, Delft university of technology, Delft, The Netherlands

Tuning viscoelasticity in colloidal nanoparticle hydrogels towards enhanced cell activity

Pascal Bertsch[1], Lea Andrée[1], Negar Hassani Besheli[1], and Sander C. G. Leeuwenburgh[1]

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INTRODUCTION

Viscoelastic hydrogel properties such as stress relaxation and plasticity have recently been recognized as important mechanical cues that govern the migration, proliferation, and differentiation of embedded cells.[1,2] Traditional monolithic polymeric hydrogels often exhibit a limited stress relaxation capacity which can impede cellular activity. In contrast, colloidal hydrogels assembled from nanoscale particles provide a new class of biomaterials with highly tunable viscoelasticity. Due to the non-covalent interactions within individual particles, colloidal hydrogels are inherently injectable and self-healing.[3] Furthermore, the viscoelastic properties of colloidal hydrogels can be fine-tuned via particle surface charge and combination with different organic/inorganic particles.[4,5] Here, we will discuss our recent work on the strain-sensitive stress relaxation in colloidal gelatin nanoparticle hydrogels.[6]

EXPERIMENTAL METHODS

Monodisperse gelatin nanoparticles were prepared based on desolvation of gelatin with acetone as described in detail before.[3] Colloidal hydrogels were prepared by dispersing 18 wt% gelatin particles in demineralized water. 18wt% physical gelatin gels were prepared from the same gelatin by heat-setting directly in the rheometer. Colloidal and physical gelatin hydrogels were characterized rheologically using a TA AR2000ex rheometer by performing strain sweeps and stress relaxation experiments. Stress relaxation was determined in step strain experiments at stepwise increasing strain following a newly introduced protocol.[6]

RESULTS AND DISCUSSION

We have designed a colloidal hydrogel assembled from gelatin nanoparticles which exhibits a comparable rheology to a monolithic physical gelatin hydrogel at rest (i.e. small deformations), but is highly strain-sensitive and fluidizes at strains relevant for cell activity, i.e. 10-50%, as visualized by strain sweeps in Figure 1A. The strain-induced fluidization is associated with a fast exponential stress relaxation in colloidal hydrogels which is strain-dependent and further accelerated at increasing strain (Figure 1B). Up to 50% of the induced stress is relaxed within a few seconds in colloidal hydrogels at strains typically induced by cells. In contrast, the physical hydrogels exhibit a slower stress relaxation limited to about 20% independent of strain (Figure 1C). Colloidal hydrogels with added surfactant and at high ionic strength were prepared to screen hydrophobic and electrostatic interactions, respectively. Thereby, we found that interparticle hydrophobic interactions are the driving force for stress build-up and limit stress relaxation in colloidal hydrogels.

CONCLUSION

Colloidal hydrogels formed from gelatin nanoparticles pose a promising biomaterial platform for hydrogels with tunable and strain-sensitive stress relaxation for enhanced cell activity.

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ACKNOWLEDGMENTS

The Netherlands Organization for Scientific Research (NWO, VICI grant #17835) is acknowledged for funding.

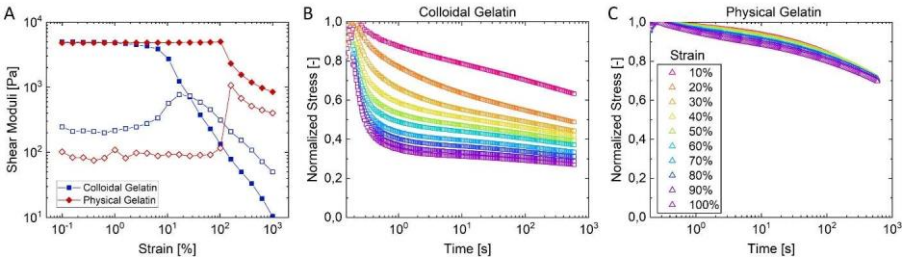

Figure 1: A) Strain sweep revealing strain-dependent rheology of 18wt% colloidal and physical gelatin hydrogels and normalized stress relaxation of B) colloidal and C) physical gelatin hydrogels showing strain-dependent stress relaxation.

Self-assembly of Peptide Based Double Network Hydrogels

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INTRODUCTION

Exploiting the potential of molecular self-assembly to produce structured and functional nanomaterials has attracted significant interest over the past two decades [1]. This method of material preparation typically involves the design of individual molecular building blocks to enable their spontaneous self-assembly across the length scales in response to certain triggers, including changes in environmental conditions, such as changes in pH, temperature or the presence of enzymes. This method is accepted to be superior in comparison with the more traditional method of material construction because the self-assembled structure usually stays in a highly ordered structure, rather than a random like state [2].

Of particular interest over the past few years has been the self-assembling behavior of oligopeptides [3], due primarily to their building block being composed of natural amino acids. This means the nanostructures formed are typically inherently biocompatible. These self-assembling peptides (SAPs) go onto form a range of nanostructures depending on their primary structure: from alpha-helices, to beta-sheet fibrils. The latter has attracted significant interest as when above a critical concentration the fibrils associate and/or entangle to form 3-dimensional (3D) hydrogels whose structure is reminiscent of the natural Extracellular Matrix. As a consequence, this has opened up a number of biomedical application opportunities including for tissue regeneration or substrates for the growth of organoids [4]. One exemplar peptide sequence in this family is FEFKFEFK (F8) where F, E and K represent phenylalanine, glutamic acid and lysine respectively. When exposed to a suitable pH and concentration of the F8 peptides come together to form anti-parallel beta-sheet structures, grow to form fibrils which come together and entangle to for a self-supporting transparent hydrogel (Figure 1A). Excellent biological properties, recoverability and easy preparation are among such hydrogels many advantages of for example synthetic polymer hydrogels. One disadvantage of using the F8 peptide is that the pH at which it self-assembles and forms a hydrogel is ~4. To make the hydrogel preparation more robust, additional charges have been introduced to the peptide sequence, i.e. FEFKFEFKK (F9) and KFEFKFEFKK (KF8K) (Figures 1B&E) which hold a net position charge of one and two respectively at pH 7.

Here, we will explore the potential to form double peptide networks and our ability to tune both hydrogel structure and mechanical strength by combining different ratios of KF8K with F9 peptides and monitoring hydrogel behavior. The overarching aim of this work is to prepare robust and reproducible hydrogels that can be used as bio-inks for 3D bioprinting for cell culture applications.

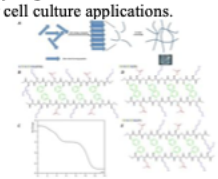

Figure 1 (A) Schematic highlighting the process of peptide self-assembly, (B,D,E) chemical structure of SAPs respectively, and (C) net charge of KF8K as a function of pH

EXPERIMENTAL METHODS

The peptide labelled FITC (florophore) and Dabcyl (quencher) quenching reaction was designed to expose the distribution of peptide chains within self-assembled hydrogels composed of single or double peptide sequences. The quenching reaction of FITC-Dabcyl will only occur when they are close together, i.e. when the distance between the two different functional molecules is between 1 and 10 nm. The control sample in these experiments is when the KF8K-FITC and KF8K-Dabcyl labelled peptides are introduced into a pure KF8K sample. Subsequent experiments involved mixing the same concentration of the KF8K-florophore and quencher peptides into different ratios of KF8K and F9 peptide samples and monitoring for any change in the quench percentage. If peptides of the same sequence prefer to accumulate and self-assemble together, then the quench ratio will increase. If the self-assembly process is random, as shown below, then the quench percentage will not vary with varying peptide ratio.

RESULTS AND DISCUSSION

F8 and F9 peptide sequences were independently mixed with KF8K peptide containing KF8K-FITC and KF8K-Dabcyl in a 1 to 1 ratio. No change in the quenching ratio was observed in either case when compared with the pure KF8K system. This indicates that the peptides in each system formed a random distribution, highlighting that the slight difference in net charge between F8, F9 and KF8K and the presence of the additional lysine/s do not introduce a tendency for similar peptides to accumulate together. The same ratio of F8/F9 peptide was subsequently replaced by the alanine equivalent peptide, AEAKAEAK (A8). This led to an improvement in the quenching ratio which is likely due to the A8 not being susceptible to self-assembly which led to an increase in the density of KF8K in the fibre. One further example was the mixing of fibres of different chirality. The quenching percentage of the standard L-KF8K blended hydrogel was 45%. When 50% of the L-KF8K peptide was replace with the D version of the KF8K peptide, there was a significant increase in the quenching (up to 60%), indicating there was an obvious accumulation, and hence separate L and D fibres forming.

CONCLUSION

In summary, we have investigated the distribution of different peptide sequences within the final self-assembled structure through the comparing the FITC-Dabcyl quenching ratio between pure KF8K system and KF8K contained blending system. Our results show the random distribution of F8 and F9 in their 1 to 1 ratio blending system with KF8K while an accumulation tendency was proved when the same ratio of F8 or F9 were replaced by D-KF8K. One further example is the present of A8 will not attend the self-assembly and form fibre process when they are blending with KF8K.

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Enzyme-controlled, nutritive hydrogel for mesenchymal stromal cell survival and paracrine functions

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[4] Centre de Recherche en Neuroscience de Lyon, TIGER team et plateforme technique AniRA-Beliv, Inserm U2018, CNRS 5292, Université Lyon 1, Lyon, France.
[5] Sorbonne Université, INSERM, AIM, Centre de Recherche en Myologie, UMRS 974, AP-HP, Hôpital Pitié Salpêtrière, 75013, Paris, France
[=] Contributed equally to this work and should be considerate as co-first
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INTRODUCTION

Human Mesenchymal stromal cells (hMSC) are appealing candidates for regenerative medicine applications. However, upon implantation, they encounter an ischemic microenvironment depleted of oxygen and nutrients responsible for their massive death post-transplantation, a major roadblock to successful cell-based therapies. To date, various approaches have been proposed to address this issue, albeit with limited clinical success. Interestingly, previous studies established that an *in situ* supply of glucose that acts as the main metabolic fuel in hypoxic conditions, increases the survival of MSCs[1,2]. We hereby propose a paradigm shift for enhancing hMSC survival by developing an enzyme-controlled, nutritive hydrogel with an inbuilt glucose delivery system for the first time. This novel hydrogel comprises fibrin, wheat starch (a glucose polymer), and amyloglucosidase (AMG), an enzyme that catalyzes the hydrolysis of starch into glucose.

EXPERIMENTAL METHODS

In vitro: Glucose concentration at the core of hydrogels was determined using a custom-made glucose electrode biosensor. hMSC survival was assessed by cytometry after releasing cells from cell-loaded hydrogels exposed at 0.1% oxygen for up to day 14 (n=9). The chemotactic potential of hMSCs towards hMSC (n=6) and Human Umbilical Vein Endothelial Cells (HUVEC) (n=3) was assessed by collecting conditioned Media (CM) from these hMSC-loaded hydrogels and evaluating migration in Boyden chambers. Moreover, chemotactic and angiogenic cytokines in CM were quantified using Luminex® (n=6). ***In vivo:*** fluorescent-labeled AMG leakage from cell-free fibrin hydrogels was monitored using Xenogen live imaging until day 14 after ectopic implantation in nude mice (n=6). Luciferase-labeled hMSC survival within fibrin/starch/AMG and fibrin hydrogels was assessed in an ectopic nude mice model by bioluminescence imaging until day 14 (n=8). New blood vessel formation in the hydrogel vicinity was determined by µCT scanner, using a radiopaque agent Microfil® perfused within blood vessels at days 7 and 14 (n=16). ***Statistics:*** Data were mean and SEM. Statistical analysis has been made using analysis of variance and the Bonferroni post-test and the Mann-Whitney test, respectively, for the parametric and non-parametric test. For all studies, the confidence interval was set at 95% and the significant level at $p < 0.05$.

RESULTS AND DISCUSSION

The Results and In vitro fibrin/starch/AMG hydrogels released glucose at physiological concentrations and exhibited a 95-fold increase in hMSC survival compared to fibrin hydrogels after 14 days. CM collected from hMSC loaded fibrin/starch/AMG hydrogels showed (i) a 9- and a 4-fold increase in chemotactic potential towards hMSCs and HUVECs and (ii) a statistically significant rise in most but not all chemotactic and angiogenic cytokines amount compared to hMSC loaded fibrin hydrogels. In vivo glucose concentration within cell-free fibrin/starch/AMG hydrogels was within physiological ranges at days 7 and 14. Fluorescence monitoring revealed that AMG had completely disappeared within 7 days. hMSCs viability (measured by bioluminescent signal intensity compared to day 1) was 76.4% within fibrin/starch/AMG hydrogels and 22.1% within fibrin hydrogels at day 7. The hMSCs viability decreased drastically between days 7 and 14, corroborating the AMG time course. Finally, the formation of new blood vessels in the hydrogel vicinity exhibited a 4-fold increase when using fibrin/starch/AMG hydrogels compared to fibrin hydrogels at day 21.

CONCLUSION

The Conclusion section presents the outcome of the work by interpreting the findings at a higher level of abstraction than the Discussion and by relating these findings to the motivation stated in the Introduction. Explain the significance of your findings / outcomes and future implications of the results

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ACKNOWLEDGMENTS

The authors would like to thank l'Agence Nationale de la Recherche (ANR-16-ASTR-0012-01 and ANR-12-BSV5-0015-01) and the China 501Scholarship Council (No. 201600160067) for providing financial support to this project.

A multifunctional and programmable micro-platform for localized tumor invasion studies

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INTRODUCTION

Dissemination and invasion of cancer cells from a primary solid tumor (e.g. lung and breast tissue) is a lethal and complex process that is associated with the largest contributor to cancer-related deaths globally. It has been shown that both physical and cellular cues in the tumor microenvironment regulate and coordinate the modes of cell migration into extracellular matrices and surrounding tissues. So far, 3D multicellular aggregates of tumor cells have become a popular in vitro model to investigate the cancer cell invasion in the tumor microenvironment. Yet little is known about the cancer cell invasion in the dynamically changeling, complex and heterogenous tumor microenvironment [1-2]. To address this issue, we created a novel micro-scale platform by integrating multi-functional micro-buckets with soft biomimetic hydrogels to introduce cellular and physical clues in a controlled manner [3].

EXPERIMENTAL METHODS

In this work, we have created a programable micro-buckets-hydrogel platforms to mimic the complexity of the tumor microenvironment for the 3D cellular migration studies. The multifunctional micro-buckets have been generated on a chip based on droplet microfluidics approaches. Both lung and breast cancer cells have been successfully loaded inside these micro-buckets with perfect cell viability.

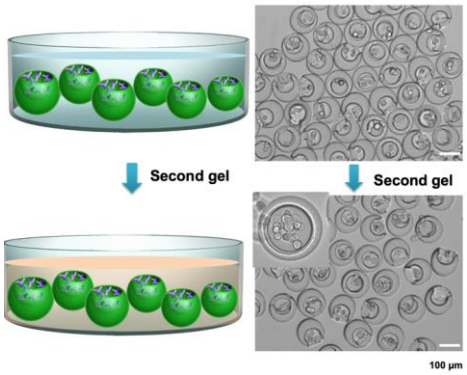

Fig. 1. The novel micro-buckets have been developed and integrated with biomimetic hydrogels for 3D cancer cell migration studies.

Next, several biomimetic hydrogels (e.g. dextran methacrylate, gelatin methacrylate) with different stiffness have been introduced on the top of the micro-bucket to mimic the extra cellular matrices for cell invasion studies.

RESULTS AND DISCUSSION

We have established a unique micro- platform allows for controllable 3D tumor cell migration inside a complex microenvironment for mechanistic studies and biomedical applications.

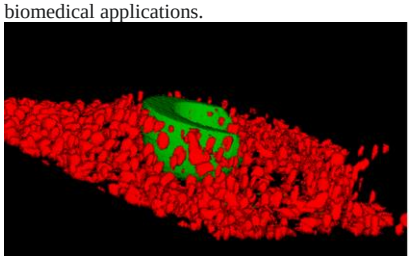

Fig. 2. The confocal images of cancer Cell invasion inside micro-bucket with cellular cues. The cell migration has been triggered and coordinated by using functionalized micro-buckets.

CONCLUSION

In this work, a new micro-platform has been obtained to produce 3D cellular aggregates with controlled volume for long-term cell cultures. Importantly, this programmable and tunable 3D cancer cell invasion micro platform takes a new step towards mimicking dynamically changing tumor microenvironment and exhibits wide potential applications in biomedical field from cancer metastasis research, and cell migration to drug screening.

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ACKNOWLEDGMENTS

The authors would like to thank the European Research Council (ERC) (Grant no: 819424) for providing financial support to this project.

>> 11:00 - 12:00 | PSOP-25 - CELL TISSUE BIOMATERIAL INTERACTIONS II
Chairpersons: Karine Anselme & Riccardo Levato
Location: Room F

11:00 | O1 Cell & Tissue interactions II - Single Cell Artificial Niches (SCANs) to study mechanotransduction guiding stem cell fate
Castro JOHNBOSCO, Leijten Lab, Dept. of Developmental BioEngineering, TechMed Centre, University of Twente, The Netherlands

11:15 | O2 Cell & Tissue interactions II - The Role of Vascularization in Adhesion to Biological Tissues
Estelle PALIERSE, Chimie Moléculaire, Macromoléculaire et Matériaux, ESPCI Paris, PSL University, Paris, France

11:30 | O3 Cell & Tissue interactions II - 3D-bioprinted bionic pancreas as an innovative method of treating and preventing diabetes - how far we are from clinical application?
Michał WSZOŁA, Polbionica Ltd; Foundation of Research and Science Development; CM Medispace, Warsaw, Poland

Single Cell Artificial Niches (SCANs) to study mechanotransduction guiding stem cell fate

Castro Johnbosco[1], Malin Becker[1], Kannan Govindaraj[1], Tom Kamperman[1], and Jeroen Leijten[1]
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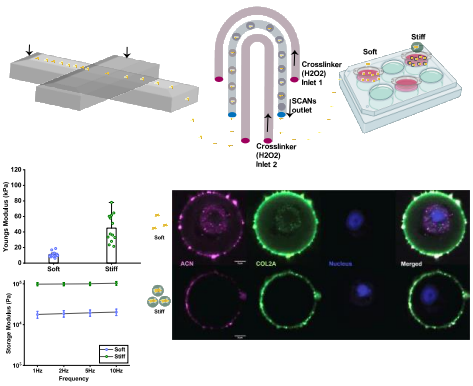
Introduction
Extracellular matrix (ECM) mechanics determine cellular processes including proliferation, differentiation, and migration. However, the role of mechanotransduction on chondrogenic differentiation of mesenchymal stromal cells (MSCs) has remained poorly understood and is notoriously difficult to study due to inter-cell heterogeneity. Indeed, single-cell resolution data on cellular heterogeneity in mechanobiology has remained scarce as current state-of-the-art systems are mostly geared towards multi-cellular cultures and population-level analyses. Hence, a robust system to study mechanoregulation altering the differentiation process in 3D microenvironments at the single-cell level is here proposed. Specifically, we here present a microfluidic system to center individual cells within an engineered matrix effectively creating single cell artificial niches (SCANs). SCANs were demonstrated to be able to scrutinize the mechanistic interaction of mechanotransduction on stem cell fate within 3D environments at single-cell resolution.

Methods
Microfluidic devices were generated using standard photolithography techniques. Dextran tyramine conjugates containing MSCs were emulsified in an oil phase creating microdroplets containing a single cell. The single-cell-laden microdroplets consisting of the polymer networks were crosslinked via diffusion-based supplementation of H2O2 which yielded SCANs. The elasticity of SCANs was controlled by tuning the crosslinker concentration. Elastic moduli were determined using interferometry-based micro-indentation. Soft and stiff SCANs were exposed to a 10 ng/ml TGFB-containing chondrogenic differentiation medium to stimulate chondrogenesis. Fluorescent confocal microscopy imaging was performed to study mechanotransduction-related transcription factors, to determine cytoplasmic/nuclear volume, and to semi-quantify differentiation markers expressed on soft and stiff SCANs after 14 days of culture. Gene expression analysis relating to the chondrogenic genes was analyzed using PCR.

Results
We successfully produced dextran-based SCANs **(Fig.1a)** with an elastic modulus of ~14 kPa (i.e., soft) and ~60 kPa (i.e., stiff), **(Fig.1b)** which were both characterized by elastic behavior. Chondrogenic differentiation markers such as collagen type 2 (COL2A) and aggrecan (ACN) were more abundantly expressed in soft as compared to stiff SCANs **(Fig.1c)**, which was corroborated by gene expression analysis. Activation of transcriptional mechanoregulator Yes-associated protein (YAP) translocation to nucleus was hardly observed, even in stiff SCANs, which is in sharp contrast to MSCs cultured atop 2D mechanically instructive hydrogels. Interestingly, we did observe a change in the nuclear volume of cells depending on the cells residing in soft or stiff SCANs. This suggested that single-cell chondrogenesis of MSCs is been influenced by nuclear volume changes in a manner that is independent of YAP activity.



Conclusion
SCANs are a promising platform with on-demand tunable mechanical properties to study mechanotransduction within 3D microenvironments at the single cell level in a high throughput manner. It is therefore anticipated that SCANs are of high value to study, probe, and elucidate population heterogeneity of mechanotransduction-guided cellular behavior.

Keywords: Single cell, mechanotransduction, stem cell fate

Acknowledgements
Financial support was received from the Dutch Research Council (NWO, Vidi Grant, #17522).

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The Role of Vascularization in Adhesion to Biological Tissues

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INTRODUCTION

Fastening hydrogels and elastomers to the surface of soft internal organs is of great interest for the development of implantable devices. However, the achievement of adherence is greatly challenged by the highly hydrated nature of biological tissues.[1] In the case of adhesion to the liver capsule, a recent study using model PEG films has shown that the dramatic loss of adhesion observed when going from ex vivo to in vivo conditions is explained by the continuous wetting of the film-tissue interface when the tissues are actively vascularized.[2] These results suggest that the performance of tissue adhesives can be modulated in vivo depending on the vascularization of the underlying tissue. Here, we explore this hypothesis using an in vivo model where we reduce tissue vascularization by temporarily excluding a liver lobe from the internal blood circulation. In this approach, weakly cross-linked poly(β-thioester) (PBT) films were used as adhesive films able to bind with tissue proteins through hydrogen bonding and hydrophobic interactions. We designed a portable peeling protocol and measured the adhesion energy between these films and the capsule of porcine liver while simultaneously monitoring the evolution of the exudation at the liver surface.

EXPERIMENTAL METHODS

Materials: Soft poly(β-thioester) films having a 0.5 mm thickness and low Young's modulus (0.4 kPa) were fabricated by photo-cross-linking of PBT diacrylates in presence of multifunctional thiols.[3]

Animal care: Three healthy Pré-Alpes pigs (1y.o., 60-70 kg) were obtained from a licensed vendor. They were cared for in accordance with the European guidelines. The study was approved by the French national ethical committee (ref: C2EA-16/18-029, 2018030517071534).

Surgical protocol: Each pig underwent a ventral midline laparotomy using standard surgical techniques. One hour after the laparotomy, a vascular occlusion of the left lateral lobe of the liver was performed with the establishment of a vascular lac. After 40 min, the lac was loosened to allow revascularization of the lobe.

Adhesion measurements: For each measurement, a 1 cm-wide 10 cm-long PBT ribbon was placed onto the surface of the liver or spleen capsule. Pressure was applied digitally during 30 s. Peeling at 90° angle was performed after 5 min contact with a manual dynamometer (Imada ZTA-DPU-10N) at a speed of 10±5 mm.s^{-1}.

Exudation measurements: During each experiment, the flux of exuded fluid at a given time was measured by weighing the mass uptake of squares of filter paper (4 cm²) deposited on the liver surface for 5 min.

RESULTS AND DISCUSSION

In ex vivo conditions, control peeling experiments confirmed that the studied PBT films adhere significantly to the liver capsule (20±3 J.m^{-2} @ 10 mm.s^{-1}). For in vivo conditions, the situation differs greatly as shown in Fig. 1A. In the normally vascularized state, exudation was observed at the surface of the liver. The adhesion energy between PBT films and the liver after a 5 min contact was hardly measurable (0-4 J.m^{-2}). A rapid decrease of the liver exudation was then produced by performing a vascular occlusion. Concomitantly, the film-liver adhesion energy increased remarkably up to 20-30 J.m^{-2}. This effect was reversible: after revascularization of the lobe, surface exudation resumed and the adhesion energy was strongly reduced again. These results show that the establishment of adhesion strongly depends on the exudation at the tissue surface. For the studied PBT films, a transition from adhesive to lubricated contact is observed for a flux of 0.4 mg.cm^{-2}.min^{-1} (Fig. 1B). Interestingly, these results also suggest that adhesion depends on the vascularization of each organ. Accordingly, a much stronger adhesion (19±10 J.m^{-2}) were obtained on the spleen capsule which has a very different vascularization from the liver (Fig. 1A&B).

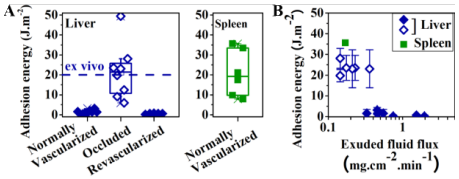

Fig.1 A: In vivo adhesion energies of PBT films on the liver in different vascularized states (left) and on the spleen in its naturally vascularized state (right). B: Adhesion energy as a function of the flux of exuded fluid for the liver and the spleen.

CONCLUSION

This study reports an original in vivo protocol combining peeling test and monitoring of tissue exudation to characterize tissue adhesives. We demonstrate that tissue vascularization plays a crucial role in the establishment of adhesion. These findings provide new insights in the design of tissue specific adhesives.

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ACKNOWLEDGMENTS

Financial support of the French National Research Agency (ANR-18-CE19-0022-04) is acknowledged.

3D-bioprinted bionic pancreas as an innovative method of treating and preventing diabetes - how far we are from clinical application?

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INTRODUCTION

Type 1 diabetes (T1D) is a disease, which affects millions of patients. Islet or pancreatic transplantation is a method of treating complicated T1D. The limitation of these methods is the lack of organs for transplantation. 3D-bioprinting using living cells could be a solution. We present results of bioprinted bionic pancreas on mouse and pig model.

EXPERIMENTAL METHODS

Research was carried out on 60 mice (SCID) and 24 pigs. The mice were divided into 3 groups: control; IsletTx in which porcine pancreatic islets were transplanted under the renal capsule; 3D-bioprint in which bioink petals consisted of bioink A and porcine islets. The bioprinted petals were transplanted into the dorsal part of the muscles under the skin in mice. Daily glucose measurement was performed, and the level of C-peptide was tested every 7-days. The pigs were divided into 4 groups: control; diabetic group (pancreatectomy-T1D); with transplanted 3D-bioprinted bionic petals (Tx-with previous pancreatectomy); pigs with transplanted 3D-bioprinted bionic organ with full vasculature. The animals were measured daily with blood glucose levels (from 5-20 measurements per day). 3D-bioprinted bionic pancreas were transplanted in some animals to the iliac vessels and in other subgroup to the aorta and vena cava.

RESULTS AND DISCUSSION

The results obtained in mice initially showed no differences in the concentration of C-peptide and glucose between groups. However, as early as 7-days after transplantation, both parameters analysed in the fasting state were significantly lower in the IsletTx and the 3D-bioprinted groups compared to the control group. On the day 14, decreased values of C-peptide and glucose were observed only in the group with petals transplants.
Mean glucose levels were two times lower, compared to the period before petals transplantation. In addition, TX pigs required lower dose of insulin after petals implantation. After transplantation of 3D-bioprinted bionic pancreas, a stable flow through the organ was observed in vivo and after the excision of the organ.

CONCLUSION

Transplantation of bionic petals in mice and pigs resulted in a decrease in mean glucose levels. None of the animals died due to postoperative complications or the lack of biocompatibility with the bionic structure.
Transplantation of fully vascularized organ created with 3D-bioprinting technology is feasible.

ACKNOWLEDGMENTS

The authors would like to thank the Research Frontiers Programme (Grant no: STRATEGMED3/305813/2/NCBR/2017) for providing financial support to this project".

CONFLICT OF INTEREST STATEMENT:

Michal Wszoła, Andrzej Berman, and Marta Klak are the co-founders of Polbionica Ltd.

>> 11:00 - 12:00 | PSOP-26 - DRUG DELIVERY II
Chairpersons: Hugo Oliveira & Frédéric Velard
Location: Room E

11:00 | O1 Drug delivery II - Immunotoxicity caused by near-infrared light-responsive nanoparticles on the integrity of the blood-brain barrier
Akhilesh RAI, Faculty of Medicine, University of Coimbra, Portugal

11:15 | O2 Drug delivery II - An eye-drop formulation of Fas-mediated apoptosis inhibitor attenuates age-related macular degeneration
Sang-Kyung LEE, Department of Bioengineering and BK21 FOUR Education and Research Group for Biopharmaceutical Innovation Leader, Hanyang University, Seoul

11:30 | O3 Drug Delivery II - Glucose Promotes Transplanted Human Mesenchymal Stromal Cell Paracrine Function Pertinent To Angiogenesis
Pauline WOSINSKI, Université de Paris, CNRS, INSERM, B3OA, Paris, France & Ecole Nationale Vétérinaire d'Alfort, B3OA, Maisons-Alfort, France

11:45 | O4 Drug delivery II - A Biomaterial Drug-Eluting Composite for Reducing Post-Surgical Inflammation and Fibrosis in Glaucoma
Alan HIBBITTS, 1. Tissue Engineering Research Group, Dept of Anatomy and Regenerative Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland

Immunotoxicity caused by near-infrared light-responsive nanoparticles on the integrity of the blood-brain barrier

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INTRODUCTION

Currently, there are no effective therapies available to completely reboot the brain function after occurrence of neurodegenerative diseases[1]. BBB is a bottleneck in development of drugs or drug carriers for the treatment of brain diseases[2]. The transient opening of the BBB using the heat generating nanoparticles (NPs) upon near-infrared (NIR) light exposure has been proposed to target the brain[3]. However, it remains elusive if the local heat can activate pro-inflammatory conditions, which affect the integrity of BBB. Here, we demonstrate that a library of cytokines and chemokines released by macrophages after the exposure of PDA NPs and NIR light do not compromise the integrity of BBB model.

EXPERIMENTAL METHODS

PDA NPs (63 nm) were synthesized using dopamine under the alkaline condition. Different densities of transferrin (Tf) peptides were covalently conjugated on PDA NPs using amine-PEG-maleimide (5kDa) linker**.** *In vitro* Mouse BBB model was used to analyze the permeability of Tf conjugated PDA NPs. Mouse brain endothelial cells (bEND.3) was used to prepare monoculture BBB model. ICP-MS measurement was done to quantify the amount of NPs, which transcytosed across *in vitro* BBB model. Moreover, the integrity of BBB after exposure of NPs and NIR light was evaluated by analysis of permeability of Lucifer yellow and electrical resistance (TEER) measurement. To study the immunotoxicity effect, the conditional media were collected after the incubation of PDA NPs with mouse macrophages and the exposure of NIR light. Conditioned media was added in the mouse BBB model for 24 h and the integrity of BBB was evaluated by permeability and TEER measurement and immunofluorescence analysis. 1-way ANOVA and unpaired t-testes were performed to do statistical analysis of the data.

RESULTS AND DISCUSSION

We have synthesized 63±3 nm PDA NPs, which is conjugated with transferrin (Tf) peptides. There are 180 Tf peptides per PDA NP. Photothermal measurement shows that 50 mg/mL of PDA NPs increase the temperature of solution to 5ºC after the exposure of NIR light (wavelength 785 nm, power density 2W/cm^2) for 5 min. Results obtained from *in vitro* BBB model showed that 50 mg/mL of Tf_{180}-PDA NPs crossed the BBB more efficiently than the PDA NPs after the exposure of NIR light. Importantly, 50 mg/mL Tf_{180}-PDA NPs and PDA NPs do not affect the integrity of the BBB after the exposure of NIR light, which is confirmed by immunofluorescence and qRT-PCR analyses of tight junction markers. Moreover, PDA NPs or Tf_{180}-PDA NPs do not induce cytotoxic effect to b.END3 cells after the NIR light exposure. qRT-PCR and cytokine microarray analyses showed the presence of a library of pro-inflammatory cytokines in the conditioned media. There is no significant change in the integrity of *in vitro* BBB model after incubation with the conditional media (containing cytokines/chemokines secreted by mouse macrophages after incubation with Tf_{180}-PDA NPs and the exposure of NIR light) compared to the conditioned media obtained from LPS exposure.

Conclusions

We show that light responsive Tf_{180}-PDA NPs can transiently open the BBB upon the exposure of NIR light and thereby promoting greater crossing of NPs. There is no harmful effect of cytokines screted by macrophages after the NIR light exposure on the integrity of *in vitro* BBB model. Overall, our results open the possibility of targeting effectively the brain by modulating the physicochemcial property of NPs.

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ACKNOWLEDGMENTS

The authors would like to thank the support of FCT (IF/00539/2015) grant.

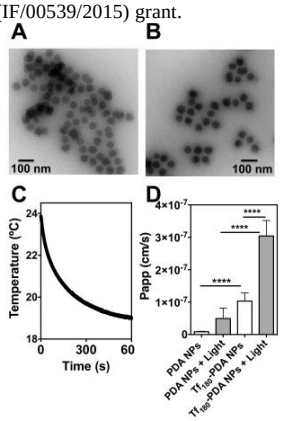

Figure 1: TEM images of PDA NPs (**A**) and Tf_{180}-PDA NPs (**B**). The temperature decline profile of PDA NP solution after NIR light exposure (**C**). Apparent permeability of NPs across the BBB model (**D**).

An eye-drop formulation of Fas-mediated apoptosis inhibitor attenuates age-related macular degeneration

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INTRODUCTION

Age-related macular degeneration (AMD) is a degenerative disease that occurs in the macula of the retina by Fas- mediated apoptosis. While dry AMD accounts for ~90% of macular degeneration, wet AMD accounts for approximately 10% of people with macular degeneration and can be treated with anti-VEGF therapies. However, current pharmaceuticals are unresponsive in about 40% of patients and intravitreal injection treatments are inaccessible, complex to use, and prone to side effects. In this study, we used Fas-blocking peptide (FBP) to inhibit Fas-mediated apoptosis in retinal cells, inhibiting AMD progression in sodium iodate (NaIO3)-induced dry AMD mice models.

EXPERIMENTAL METHODS

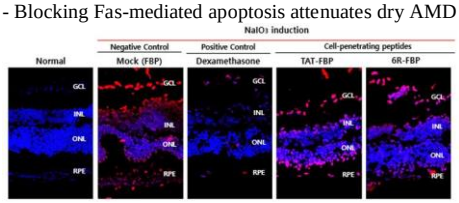
Animal studies
All experiments were performed in compliance with guidelines and using protocols approved by Institutional Animal Care and Use Committee (IACUC) of the Hanyang University. AMD was generated by intra-peritoneal injection of sodium iodate in Balb/c mice weighing 18-22g (Orient Bio, Seoul, Korea).

Evaluation of therapeutic efficacy
To confirm the delivery of FBP to retina, we conducted Immunohistochemistry, TUNEL and H&E staining were performed. Optical coherence tomography (OCT) was performed to test therapeutic effect of Fas-binding peptide. Molecular level of cytokines was evaluated by RT-PCR.

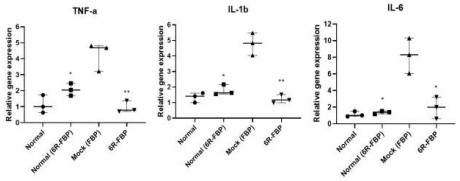
RESULTS AND DISCUSSION

Intravitreal inoculation of FBP successfully binds to Fas and inhibits Fas-mediated apoptosis in retinal inhibiting AMD progression in vivo. However, an eye-drop formulation of FBP could not deliver to the posterior segment of the retina. To address this limitation, we used an eye-drop formulation of FBP conjugated with hexa-arginine (FBP6R) for ocular delivery of FBP to the retinal segment of the eye. An eye drop formulation of FBP6R delivered to the posterior ocular regions of the macula of the retina segment of the eye and attenuated AMD by blocking Fas-mediated apoptosis. Our results indicate that an eye-drop formulation of FBP6R is a convenient and patient-compliant route of drug administration as a novel therapeutic intervention in treating AMD.

- Blocking Fas-mediated apoptosis attenuates dry AMD



- FBP6R therapeutic effect in molecular level by evaluation of cytokine level in RT-PCR from AMD mouse eye.



CONCLUSION

Application of an eye-drop formulation attenuates Fas-mediated apoptosis in retinal cells and downregulates inflammation in dry-AMD. Our data demonstrates that the inhibition of Fas-mediated signaling results in a reduction of retinal cell death and provides a therapeutic treatment for dry-AMD.

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Glucose Promotes Transplanted Human Mesenchymal Stromal Cell Paracrine Function Pertinent To Angiogenesis

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INTRODUCTION

Massive cell death post-implantation is a roadblock for the application of human mesenchymal stromal cell (hMSC) for large bone defect repair. We previously established that glucose is instrumental for hMSC survival post-implantation[1,2,3]. Because osteogenesis and angiogenesis are intrinsically linked in the process of bone repair, this study aims to determine whether glucose affects hMSC-mediated angiogenesis.

EXPERIMENTAL METHODS

***In vitro* experiment:** *Chemotactic cell migration*: The *in vitro* chemoattractive potential of conditioned media (CM) from hMSCs towards human umbilical vein endothelial cells (HUVECs) was determined using the Incucyte® Live Cell Analysis System. The CM was obtained by exposure of hMSC to near-anoxia in the presence of glucose at 0, 1, or 5 g/L for 3 days (n=6). *Angiogenic potential of CM*: The *in vitro* angiogenic potential of CM from hMSCs was assessed using an in vitro angiogenesis assay on Matrigel and quantification of the formation of vascular-like structures. *Released bioactive mediators*: The release of chemotactic and pro-angiogenic bioactive mediators was determined using Luminex technology on CM collected from hMSC exposed to near-anoxia in the presence of glucose at 0, 1, or 5 g/L for 3 days. *MSC tolerance to supra-physiological glucose level:* Because of glucose high diffusibility in hydrogels, MSC tolerance to glucose was assessed in order to load hydrogels with as much glucose as possible without jeopardizing cell viability. Briefly, the glucose toxicity assessments towards hMSCs was performed using a flow cytometer after exposure of hMSCs to glucose at 1, 5, 10, 20, 40, or 80 g/L under near-anoxia for 3 and 8 days. ***In vivo* experiment:** *New blood vessels formation in a mouse ectopic model*: To assess the influence of glucose on hMSCs pro-angiogenic potential, fibrin hydrogel loaded with hMSCs and glucose at either 0, 1, 5, 10, or 20 g/L were placed at the core of silicone cylinders whose both extremities were sealed with additional fibrin gels. Fibrin hydrogels loaded with 20 g/L glucose without hMSCs were included as cell-free glucose control. These devices were implanted ectopically in nude mice. The formation of new blood vessels was visualized at sacrifice by injecting a contrasting agent, Microfil®, in the bloodstream at 21 days post-implantation (n=8). The explanted silicone cylinders were imaged using a Skyscan1172 CT-scanner for quantifying new blood vessel formation. Statistics: Data were analyzed statistically using parametric tests, analysis of variance and the Bonferroni post-test, and, for non-parametric tests, the Mann-Whitney test. For all analyses, the confidence interval was set at 95% and the significant level at $p < 0.05$.

RESULTS AND DISCUSSION The Results and ***Glucose improves the angioinduction of MSCs in near anoxia***: Supernatant CM collected from hMSCs cultured with either 1 or 5 g/L glucose in near-anoxia for 3 days increased HUVECs migration and formation of vascular-like structure compared to that in the CM collected from hMSCs cultured without glucose. These data were corroborated by the increased amounts of pro-angiogenic factors (Angiogenin, VEGF-A, VEGF-C, Angiopoietin-1, Endostatin, and CCL2) in the CM collected from hMSCs cultured with glucose. *In vitro* exposure of hMSCs to glucose at 1, 5, 10, and 20 g/L did not affect hMSC viability. In contrast, the exposure of hMSCs to glucose at 40 or 80 g/L induced a statistically significant decrease in hMSCs viability when cultured under near-anoxia for 3 and 8 days. ***Glucose improves new blood vessels formation post-implantation***: Upon ectopic implantation into nude mice, the volume of newly formed blood vessels within hMSCs-containing hydrogels loaded either 5, 10, and 20 g/L glucose exhibited a 2.4, 2.8, and 2.4 fold increase compared to hMSCs-containing hydrogels without glucose at days 21 post-implantation, respectively. This study demonstrates for the first time its critical impact on MSC-mediated angiogenesis, both *in vitro* under near-anoxia and *in vivo* using an ectopic mouse model.

CONCLUSION

The *in vivo* delivery of hMSCs in the presence of glucose strategy may be broadly useful to improve the efficiency of current MSC-mediated therapeutic angiogenesis applications by providing pro-angiogenic growth factors.

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A Biomaterial Drug-Eluting Composite for Reducing Post-Surgical Inflammation and Fibrosis in Glaucoma

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INTRODUCTION

Drainage tube or trabeculectomy surgeries in late-stage glaucoma patients remain beset by unacceptably high failure rates due to post-operative inflammation and fibrosis. Therefore, we describe a proteoglycan (PG)-modified hyaluronic acid (HyA) hydrogel-Polycaprolactone (PCL) composite drug-device that aims to address post-surgical inflammation and remove patient compliance issues (Figure 1A+B).

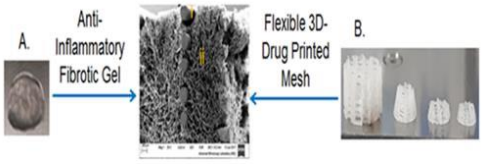

Figure 1. The component pieces of the HyA-PG-PCL insert. A) An outer PG-modified hyaluronic acid hydrogel that surrounds and provides a soft surface to B) a tapered, drug loaded PCL supporting mesh.

EXPERIMENTAL METHODS

Proteoglycan-HyA hydrogels were chemically crosslinked, freeze-dried and assessed for percentage crosslinking, swelling speed, degradation and ease of insertion into cadaveric rabbit eyes. Following drug loading and 3D printing, prednisolone drug release over 12 weeks in PBS was assessed. Bioactivity of the released drug and the effect of proteoglycan was investigated in primary rabbit conjunctival fibroblast (RconF) cells stimulated with Transforming Growth Factor beta (TGF-β). Furthermore, anti-angiogenic effect evaluated in a chick embryo model of angiogenesis.

RESULTS AND DISCUSSION

HyA hydrogels were capable of rapid swelling with full hydration in <30 min and stability up to 4 weeks minimum. Enzymatic degradation was also shown to be and related to cross-linking efficacy. Sham surgeries found devices were easily implanted in the sub-conjunctival space (Figure 2A). Release of prednisolone from was found to highly dependent on both the mesh and gel being present (Figure 2B).
Direct implantation of meshes into chicken embryos were found to effectively inhibit angiogenesis up to 5 days in the chick embryo model compared to blank inserts (Figure 2C+D). Primary isolates of rabbit conjunctival fibroblasts demonstrated a strong fibrotic response in the presence of TGF-β (Figure 2E). When release samples were applied to cells at each timepoint, a significant reduction in collagen deposition were found in PG and drug-loaded inserts compared to blank controls (Figure 2F).

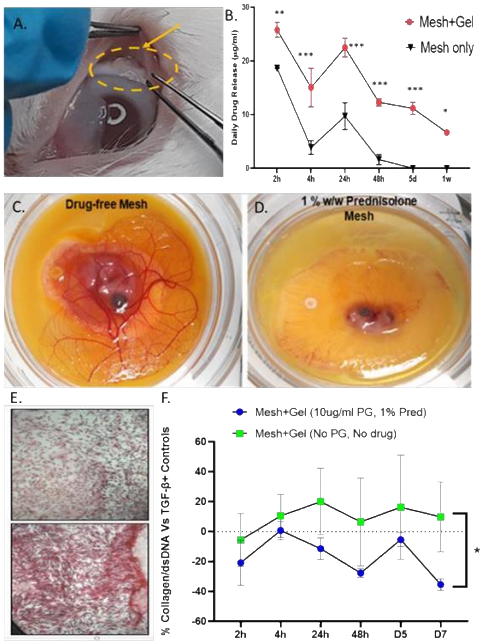

Figure 2.A) Inserts were easily inserted sub-conjunctively. B) Gel elicited significant improvements in the drug elution profile compared to meshes alone. C) Chicken embryos showed no decreases in angiogenesis using blank meshes. D) Near-total inhibition of angiogenesis was visible using drug-loaded meshes. E) RconF cells without (top) and with (bottom) TGF-β. F) Significant decreases in collagen deposition were achieved in finalized inserts compared to blank controls (n=3 ±SEM, 2-way ANOVA, *P ≤0.05, **P ≤0.01, ***P ≤0.001).

CONCLUSION

From this work, a highly scalable and easily implanted device was created. The device demonstrated significant reductions in key markers of fibrosis, namely collagen deposition and angiogenesis. Current work now focuses on advanced testing with an in-vivo pilot study in post-trabeculectomy rabbits currently ongoing.

ACKNOWLEDGMENTS

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>> 11:00 - 12:00 | PSOP-27 - POLYMER II
Chairpersons: David Eglin & Joanna Babilotte
Location: Room C

11:00 | O1 Polymer II - Optimization and characterization of Gellan Gum/Alginate microspheres produced via coaxial airflow
Henrique CARRÊLO, Departamento de Ciência dos Materiais, CENIMAT/I3N, NOVA/FCT, Almada, Portugal

11:15 | O2 Polymer II - Increasing birefringence in 3D printed PLLA single layers
Luke MALONE, Department of Materials Science and Metallurgy, University of Cambridge, Cambridge, UK

11:30 | O3 Polymer II - Biodegradable PLA-PEU-PLA membranes: A new solution for the reduction of post-operative peritendinous adhesions
Hélène VAN DEN BERGHE, Polymers for Health and Biomaterials/IBMM/Univ Montpellier, France

11:45 | O4 Polymer II - Designing Photo-responsive Double Network Hydrogels for Tissue Engineering
Ana Agustina ALDANA, Department of Complex Tissue Regeneration, MERLN Institute for Technology Inspired Regenerative Medicine, Maastricht University, P.O. Box 616, 6200 MD Maastricht, The Netherlands

Optimization and characterization of Gellan Gum/Alginate microspheres produced via coaxial airflow

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INTRODUCTION

Breast cancer is still a deadly disease despite the great advances of our time. Chemotherapy is an effective cancer treatment but its side effects are known to significantly degrade the patient's quality of life. In situ Drug Delivery Systems (DDS) are systems that can deliver chemotherapeutic drugs around the tumour area avoiding the general and direct administration of these drugs to the blood flow. Microspheres have been used as DDS and also for other bioactive agents' delivery, like cells [1,2]. This work was focused on the development, optimization and characterisation of microspheres made of a blend of Gellan Gum and Alginate. The microsphere's production was via coaxial airflow and the optimization of this process was carried out to define the most significant factors. Methylene Blue was used as a model drug to characterize the microsphere's release.

EXPERIMENTAL METHODS

This work focused on the development of Gellan Gum/Alginate microspheres produced via coaxial airflow with the aid of a design of experiments. The chosen factors in the development of the microspheres were airflow, pump flow, Gellan Gum/Alginate ratio and nozzle-bath height. The aim was to reduce the diameter and the dispersibility of the microspheres. These were hardened with ionic gelation through their drop to a $CaCl_2$ solution. Then, their diameter was determined. After production optimization, the microspheres were dried and characterized by Swelling in Mass and Volume, *In vitro* biodegradation, TGA and SEM. Methylene blue was then embedded within the microspheres and it's release was analysed in PBS with pH 7.4 and 6.5, with batches with different quantities of microspheres. pH 6.5 was chosen due to the acidic nature of cancerous regions. Mathematical model fittings were done to characterise the type of release from the microspheres.

RESULTS AND DISCUSSION

Using the coaxial airflow technique, it was found that airflow was the most statistically significant factor for the microsphere's diameter and airflow and the Gellan Gum/Alginate ratio were both statistically significant for the diameter's dispersibility. The optimized process produced microspheres with a diameter of around 400 µm. After drying, particles had an average diameter of around 150-200 µm. Swelling within PBS with pH 7.4 made the microspheres regain their wet diameter. However, with pH 6.5 the microspheres did not regain their wet diameter. This was most likely due to the higher interparticle repulsion that anionic polysaccharides suffer at more acidic pH [3]. The higher pH, 7.4, allowed higher Methylene blue, MB, dosages. In biodegradation tests, the microspheres were withheld for 60 days in PBS at pH 6.5 and 7.4. They presented a higher biodegradation rate with pH 7.4 with a mass loss of around 50 % within this period. The release was affected by the pH and the quantities of particles on each batch. The release was faster at pH 6.5 and with smaller amounts of microspheres. With higher content of microspheres, the release was more prolonged. This might be due to the saturation release medium. Using mathematical model fitting, it was found that the microspheres had a Fickian release profile and that Korsmeyer-Peppas and Peppas-Sahlin models modified with T_{lag} had the most suitable fittings.

CONCLUSION

The production of these microspheres was optimized, and it showed that airflow was the most significant factor in particles production. They had different release responses with pH 7.4 and 6.5, making it possible to adapt the cargo's release in cancerous areas. Faster releases were obtained within pH 6.5, however, the release profile could be adjusted with the quantity of particles. These microspheres were found to be suitable candidates for DDS in cancer treatments.

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Increasing birefringence in 3D printed PLLA single layers

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INTRODUCTION

Despite the favourable degradation time-period of poly-L-Lactic acid (PLLA)[1], its compliance limits its use for medical applications[1,2]. 3D printing has the potential to increase molecular alignment within PLLA, increasing its stiffness. Previous work has shown that as the polymer accelerates out the nozzle there is an increase in chain alignment[3]. However, the birefringence measured is below that previously reported for melt spinning[4]. The printing process is therefore limiting the alignment. There has been no investigation into the effect of other print parameters on birefringence. This work investigates the effect of changes in nozzle temperature, bed temperature and nozzle diameter on the alignment within the printed layer.

EXPERIMENTAL METHODS

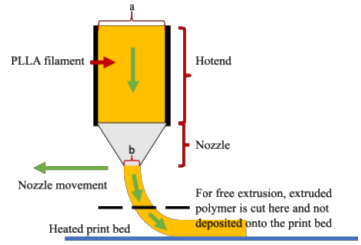

Figure 1. Schematic of FFF printing set-up. 'a' is filament diameter, 'b' is nozzle diameter

PLLA preparation: NatureWorks (2500HP) PLLA pellets were extruded at 182^{0}C, 1200rpm with a 1.75mm nozzle. ***PLLA-PVA printing:*** Prior to printing, all material was vacuum dried for 24 hours. A Prusa MK3s+ Fused Filament Fabrication (FFF) printer, with mounted Titan Extruder, was used. The standard print conditions were 60^{0}C bed temperature, 215^{0}C nozzle temperature, 400µm nozzle diameter and 6000mm min^{-1} print speed. Each parameter was then changed independently. Nozzle temperature was varied between 180-230^{0}C, bed temperature 30-60^{0}C and nozzle diameters of 150, 400 and 800µm were applied. A 200µm PVA sacrificial support layer was used, as defined in previous work[5]. For free extrusion, the nozzle was stationary and the polymer was cut before touching the bed. Figure 1 shows a printing set-up schematic. ***Birefringence measurement:*** After peeling the PLLA from the PVA, sample retardation (used to find the birefringence) was measured using an optical microscope with 6.5x magnification. Two perpendicular polarisers were inserted and rotated to 45^0.

RESULTS AND DISCUSSION

Nozzle temperature: As nozzle temperature increased from 180^{0}C to 230^{0}C, the birefringence decreased. With an increasing temperature, it is suggested the polymer chains undergo increased inter-chain sliding and a decrease in conformational changes along their backbone. Figure 2 shows the birefringence results.

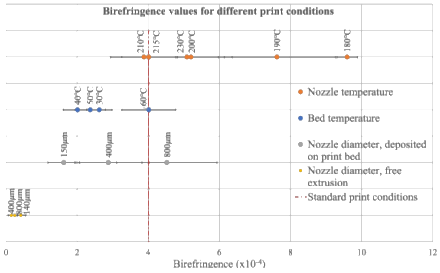

Figure 2. Effect on birefringence of nozzle temperature (orange), bed temperature (blue), nozzle diameter deposited on the print bed (grey), nozzle diameter free extrusion (yellow), standard print conditions line- 215^{0}C nozzle temperature, 400µm nozzle diameter, 60^{0}C bed temperature, 6000mm min^{-1} print speed- (red) shown for comparison

Bed temperature: Below the glass transition (Tg), approximately 60 ^{0}C, the bed temperature has no effect on the chain alignment.

Nozzle diameter: The effect of nozzle diameter on birefringence is suggested to be two-fold. Firstly, if all print and extrusion parameters are kept constant, a larger nozzle diameter decreases the speed that the PLLA exits the nozzle. This increases the strain rate due to the difference between nozzle exit and translation speed, explaining the resultant increase in birefringence for material deposited on the print bed. However, if the polymer is freely extruded, then with a smaller nozzle diameter the increased shear stress within the nozzle results in a small increase in birefringence. This second effect is smaller, with the overall alignment appearing to be dominated by the first term.

CONCLUSION

Of the three techniques, nozzle temperature is shown to have the greatest effect on birefringence. Bed temperature has little effect below the Tg. For changes to nozzle diameter, it is suggested that shear stresses within the nozzle are small compared with the alignment generated from acceleration out of the nozzle. Acceleration appears key to the alignment.

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Biodegradable PLA-PEU-PLA membranes: A new solution for the reduction of post-operative peritendinous adhesions

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INTRODUCTION

Peritendinous adhesions are complications known to occur after surgery and cause chronic pain and disability. Anti-adhesive biodegradable membranes, based on a new poly (lactic acid)-poly (ether urethane)-poly (lactic acid) (PLA-PEU-PLA) copolymer, were designed to be easily applied as barrier during tendon surgery to effectively prevent adhesions.[1] These membranes were fully characterized in terms of thermal and mechanical properties, degradation rate, water uptake and *in vitro* cytotoxicity.

A preliminary *in vivo* study in a rat model of peritendinous adhesions was conducted to evaluate the membranes' degradation rate, tendon healing and anti-adhesion effect.[2]

EXPERIMENTAL METHODS

Poly (ether urethane) (PEU) was synthesized by polycondensation of low molecular weight poly(ethylene glycol). Then, PLA-PEU-PLA triblock copolymer was synthesized from PEU by ring opening polymerization of D,L-lactide. PEU and PLA-PEU-PLA were characterized by SEC, FT-IR, 1H-NMR, DSC and TGA.

Membranes based on PLA-PEU-PLA were prepared by hot molding and characterized by DMA, SEM and contact angle measurements. The membrane degradation kinetics were studied by water uptake, mass loss and molecular weight determination. The *in vitro* cytotoxicity of membranes was also evaluated.

A preliminary *in vivo* study of membranes was performed on a rat model of Achilles tendon peritendinous adhesions by histologic and biomechanical evaluations as well as degradation rate evaluation. Membranes were compared to a control group (no surgery) and an adhesion group (surgery without treatment) at 2 and 10 weeks post-surgery.

RESULTS AND DISCUSSION

Characterization of PLA-PEU-PLA membranes showed that were characterized in terms of structure, thermal and morphological properties. These membranes were non porous, flexible, easy to handle, with a suitable suturability, making them adapted to clinical settings (Fig. 1). Membranes have also proven to be cytocompatible.

Macroscopic and histological evaluations of membranes showed anti-adhesion efficacy in the rat Achilles tendon model after 2 weeks' surgery and a reduction of the adhesions after 10 weeks.

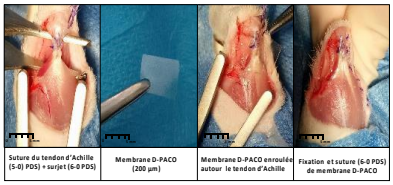

Figure 1. Flexibility and suturability of PLA-PEU-PLA membranes.

Histological and biomechanical results showed that membranes do not interfere with tendon healing. Moreover, *in vivo* degradation study by SEC showed that membranes filmogenicity was maintained at 2 weeks, whereas they were significantly degraded after 10 weeks.

CONCLUSION

The thermal behavior and stability of PLA-PEU-PLA copolymers allowed the design of membranes by hot molding. Thanks to the urethane bonds in PLA-PEU-PLA copolymer structure, membranes showed an optimal *in vitro* degradation rate. Membranes are non toxic and adapted to clinical settings in terms of flexibility and suturability. *In vivo* study showed that membranes acted as an effective physical barrier at the beginning of tendon healing (>2 weeks) and in 10 weeks underwent significant biodegradation. Membranes could also promote tendon healing.

The study also highlights the need to increase the degradation rate of PLA-PLU-PLA membranes up to 10 weeks as adhesions are still forming beyond the 6 weeks cited in literature (up to 10 weeks after surgey).

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Designing Photo-responsive Double Network Hydrogels for Tissue Engineering

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INTRODUCTION

Recent works on dynamic hydrogels, using non-covalent and/or dynamic covalent linkages, have shown that dynamic and spatial-temporal complex materials are a promising step-towards the recapitulation of extracellular matrix (ECM) functionality and structure[1,2]. However, the uncontrollable network properties across timescales makes these dynamic hydrogels insufficient for fully recapitulating the native ECM.

Double network (DN) hydrogels are a promising step towards recapitulating the multicomponent ECM and have enhanced mechanical properties[3,4]. In order to control the mechanical and biological cues provided to cells through the biomaterial at each stage, we investigated the combination of two networks: one dynamic covalent cross-linked, and the other one photo-responsive network. The second network based on light-responsive linkages could enable reversible control of cross-linking degree with spatiotemporal resolution. Here, we first synthesized photo-responsive polymers based on polyethylene glycol with coumarin derivate as end group.

EXPERIMENTAL METHODS

Synthesis of PEG-cou: 40.5 mg (0.18 mmol, 4.61 equiv.) of epoxycoumarin was added into 10 mL TBAF (184.9 mg, 0.71 mmol, 17.56 equiv.) solution in THF, and stirred 30 minutes under nitrogen atmosphere. 201.2 mg (0.04 mmol, 1.0 equiv.) of 4-arm PEG thiol (5 kDa) was added and the reaction mixture was stirred overnight. The product (4-arm PEG coumarin, 4PC) was purified by precipitation in cold diethyl ether and drying under vacuum (yield: 81.9%).

Hydrogel preparation: 4PC was evaluated for preparing single (SN) and double network (DN) hydrogels. For DN formulations, a hydrogel precursor solution, which is composed of 4PC (5 or 10% w/v) and oxidized alginate (OA; 2.5% w/v), was prepared in PBS. Then, ADH solution was added into the precursor solution and transferred to molds to prepare dynamic cross-linked hydrogels with equimolar concentrations of aldehyde/hydrazide groups. To form the second network (4PC), the hydrogels were then irradiated with UV light (365 nm UVP CL-1000 UV, 10 J/cm^{-2}) for 30 min. As controls, OA (2.5% w/v) and 4PC (20%) single networks were also prepared. All hydrogel formulations are denoted OA2.5/4PCx, where x is the weight percentage of 4PC.

Characterization: Nuclear magnetic resonance (^{1}H-NMR) was performed on a Bruker ASCEND™ 700 MHz NMR spectrometer equipped with TCI cryo probe™. *UV-Vis spectra* were measured with a Cary 60 UV-Vis Spectrophotometer (Agilent).

RESULTS AND DISCUSSION

The 4-arm-polyethylene glycol coumarin (4PC) was synthesized successfully. The thiol-epoxy reaction allow for coupling of coumarin to 4-arm-PEG-thiol, which was corroborated by NMR. Then, the dimerization-cleavage transition was analyzed by UV-Vis spectroscopy. As it known, the dimerization of coumarin (cross-linking) takes place by irradiation above 300 nm, while the cleavage of the dimers (de-crosslinking) is below 300 nm. The results showed that 10.2% and 14.1% of cross-linking happened in 20 minutes for 4PC 5 kDa and 20 kDa, respectively. The de-crosslinking rate for 4PC 20 kDa was also faster than that of 4PC 5 kDa (54% and 69% for 8 minutes, respectively). These results were in agreement with the literature with a crosslinking and a de-crosslinking rate.

Once the photo-responsive polymers were characterized, double network (DN) hydrogels were developed by combining a dynamic and a static photo-responsive cross-linked network. We investigated the combination of oxidized alginate cross-linked by Schiff-base reactions (OA, dynamic network) and photo-crosslinkable polymers (4PC, photo-responsive network). 4PC 20 kDa was discarded for preparing double networks because the hydrogels were not homogenous and phase separation was observed. The DN-precursor solutions with 4PC 5k were homogenous. The hydrogels after the first and second network formation were translucent and slightly yellow. We observed that SN were softer than DN.
Rheological characterization is carrying out to further investigate the reversibility of the hydrogel properties by UV irradiation.

CONCLUSION

We synthesized a promising photo-responsive polymer for biomedical applications. The double network strategy allow for controlling hydrogel properties. These biomaterials are promising for remotely providing to cell mechanical and biological cues and, hence, modulating cell response

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Novidia Veterinary est un laboratoire développant une gamme de biomatériaux sous forme d'hydrogels, pour un usage en orthopédie vétérinaire. La R&D est centrée sur une forte expertise vétérinaire avec des équipes pluridisciplinaires situées sur Sophia Antipolis (06). www.novidia.com Contact : Management@novidia.com

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Micromechanical properties are an increasingly important parameter in biomaterial design, as there is a strong link between the function of cells and tissues and their mechanical properties. Optics11 Life provides high-throughput micromechanical characterization of hydrogels, (engineered) tissues, organoids, and cells through nanoindentation. Our high-content platform, the Pavone, enables researchers to screen biomaterials in 96-well plates fully automatically. Analyze local functional properties such as stiffness, viscoelasticity, stress relaxation, and many more in physiological environments with the addition of advanced microscopy modes. Come meet us at our booth to see how your materials can be easily and quickly measured."

PBC Biomed

Our core focus at PBC Biomed is to enhance patient wellbeing by accelerating medical innovation from concept to commercialization. With a 10+ year track-record of bringing technologies to market and an experienced staff with broad expertise in medical devices, biomaterials and pharmaceuticals, we work with surgeon inventors, companies, and universities to bring their ideas to life. PBC BioMed offers a wide range of services to our partners, including early research, product development, manufacturing, regulatory, quality, commercialization and distribution. Every partnership is tailored to your needs, from full-service design and development, to specific project milestones helping you achieve your timelines and budget. Stop by our booth at ESB to learn how PBC BioMed can work with you to accelerate your medical innovation. PBC Biomed is certified to ISO 13485;2016 and ISO 9001:2019.

SPONSORS & EXHIBITORS PROFILES

POEITIS

Poietis is a health technology company specialized in the development and manufacturing of human tissues by bioprinting. Its main mission is to develop new therapeutic solutions based on its expertise in bioprinting technologies and in high resolution laser bioprinting in particular.

Poietis has developed the Next Generation Bioprinting (NGB) platform, which is available in a research version for tissue bioengineering (NGB-R) and a clinical grade version (NGB-C) compatible with Good Manufacturing Practices for ATMPs and the production of implantable bioprinted tissue. This multimodal and robotic platform allows the manufacturing of complex tissues while ensuring repeatability and reproducibility.

Poietis' bioprinting technology is the result of innovative research conducted over ten years at Inserm and the University of Bordeaux.

Created in September 2014, Poietis operates a portfolio of over 75 patents and currently employs 33 people. More info: www.poietis.com

Polbionica

Polbionica is not a typical manufacturer of biomaterials. Polbionica is carrying out advanced studies on bionic organs. Our daily research and implementation practice using our own biomaterials enabled us to achieve high and reproducible quality parameters. We carry out production in our specialised laboratories in Warsaw(Poland). Continuous inspection of components and bio-physico-chemical properties of the manufactured bioink precursors makes it possible to use them in the innovative 3D bioprinting technology. We are happy to share our best experiences with scientists. We have prepared the Tintbionic® product line to meet their needs: bioink precursors, methacrylates based on natural polymers, photoinitiators and additives used in 3D bioprinting, dECM (decellularised extracellular matrix from the porcine pancreas), equipment UV–Vis 365/405 nm laboratory crosslinking lamp. Visit www.polbionica.com

Readily3D

Readily3D commercializes and develops volumetric bioprinters. This novel biofabrication technique enables the design of complex bioconstructs with high porosity and vasculature networks. Volumetric biofabrication is both organoid and cell friendly with over 90% viability post-printing, owing to its contactless and fast process (< 30s). Its high repeatability allows conducting statistical studies and drug screenings on bioconstructs replicas produced within minutes.

SPONSORS & EXHIBITORS PROFILES

Regemat 3D

Regemat 3D, at the forefront of personalized bioprinting solutions, makes available to research and science its tailor made technology, designed to unlock the potential of additive manufacturing applied to regenerative medicine.The personalized configuration of each bioprinter taking into account the specific application and the support of our engineer team, make possible daily, the achieving of reliable results, which facilitate the path of each research. We have recently developed a whole new series of bioprinting tools and systems and also validated performing biomaterials to be integrated within the experimental workflow of investigations related to disease modelling, tissue engineering or drug testing driving the researches one step forward. REGEMAT's modular systems, the BIO V1 and REG4LIFE, have been now validated from researchers in more than 25 countries working in a wide variety of research lines, including skin bioprinting, bone regeneration, and 3D models of cancer.

Région *Nouvelle-Aquitaine*

Largest region in France, *La Nouvelle-Aquitaine* is an attractive French territory comprised of 720km of coastline, an exceptional diversity of landscapes lulled by a mild and sunny climate: *La Nouvelle-Aquitaine* can be proud to be a coveted tourist destination. The regional natural parks of the Marais poitevin, the Millevaches plateau and Périgord-Limousin, the marine natural park of the Bassin d'Arcachon or the Dune du Pilat, classified as a Grand Site de France, have everything to seduce visitors. From the Basque coast to La Rochelle via the Iles de Ré or Oléron, the "Glissicon Valley" offers an ideal playground for water sports. Winter sports and hiking enthusiasts are also delighted between the Pyrenean chain, the foothills of the Massif Central, the forests of the Landes, the Dordogne and the Creuse.

Its Regional Council holds important responsibilities for the daily life of their citizens according to 4 main lines:

- **Economy and employment** (support to innovation, agriculture, numerical transition, industry, employment, tourism, etc.)
- **Youths** (support to apprenticeship, training dedicated to young people, scholar transport, etc.)
- **Town and Country planning** (territory politics, transportation, support to health, sport, culture, etc.)
- **Energetic and Ecological transition** (biodiversity preservation, sustainable energies, zero waste; etc.)

Last but not least, *La Nouvelle-Aquitaine* is home to many local products and regional specialties: regional brands, gastronomic specialties or artisanal creations, symbols of the dynamism of companies, the excellence of the sectors and the liveliness of a heritage that knows how to reinvent itself. Among all these regional products, La Nouvelle Aquitaine offers a multitude of possibilities for consuming and buying local, and just as many gift ideas.

Rheolution

Rheolution is shaping the future of Soft Matter Analytics™, delivering next-gen solutions to help our users bring soft materials to life. We're on the mission of creating Soft Matter Analytics as a unique data set that empowers our users in their quest for innovation, quality and efficiency. Our cutting-edge analytical instruments reproduce real-life environments and capture the dynamic evolution of live soft materials. We design simple-to-use, highly effective and modular products to provide a unique testing experience to our customers. In Life Sciences, we make smart, non-invasive and patented analytical instruments that unlock the access to crucial information on the viscoelasticity of biomaterials and medical devices for R&D, quality insurance and process control.

coexhibiting with **I&L Biosystems France**

I&L Biosystems France distributes innovative solutions for research in the life sciences, cell culture (upstream bioproduction), purification processes (downstream), transposition to industrial scale and microbiological controls. We put our skills and expertise at the service of academic and hospital laboratories, the food industry, cosmetics, biotechnology and pharma (small and large companies) with a need for robust equipment meeting the expectations of traceability, reliability, productivity... We favor close relationships with our users. A dedicated contact person supports you in your projects by advising you and offering the solution best suited to your needs and requirements before, during and well after the purchase. We provide installation, training, IQ QO qualifications and preventive / curative maintenance on our instruments.

REGENHU

REGENHU started in 2007 with a goal to create and develop bioprinting technologies that will positively impact many medical fields. Medicine is evolving and those at the frontline who are pushing boundaries, making remarkable discoveries and changing lives, need the right tools and support to take them into the future. As a Swiss Bio Tech company, REGENHU's mission is to enable our users to reach the next level in their work, goals and ambitions.

SPONSORS & EXHIBITORS PROFILES

Rousselot

Rousselot® is the global leader in collagen-based solutions, pioneering the development and production of pharmaceutical grade gelatins designed for diagnostics and bioabsorbable clinical applications. Since the launch of its biomedical segment, Rousselot Biomedical has expanded its portfolio with pure (Quali-Pure®) and ultra-pure (X-Pure®) gelatins. The latest range includes customized, modified gelatins, produced under controlled conditions and compliant with the most rigorous quality controls. The production process ensures standardized, high quality, pure and consistent biomaterials, thereby accelerating the translation of research to clinical practice. Rousselot is the first company producing GMP-grade GelMA, which, due to its functional equivalence to the research grade product, minimizes the need for revalidation when transitioning into clinical development. Rousselot Biomedical is committed to supporting the use of X-Pure and Quali-Pure in your applications and to help "Advancing medical science".

ROYAL SOCIETY PUBLISHING

The Royal Society, the UK Academy of Science, recognises, promotes and supports excellence in science. Our journals Interface and Interface Focus offer a range of publishing options for scientists working within biomaterials research. We offer rigorous, constructive peer review by practising experts in the field; high quality author care; efficient processing and rapid publication; open access options; and promotion by a dedicated press office. To find out more about our journals and our editorial processes, please visit booth 26 where our representative Jessica Miller will be happy to answer your questions. Alternatively, visit our website at http://royalsociety.org/journals

Science & BioMaterials

S.B.M. (Science & BioMaterials) has been specialized in the design, manufacture and distribution of biomaterials for bone reconstruction since 1991. Our priority is the development and optimization of medical devices that promote both bone healing and human tissue replacement. Thanks to a total mastery of its manufacturing techniques, the company develops complete systems based on 100% synthetic and absorbable materials, combined with adapted instrumentation.

SPONSORS & EXHIBITORS PROFILES

SEPTODONT

Since its foundation in 1932, Septodont has been at the forefront of pharmaceutical dentistry – developing, manufacturing and distributing a wide range of high quality dental products all over the world. Looking to the future, Septodont is also leveraging its competencies to bring innovative solutions beyond dental professional field into the medical field.
With its six manufacturing sites on three continents, Septodont has the technical expertise to manufacture all types of pharmaceutical forms: injectables, solutions, sprays, pastes… Each site is approved by the most stringent government health agencies such as FDA (for the USA), ANSM (for France), ANVISA (for Brazil) and MHLW (for Japan).
Beyond pain management, Septodont developed and patented a breakthrough technology platform, Active Biosilicate Technology™. It led to the launch of Biodentine™ and BioRoot™ RCS, two biocompatible and bioactive materials with indications across restoration and endodontics. Modern dentistry will restore organs up to their initial functions. We believe Regeneration is the next game changing with full healing of damaged dental tissues, bones or ligaments. More info: https://www.septodontcorp.com/

SILTISS

SILTISS is dedicated to the development, production and marketing of non-animal biomimetic biomaterials, which promote and support tissue regeneration processes. The initial applications relate to bone substitutes for maxillofacial surgery as well as for the dental and orthopedic sectors.

The company is born in 2016 from the joint brainchild between Jean Paufique, President and Founder of SILAB (www.silab.fr) and Didier Letourneur, Research Director at CNRS (French National Centre for Scientific Research), with a recognized expertise in biomaterials. A spin-off of SILAB, SILTISS is equipped with technological, human and financial resources to address the ambitious development of innovative products in regenerative medicine. For more details: www.siltiss.fr

STEMCELL

At STEMCELL, science is our foundation. Driven by our mission to advance research globally, we offer over 2,500 tools and services supporting scientists working in stem cell research, regenerative medicine, immunotherapy, and disease research. Organoids and organotypic model systems, as alternatives to animal models, are powerful new tools for research approaches in modeling and understanding human health and disease. STEMCELL Technologies supports organoid researchers with high-quality cell culture media and kits that enable robust establishment, expansion, maintenance, and differentiation of organoid cultures. To explore STEMCELL's high-quality organoid media and differentiation kits, visit: www.stemcell.com/organoid-products

SPONSORS & EXHIBITORS PROFILES

SPARTHA Medical

SPARTHA Medical is a young Strasbourg-based biotechnology company that develops invisible shields, made of natural biopolymers-based multifunctional and customisable coatings.
Our coatings are conceived to prevent hospital-acquired infections (nosocomial) or fomites-driven infections. Our main activity is therefore dedicated to the industrial sector, from the medical devices to cosmetics and electronics. To a larger scale, we aim at creating the future solutions for non-toxic and biocompatible disinfecting products.

Our technology, which respects humans and the environment, comes from a public research institute (INSERM, where the three partners are inventors).
SPARTHA Medical has obtained the full exclusive license of 4 patents related to the technology in December 2019 from SATT Conectus (Technology Transfer Office from Strasbourg university) and has been laureate of the European Innovation Council Accelerator program in 2022.

TISSIUM

TISSIUM is a privately held med tech company based in Paris, France and Boston, USA, specializing in the development and commercialization of a unique biopolymer platform to address various unmet clinical needs, including atraumatic tissue repair and reconstruction.
TISSIUM is developing a portfolio of solutions based on a proprietary family of programmable biomorphic polymers, which forms the foundation of the company's technology platform.
Currently, the company has a pipeline of seven products across 3 verticals, including peripheral nerve repair, hernia treatment, and treatment of suture line leaks in cardiovascular surgery. The company's technology is the result of research and intellectual property from the laboratories of Professor Robert Langer (MIT) and Professor Jeffrey M. Karp (Brigham and Women's Hospital), who co-founded the company in 2013

TWEVEL

Twevel takes care of distribution, application and technical support, and maintenance service in France and Africa, in the following areas:

- X-ray imaging and analyzes
- Radiation detection and associated spectrometry
- Mechanical and optical characterization of surfaces
- Electron microscopy and nuclear magnetic resonance

SPONSORS & EXHIBITORS PROFILES

University of Bordeaux

The University of Bordeaux is ranked among the top French universities for the quality of its education and research. It is a multidisciplinary, research-focused and international institution that leads an ambitious development program with its partners to further promote Bordeaux as a "Campus of Excellence". As a leading international research university, the University of Bordeaux promotes the development of training, research and knowledge transfer within its numerous Clusters of Excellence: neuroscience, medical imaging, environment/climate, advanced materials, archaeology, lasers/optics, digital certification, health and society and cardiology.

The University of Bordeaux offers students and staff a high-quality working and living environment. Located in southwest France, on the European Atlantic coast, the city of Bordeaux, also known as the "Wine Capital" of the world, is located at the very heart of Southern Europe, only a few hours from major European cities and just one hour from the Atlantic Ocean.

Urgo RID

Urgo Research, Innovation & Development (Urgo RID) is part of Urgo Medical company. Urgo RID brings together nearly 190 people representing all stages of the innovation value chain: monitoring, research, innovation, business development, intellectual property, development, clinical studies, industrial transposition. Our mission is to imagine and develop tomorrow's healing and responsible health solutions to meet the needs of patients and healthcare professionals.We invest nearly €33 million per year in research. Our research centre employs nearly 80 people (chemists, materials researchers, physicists, biologists, etc.) who work every day with the aim of improving wound healing solutions to heal, relieve patients and simplify treatments. At Urgo Medical, we are convinced that research is above all collaborative, which is why we have an active strategy of public-private partnerships. Over the last 15 years, we have signed nearly twenty partnerships ranging from start-ups to international research centres.Finally, we have made clinical research a fundamental part of our innovation to demonstrate the effectiveness of our products. Since 1997, we have conducted nearly 55 clinical and observational studies involving nearly 60,000 patients and obtained more than 40 scientific publications in indexed journals.

WILEY

Wiley, a global company, helps people and organizations develop the skills and knowledge they need to succeed. Our online scientific, technical, medical, and scholarly journals, combined with our digital learning, assessment and certification solutions help universities, societies, businesses, governments, and individuals increase the academic and professional impact of their work.

WPI

World Precision Instruments (WPI) is a leading instrument and equipment manufacturer specialised in supporting cell, tissue and electrophysiology research.

We offer a complete line of mechanical testing and cell stimulation including the flagship BioTester which remains a market leader for biaxial testing of biomaterials.

WPI's products for cell and tissue applications have 1000s of citations and include the EVOM range for TEER Measurement and our new Live Cell Imaging system AutoLCI.

For essential fluidic control, we offer solutions across pumping platforms including syringe, peristaltic, microfluidic and pneumatic microinjection pumps for picoliter to microliter flow.

Come and visit our booth to discuss your applications and discover our latest innovations in Cell Imaging and Biomaterials testing.

SOCIAL PROGRAM

Welcome Reception

The Welcome Reception will include a cocktail as well as French traditional activities.

Date: Sunday, 4 September 2022
Time: 19:00-22:00
Location: Hall of the Palais des Congrès
Price:

- Free for congress participant
- 25€ for accompanying person

SOCIAL PROGRAM

YSF's Night Out at La Belle Saison

Event mainly for undergraduate students, graduate students and scientists in early postdoc phase.

Date: Tuesday, 6 September 2022
Time: 20:15 to 00:00
Location: 5 Quai des Queyries, 33100 Bordeaux
Price : 20€ (Delegates)

Access: Located across the river La Garonne, La Belle Saison is just a 15min walk from the Tramway (line A, "Place Stalingrad" or "Jardin Botanique") and close to the historical center of Bordeaux (https://la-bellesaison.fr)

- **On foot or by bike:** Along the quays, the restaurant is on the right bank.
- **By public transportation:**
  - Tram A: 'Place Stalingrad' or 'Botanical Garden'
  - Bus: 91 and 92 stops 'Hortense' or 'Maréchal Niel' and bus 45 stop 'Hortense'
- **By car:** Between the Chaban Delmas bridge and the Pont de Pierre. Parking reserved for customers.

You will be welcomed in a typical "cabane" on the waterfront for a barbecue evening! Enjoy a panoramic view of the historical city of Bordeaux from a terrace and a garden on the natural banks of the Garonne. Especially in summer, you can relax in the cosy garden and its garden bar.

Don't miss this opportunity to make new friends or meet old ones during a nice party. Registration includes a barbecue and two drinks.

SOCIAL PROGRAM

Conference Dinner

Only with tickets. Only 20 tickets will be available on September 4th for additional attendees.

Date: Wednesday, 7 September 2022

Time:

- Bus departure: 18:15-19:00
- Return to the Congress Center or in Town: 23:00-23:59

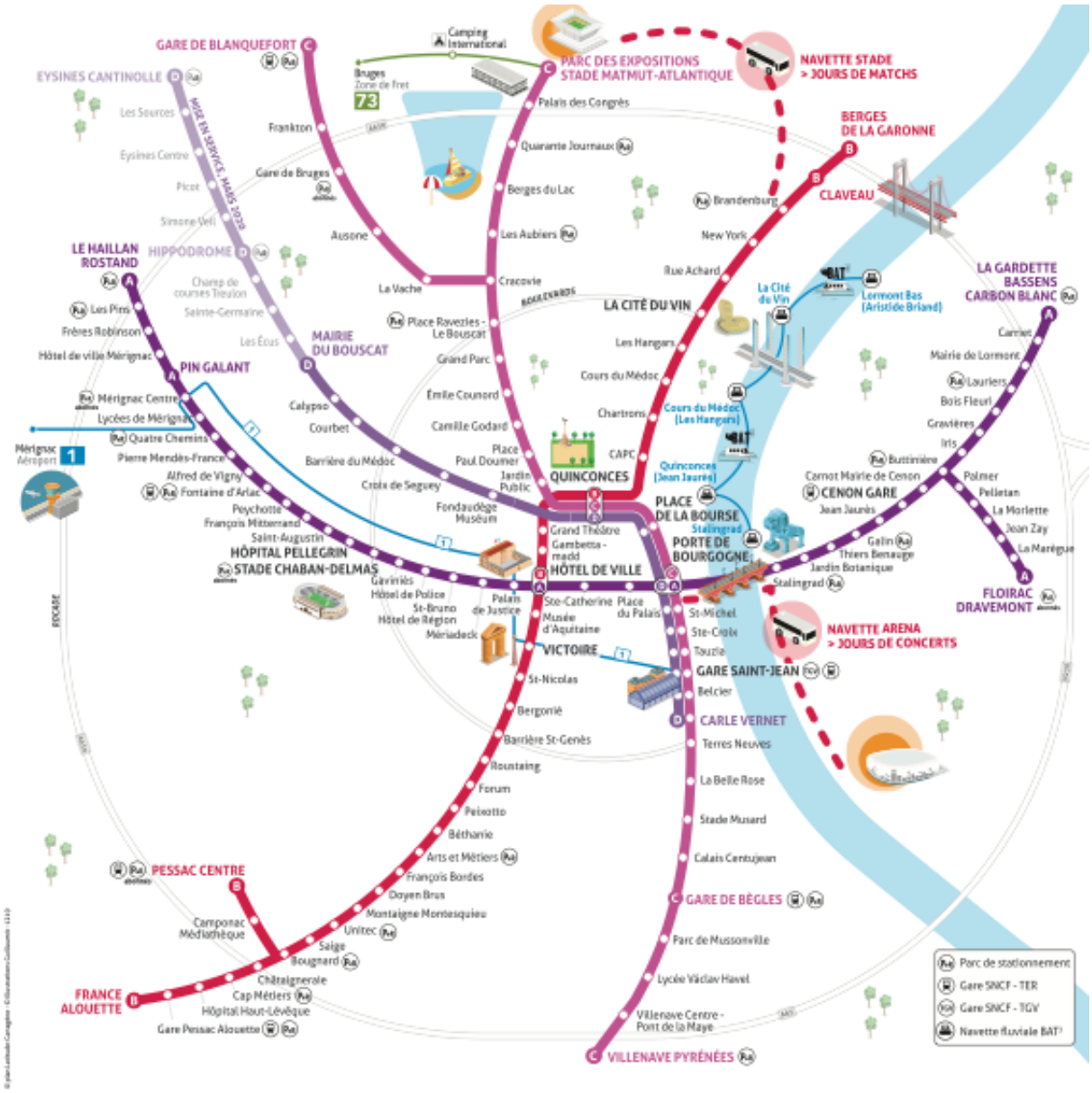
Location: Château Giscours, 10 route de Giscours, 33460 Labarde

Price:

- 100€ for congress participants
- For accompanying person, please contact ESB2022 at contact@esbbordeaux2022.org for booking

Access: By bus; departure from the Congress Center (6:15-7:00pm).

CITY MAP


GARE DE BLANQUEFORT
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Picot
Simone Veil
MISE EN SERVICE MARS 2023
LE HAILLAN ROSTAND
HIPPODROME
Champ de courses Treuilon
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PARC DES EXPOSITIONS STADE MATMUT-ATLANTIQUE
NAVETTE STADE > JOURS DE MATCHS
BERGES DE LA GARONNE
CLAVEAU
Brandenburg
New York
Rue Achard
LA CITÉ DU VIN
La Cité du Vin
Lormont Bas (Aristide Briand)
LA GARDETTE BASSENS CARBON BLANC
Carriet
Mairie de Lormont
Lauriers
Bois Fleuri
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Iris
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Tauzia
GARE SAINT-JEAN
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CARLE VERNET
Terres Neuves
La Belle Rose
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GARE DE BÈGLES
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VILLENAVE PYRÉNÉES
NAVETTE ARENA > JOURS DE CONCERTS
Parc de stationnement
Gare SNCF - TER
Gare SNCF - TGV
Navette fluviale BAT³

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e-BOOK INFORMATION

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Responsibility in terms of content

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Université de Bordeaux, France

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33070 Bordeaux

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Marjorie Dufaud, Member of the BIOMAT Young Researchers Board
Université de Montpellier, France

Advertisement references

ADOCIA

Innovative Medicine

for everyone, everywhere

Adocia is a biotechnology company based in Lyon specialized in the discovery and development of therapeutic solutions in the field of metabolic diseases, diabetes and obesity

115 Employees

80% R&D staff

25 Patent families

3 Technology platforms

4 Products in clinical trials

With 15-years of experience in biomaterials, Adocia has developed a **biocompatible** encapsulation material, **ultra-thin** and **easily manipulated** for pancreatic islet transplant with no need for immunosuppressive treatments

BIO INX®

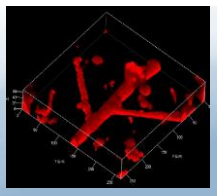
BIO INX® is a spin-off of Ghent University focusing on the commercialisation of materials and bio-inks for 3D bioprinting or biofabrication. By offering a unique material portfolio with a diverse range of material properties, the applications become nearly limitless.

Looking for materials for Biofabrication and/or Tissue engineering?
Discover our ready-to-use bio-inks for multiple printing technologies!

Deposition-based 3D printing

Digital Light Projection

Multiphoton Lithography



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www.admedsol.com

Le GDR « Réparer l'humain » (GDR-RH) est né de réflexions communes entre le CNRS et l'INSERM sur la position nationale de la France dans le domaine des biomatériaux. Il regroupe plus d'une centaine d'équipes académiques et 35 industriels et porte une démarche innovante interdisciplinaire, multi-instituts et inter-organismes dans le domaine de la médecine réparatrice.

Entre autres, le GDR « Réparer l'humain » :

- ✓ Fédère les forces et les acteurs des dynamiques déployées dans ce large domaine de recherche ;
- ✓ Met à disposition une cartographie nationale des formations autour des dispositifs médicaux ;
- ✓ Instaure un dialogue avec des associations de malades, dans le but de mieux intégrer la chaine de valeur de la recherche académique vers le patient ;
- ✓ Soutien sous la forme de mentorats des start-ups en développement.

L'inscription au GDR « Réparer l'humain » est gratuite. Pour en savoir plus, rendez-vous sur le site du GDR-RH : http://reparer-humain.insa-lyon.eu

Le GDR-RH travaille actuellement à un élargissement du périmètre d'action du groupement en abordant de nouvelles problématiques scientifiques. Les aspects « biomatériaux », la dimension SHS ainsi que le dialogue entre les laboratoires et les cliniciens seront renforcés, autour d'axes de développement qui intégreront, davantage encore, la formation et le rayonnement international.

Siltiss

DESIGNING TISSUE REGENERATION SOLUTIONS

Core business

French company specializing in the development, production and marketing of non-animal biomimetic biomaterials, which promote and support tissue regeneration processes

Applications

Bone substitutes for the orthopedic and dental sectors as well as maxillofacial surgery

Unique product offer

- Natural hydrogels of non-animal origin
- Biomaterials that are biocompatible and of variable porosity
- Biodegradable and biomimetic environment

Transfer technology

4

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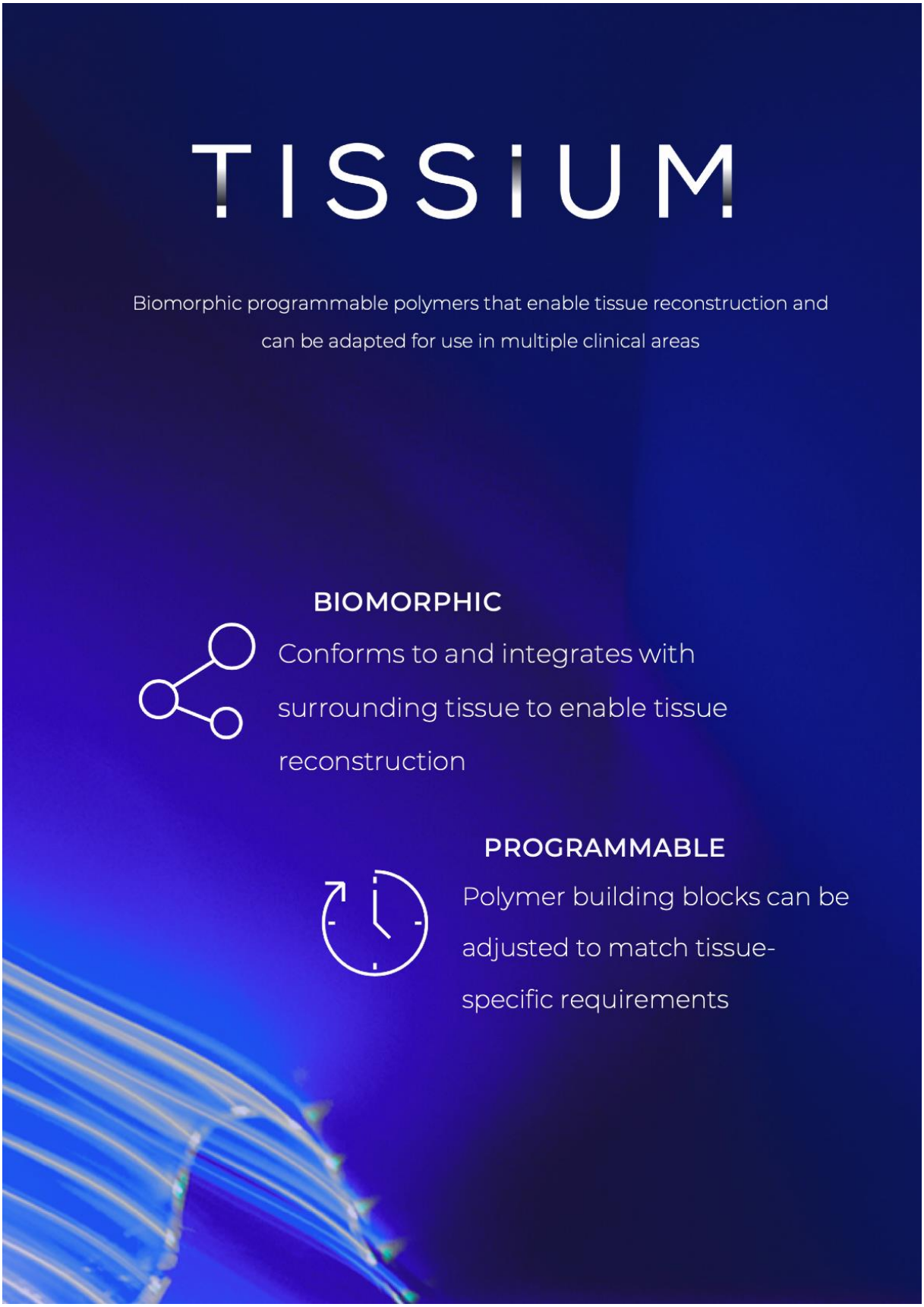
TISSIUM
Biomorphic programmable polymers that enable tissue reconstruction and can be adapted for use in multiple clinical areas
BIOMORPHIC
Conforms to and integrates with surrounding tissue to enable tissue reconstruction
PROGRAMMABLE
Polymer building blocks can be adjusted to match tissue-specific requirements


URGO RID
RESEARCH INNOVATION & DEVELOPMENT
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FOR CAREGIVERS, FOR PATIENTS
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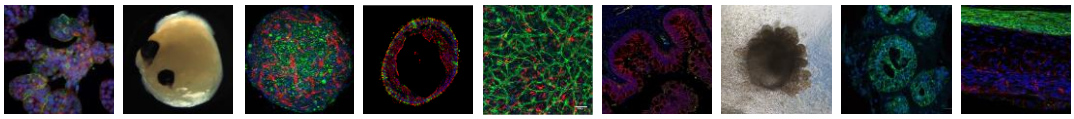
MEDICAL
Healing people

Qui sommes-nous ?

La recherche en biologie et en santé utilise des modèles pour comprendre les processus du développement, de la physiologie ou des pathologies. Le développement de systèmes modèles plus représentatifs en termes de physiologie et de prédictibilité a connu ces dernières années une innovation majeure : ***les organoïdes***.



Cette approche combine la maîtrise grandissante des outils de culture des cellules souches, du contrôle de la différenciation cellulaire, de l'édition de gènes et des technologies de culture multidimensionnelles. La combinaison de ces avancées technologiques offre ainsi un accès à des structures biologiques tridimensionnelles, reproduisant de plus en plus fidèlement l'architecture et les propriétés fonctionnelles des organes. Le développement considérable des organoïdes ouvre des perspectives dans de nombreux domaines, notamment en santé (médecine régénératrice et de précision, cibles thérapeutiques, biomarqueurs…). ***Le GDR Organoïdes (CNRS GDR2102)*** a été constitué en 2021 pour réunir dans un réseau national les équipes académiques et plates-formes s'intéressant à ce champ de recherches.

Les ateliers du GDR Organoïdes

Les actions du GDR sont subdivisées dans les axes suivants :

- *Recherche :* générer, caractériser, intégrer et exploiter les organoïdes
- *Valorisation :* soutien au transfert de technologies et aux interactions avec l'industrie
- *Plates-formes :* identification des équipes et accompagnement vers une activité de prestation
- *Formation :* recensement des enseignements universitaires et formations professionnelles ; organisation du concours étudiant INOContest
- *Ethique :* réflexion autour des enjeux éthiques et d'intégrité scientifique

Le Club des Partenaires

Rejoignez les partenaires industriels soutenant les actions du GDR Organoïdes.

NOTRE RESEAU

RENSEIGNEMENTS ET CONTACT

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https://inocontest.cnrs.fr/

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GDR Organoïdes

A VENIR

Les rencontres annuelles du GDR Organoïdes, 1-2 Décembre 2022, Biopark, Paris

Conférence plénière : Pr. Anne Grapin-Botton, Max Planck Institute (Dresden, Allemagne)

Programme complet prochainement disponible sur le site internet du GDR