REMIX Final Workshop

Biomaterials, Tissue Engineering and Regenerative Medicine: Potential, Exploitation and Validation of Natural Materials

Regenerative Medicine Innovation Crossing REMIX

3 | 4 April 2023

Vila Galé Collection, Braga, Portugal

WORKSHOP CHAIRS

Rui L. Reis

Natália Alves

LOCAL ORGANIZING COMMITTEE

- Ana Cláudia Lima
- Ana Guerra
- Ariana Santos
- Cátia Correia
- Daniela Peixoto
- Duarte Nuno Carvalho
- Helena Ferreira
- Luísa Rodrigues
- Rita Sousa
- Rui Costa

REMIX CONSORTIUM MEMBERS

UNIVERSITY OF TRENTO
Department of Industrial Engineering

Universidade do Minho
Instituto de Investigação em Biomateriais, Biodegradáveis e Biomiméticos

Chula
Chulalongkorn University

JEONBUK NATIONAL UNIVERSITY

ШУТИС

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REMIX PROJECT

The REMIX Final Workshop on "*Biomaterials, Tissue Engineering and Regenerative Medicine: Potential, Exploitation and Validation of Natural Materials*" will take place in the 3st and 4th of April, in the very hospitable city of Braga.

Regenerative Medicine Innovation Crossing -Research and Innovation Staff Exchange in Regenerative Medicine — 'REMIX' is a research and mobility project funded by the European Union under the MSCA-RISE instrument, G.A. 778078.

The project's partners are the University of Trento (Italy – BEN and Coordinator), University of Minho (Portugal - BEN), Chulalongkorn University (Thailand – OPE), Mongolian University of Science and Technology (MUST – OPE), Chonbuk National University (South Korea – OPE).

The main objective of REMIX is to actively investigate and develop natural or nature-inspired biomaterials for tissue engineering and regeneration therapy through intensive collaboration among European and Asian experts with different academic and scientific backgrounds, aiming at the build-up of a research hub of nature-derived biomaterials for TERM.

Within this overall objective, the project pursues several aims:

- exploring innovative applications of Natural materials in the biomedical field;
- benchmarking results, standards and procedures;
- comparing the different approaches and regulations driving this research sector in partners' countries;
- building in vitro models and technologies, and defining agreed procedures aimed at reducing the need of animal testing;
- equip post-docs, PhD students and younger researchers with the competence required to develop TERM products with the use of natural resources and materials. At the end of the program we expect 24 researchers to be fully trained in the field;
- train post-docs and PhD students in research methods, both through classes and through practice (including being involved in the organization of workshops);
- train post-docs and PhD students in innovation & entrepreneurship in a manner that is as hands-on as possible, by actually enabling start-up experiences (UNITN and UMINHO have realized two start-up companies on TERM materials). New ventures are very important in this field which is itself new, and we see entrepreneurship as an ideal way to exploit the project results – even more so as we hope students will also want to start initiatives in their own country, to give back to people in need.

WORKSHOP VENUE

The Workshop venue will be in the Hotel Vila Galé Collection, located very near to the heart of the city of Braga, being the ideal place also for those looking to experience the city's true essence. The beautiful city of Braga, considered one of the youngest European cities and distinguished the second Best European destination of 2019 by European Best Destinations. Founded by the Romans in the year 16 B.C and denominated "Bracara Augusta", Braga combines its bimillennial History with a youth and invigorating vitality. With more than 2000 years of History, Braga is the oldest Portuguese city and one of the oldest Christian cities in the world.

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GENERAL INFORMATION

The event will be at **André Soares Room** and the registration desk will be located at the **Rodrigo Moura Teles room** in the first day of the conference and will be open during the following days.

REGISTRATION AND INFORMATION DESK: All attendees must be registered for the Workshop. Admission to the conference and social events is permitted only to those wearing the official conference badge. If a name badge is misplaced, please contact the registration desk.
CERTIFICATE OF ATTENDANCE is available to all registered participants and will be sent by email after the event.

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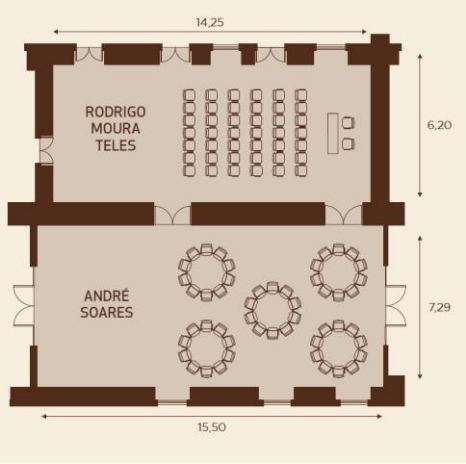
SMOKING POLICY: From 1st January 2008 legislation was introduced in Portugal, which makes it forbidden to smoke in all public places. This includes cafes, bars and restaurants (excluding those with signalized smoking areas). Smoking is only allowed outside the conference building.

PHOTOGRAPHY POLICY. Recording and photographing Workshop presentations will not be allowed.

ELECTRICITY SUPPLY: 220V is the standard power supply throughout Portugal. If you need a plug or a power adapter, you may find in electronic specialty retailers or ask in the registration desk.

TRANSPORTATION: In Braga, there is a bus that lets you travel through the city centre but, as it is not a very big city centre, you probably will prefer to walk by foot and enjoy the harmony of this city.



PROGRAM

	Day 1 | Monday | April 3	Day 2 | Tuesday | April 4
09.30 | 09.45		OP 5 - Tae Woong Kang
09.45 | 10.00		OP 6 - Chavee Laomeephol
10.00 | 10.15	Registration	OP 7 - Sunjae Park
10.15 | 10.30		OP 8 - Supamas Napavichayanun
10.30 | 10.45		OP 9 - Lekha Shah
10.45 | 11.00		Coffee-Break
11.00 | 11.15	Opening Ceremony	
11.15 | 11.30	KL 1 - Nuno Neves	KL 5 - Gilson Khang
11.30 | 11.45		
11.45 | 12.00		
12.00 | 12.15	OP 1 - Francesca Perin	OP 10 - Ilaria Corridori
12.15 | 12.30	OP 2 - Cátia Correia	OP 11 - Rita Sousa
12.30 | 14.00	Lunch	Lunch
14.00 | 14.15	KL 2 - Sorada Kanokpanont	KL 6 - Annalisa Tirella
14.15 | 14.30		
14.30 | 14.45		
14.45 | 15.00	OP 3 - Helena Ferreira	OP 12 - Ana Cláudia Lima
15.00 | 15.15	OP 4 - Francesca Agostinacchio	OP 13 - Solongo Ganbold
15.15 | 15.30	KL 3 - Miguel Alaminos	KL 7 - José António Vázquez
15.30 | 15.45		
15.45 | 16.00		
16.00 | 16.15	Coffee-Break	Coffee-Break
16.15 | 16.30		
16.30 | 16.45	KL 4 - Devid Maniglio	KL 8 - Subhas Kundu
16.45 | 17.00		
17.00 | 17.15		
17.15 | 17.30		Closing Ceremony

3B's

Universidade do Minho
Instituto de Investigação em Biomateriais, Biodegradáveis e Biomiméticos

REMIX

Horizon 2020 European Union Funding for Research & Innovation
MSCA-RISE 2019 GA 778078

SCIENTIFIC INFORMATION

KEYNOTE LECTURES

- 45 minutes presentation

ORAL PRESENTATIONS

- 15 minutes presentation

E-Poster Area

The posters will be displayed in LCD monitors available at Rodrigo Moura Teles room. Please check your poster code in the Posters List herein included.

One of the main objectives of the layout of this workshop is to allow an informal atmosphere where science can be discussed. Therefore, there are no specific Poster Sessions where you will need to present your poster.

Posters will be placed during the breaks scheduled in the program; poster presenters will have participants and Keynote Speakers circulating giving the informal opportunity to discuss their work with anyone interested and for how long you need.

SCIENTIFIC PROGRAM

Day 1 | Monday | April 3

10.00 | 11.00	Registration
11.00 | 11.15	**Opening Ceremony**
Session I – Chairs: Rui Reis & Natália Alves	
11.15 | 12.00	Nuno Neves 3B's Research Group – University of Minho, Portugal
12.00 | 12.15	OP 1 – Francesca Perin University of Trento, Italy
12.15 | 12.30	OP 2 – Cátia Correia 3B's Research Group – University of Minho, Portugal
12.30 | 14.00	Lunch
Session II – Chairs: Antonella Mota & Ana Cláudia Lima	
14.00 | 14.45	Sorada Kanokpanont Chulalongkorn University, Thailand
14.45 | 15.00	OP 3 – Helena Ferreira 3B's Research Group – University of Minho, Portugal
15.00 | 15.15	OP 4 – Francesca Agostinacchio University of Trento, Italy
15.15 | 16.00	Miguel Alaminos Granada University, Spain
16.00 | 16.30	Coffee-Break
Session III – Chairs: Annalisa Tirella & Subhas Kundu	
16.30 | 17.15	Devid Maniglio University of Trento, Italy

SCIENTIFIC PROGRAM

Day 2 | Tuesday | April 4

Session IV – Chairs: Devid Maniglio & Helena Ferreira

09.30 | 09.45	OP 5 – Tae Woong Kang Chonbuk National University, South Korea
09.45 | 10.00	OP 6 – Chavee Laomeephol Chulalongkorn University, Thailand
10.00 | 10.15	OP 7 – Sunjae Park Chonbuk National University, South Korea
10.15 | 10.30	OP 8 – Supamas Napavichayanun Chulalongkorn University, Thailand
10.30 | 10.45	OP 9 – Lekha Shah University of Trento, Italy
10.45 | 11.15	Coffee-Break

Session V – Chairs: Nuno Neves & Francesca Agostinacchio

11.15 | 12.00	Gilson Khang Chonbuk National University, South Korea
12.00 | 12.15	OP 10 – Ilaria Corridori University of Trento, Italy
12.15 | 12.30	OP 11 – Rita Sousa 3B's Research Group – University of Minho, Portugal
12.30 | 14.00	Lunch

Session VI – Chairs: Sorada Kanokpanont & Ilaria Corridori

14.00 | 14.15	Annalisa Tirella University of Trento, Italy
14.45 | 15.00	OP 12 – Ana Cláudia Lima 3B's Research Group – University of Minho, Portugal
15.00 | 15.15	OP 13 – Solongo Ganbold Mongolian University of Science and Technology, Mongolia
15.15 | 16.00	José António Vázquez Spanish National Research Council (CSIC),
16.00 | 16.30	Coffee-Break

Session VII – Chairs: Gilson Khang & Lekha Shah

16.30 | 17.15	Subhas Kundu 3B's Research Group – University of Minho, Portugal
17.15 | 17.30	**Closing Ceremony**

KEYNOTE LECTURE

Nuno M. Neves
3B's Research Group – University of Minho

Nuno M. Neves is an Associate Professor with Habilitation at the I3Bs Research Institute of the University of Minho, Portugal. He has been involved in biomaterials research since 2002 and is currently Director of the 3B's Research Group. Nuno M. Neves background includes: (i) BSc in Polymer Engineering, University of Minho, (ii) Master degree by research on Polymer Engineering and (iii) PhD in Polymer Science and Engineering, Univ. Minho, Portugal, degree that was prepared in cooperation with the University of Twente, Netherlands and (iv) Habilitation in Tissue Engineering, Regenerative Medicine and Stem Cells. He has worked several periods abroad at the University of Twente, in a sabbatical leave at the University of Tokyo, Japan and in a Visiting Professor position at the University of Trento in Italy.

His main area of research is focused on tissue engineering and regenerative medicine strategies using stem cells and biodegradable biomaterials for advanced drug delivery systems, scaffolds and medical devices. He is supervising or co-supervising the work of more than 20 post-graduation researchers. As of February 2023, he is the author of 245 publications in WoS (170+ peer reviewed international papers), with h-factor of 48 and a total number of citations of over 7800 (h:50;8900+ in Scopus) and is the co-inventor of 7 patents. He was invited and currently serves as editorial board member of Biomolecules, Frontiers in Bioengineering and Biotechnology and the peer-reviewed Elsevier Journal on Regenerative Therapy.

He is an elected member of the Board of Governors of the European Society for Artificial Organs and is currently the responsible for the Tissue Engineering Working Group of the ESAO. He is routinely invited to review research grants and research proposals for the European Commission and for various funding agencies namely in Portugal, Argentina, Austria, Czech, France, Georgia, Germany, Netherlands, New Zealand, Singapore, Slovakia, Slovenia and USA and advisory panels of research labs in France and Croatia.

Surface Functionalised Biomaterials and Nanostructures for Advanced Therapies

Many biomaterials have been proposed to produce porous scaffolds, nanofibers and nanoparticles for different medical treatments and applications. Systems combining natural polymers and synthetic biodegradable polymers offer particular properties adequate for those demanding applications. Those biomaterial systems can be tailored with enhanced mechanical properties, processability, cell-friendly surfaces and tunable biodegradability. Those biomaterials can be processed by melting or solvent routes into devices with wide range of applications such as biodegradable scaffolds, films or particles and adaptable to many other high performance biomedical applications.

Non-woven meshes of polymeric ultrafine fibers with fiber diameters in the nanometer range can be produced by electrospinning. Those meshes are highly porous and have a high surface area-to-volume ratio. Furthermore, they can mimic the fibrous structure of the extracellular matrix of human tissues and can be used as scaffolds for Tissue Engineering (TE). There is a great interest in developing also nanoparticles and hydrogels from those polymeric systems for injectable treatment modalities. All those structures can be used as substrates for specific surface functionalization having fine-tuned bioactivity and biological performance. This strategy enables developing highly controlled devices for exposure, capture and, whenever needed, inactivation of biological biomolecules. Those high-performance devices offer the specificity and local bioactivity that enable to design novel treatment modalities in various disease conditions. This talk will review our latest developments in biomaterials, nanoparticles and nanofibre meshes in the context of novel therapeutic applications.

KEYNOTE LECTURE

Sorada Kanokpanont
Center of Excellence in Biomaterial Engineering in Medical and Health,
Department of Chemical Engineering,
Faculty of Engineering, Chulalongkorn University,
Bangkok, Thailand

Dr. Sorada Kanokpanont is currently an Associate Professor at the Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University, Bangkok, Thailand. She received her Ph.D. (specialized in Biochemical Engineering) from the Department of Chemical Engineering, Drexel University, PA, USA in 2002.

She was one of the committee to found the Biomedical Engineering program and the Center of Excellence in Biomaterial Engineering in Medical and Health, Chulalongkorn University. She has been continuously received the European Union grants from Erasmus Mundus-Mobility grant funded by the European Commission, Action 2 One More Step from 2013 into the leader of Thailand's Marie Skłodowska-Curie Actions, Research and Innovation Staff Exchanges 2017 – 2025, the REMIX and the SHIFT projects.

Her expertise is in Biochemical Engineering, in the field of Biomaterial Applications. Her research which has been translated to commercialized products included the Edible coating for tropical fruits, Anthocyanin microencapsulating system, and the Sterile Thai silk fibroin solution.

In vitro 3D formation of solid tumors on gelatin/silk fibroin microspheres

In developing an *in vitro* system to mimic the formation of different solid tumor for chemo-sensitivity testing, Chulalongkorn University has been working in collaboration with the Department of Medical Science, Ministry of Health, Thailand, and the Center for Medical technology, Trento University, to develop gelatin/Thai silk fibroin based microspheres with the optimal protocol of cancer cell culturing on them. Series of experiments were test on various compositions, sizes, and morphologies of the microspheres, with the different cancer cell lines. For the project partly supported by the REMIX – H2020 - Marie Skłodowska-Curie Actions, Research and Innovation Staff Exchanges 2017. We successfully developed an efficient method of producing the microspheres at the size range of 30 – 100 microns, with a target degradation time of 2-3 weeks *in vitro*. Breast cancer cell line was used as a model to test 3D solid tumor formation. We could product significantly large spheroids within a week. The continuing testing is ongoing on in evaluating the solid tumor gene expression.

KEYNOTE LECTURE

Miguel Alaminos
Tissue Engineering Group, University of Granada, Spain, and Instituto de Investigación Biosanitaria ibs.Granada, Spain

Doctor in Medicine and Surgery, Doctor in Biology and Specialist in Paediatric Surgery. Full professor of Histology and Tissue Engineering at the University of Granada, Spain. More than 200 original articles in journals. Participation as PI in more than 20 research projects in the field of tissue engineering, including a European project, as coordinator. 12 patents related to biomaterials and tissue engineering. Participation in the design and generation of different types of artificial human tissues using novel biomaterials and nanostructuration protocols. Two of these artificial tissues (human cornea and skin) are being generated as Advanced Therapies medical products in GMP facilities of the Public Health System and are being clinically used in patients with the approval of the Spanish Agency of Medicines and Health Products. The clinical use of both types of artificial tissue has reached great scientific, sanitary and social relevance.

Generation of bioartificial tissues using natural biomaterials
Our experience in Granada

Development of functional bioartificial tissues and organs is strictly dependent on the availability of a suitable biomaterial that can act as a scaffold for cultured cells. Among the multiple biomaterials described to the date, naturally-derived biomaterials typically offer excellent biocompatibility and accessibility. During the last years, our research group has generated several types of bioartificial tissues using different sources of natural biomaterials with promising results.

On the one hand, we have evaluated the potential usefulness of five different types of agaroses extracted from several species of Gracilaria and Gelidium red algae. Results showed that all these agaroses were highly biocompatible both ex vivo and in vivo, and each type of agarose had specific biomechanical properties that can be tuned to generate a bioartificial tissue with definite rheological behavior. Combination of specific types and concentrations of agarose with human plasma allowed us to generate fibrin-agarose tissues displaying particular properties for use in cornea, scleral limbus, skin, and other applications. In this regard, we used a biomaterial based on human fibrin and type VII agarose at the concentration of 0.1% subjected to plastic compression nanostructuration methods to generate a bioengineered substitute of the human skin that is currently used in severely burnt patients, and a substitute of the human cornea that was evaluated in a clinical trial with good results.

On the other hand, we generated a decellularized biomaterial based on sturgeon cartilage. Evaluation of these biomaterials confirmed their excellent biocompatibility and the capability of different types of cells to grow and proliferate on the surface of these scaffolds, suggesting that surgeon cartilage-derived biomaterials could be potentially useful for cartilage or cornea tissue engineering.

Finally, a novel biomaterial obtained from the skin of holothurian marine invertebrates was extracted and evaluated. This biomaterial consists in small ossicles whose 3D structure and morphology is adequate for cell attachment and cell growth, and its composition is similar to the human bone. Regeneration studies were carried out on the rat mandible bone, demonstrating that these particles are able to promote bone regeneration in vivo.

These results suggest that the different natural biomaterials evaluated here were biocompatible and allowed the efficient generation of different types of bioartificial tissues, such as the human cornea, limbus, skin, cartilage and bone. In vivo evaluation suggests that these biomaterials were biocompatible and able to promote tissue regeneration.

Supported by the Spanish Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) of the Spanish Ministry of Science and Innovation (Instituto de Salud Carlos III), grants FIS PI20/0317, FIS PI21/0980, ICI19/00024 and ICI21/00010, and by grants PE-0395-2019 and PI-0086-2019 from Consejería de Salud y Familias, Junta de Andalucía, Spain. Cofunded by the European Regional Development Fund (ERDF) through the "Una manera de hacer Europa" program.

NOTES

KEYNOTE LECTURE

Devid Maniglio
1 Department of Industrial Engineering and BIOtech Center for Biomedical Technologies, University of Trento, Italy
2 European Institute on Tissue Engineering and Regenerative Medicine, Trento, Italy

I received my Master degree in Physics and my Ph.D. in Materials Engineering at the University of Trento (Italy), where I am associate professor in industrial bioengineering.

My research centers around biomaterials used for tissue engineering, cell-materials interactions, natural origin biomaterials treatment and processing, with particular focus on silk, finalized to scaffolds fabrication, bioinks formulation, tissue bioprinting, and synthesis of biomimetics for molecular recognition.

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Gas-based methods for producing fibroin scaffolds with controllable pore size and structure

Silk fibroin has attracted increasing attention for its potential biomedical applications, particularly in scaffold fabrication for tissue engineering due to its favorable biological properties and the ability to adapt to various application requirements through different fabrication methods. These methods can be utilized to produce films, sponges, fibers, nets, or gels with predictable degradation times. In tissue engineering, porous scaffolds are often necessary, sometimes requiring in-situ formation, and thus, mild body-compatible conditions must be employed.

We propose novel methods for the creation of silk fibroin foams and dry sponges through two alternative approaches. Firstly, fibroin foams are created using low-pressure N2O gas as the foaming agent, starting from the protein solution. This technique enables the production of fibroin porous scaffolds with tunable porosity, under mild processing conditions, and the use of an inert foaming agent that can saturate a fibroin water solution. The solution can be occasionally injected through a thin needle at the implantation site, where expansion and foaming occur.

In the second approach, air bubbles are mechanically integrated into a chemically modified fibroin (Sil-MA) solution. The incorporation of pendant double bonds into the fibroin chain allows for the stabilization of the foam through the addition of a photoinitiator (LAP) and the application of UV radiation, which triggers radical polymerization.

Optimal foaming processing conditions were studied, and the foams produced were characterized using Fourier Transform Infrared Spectroscopy (FTIR), compressive mechanical and rheological properties measurements, scanning electron microscopy (SEM), and microCT. In-vitro assessments were conducted to evaluate the potential of the foams as scaffolds for tissue engineering.

KEYNOTE LECTURE

José Antonio Vázquez
Spanish National Research Council- CSIC, Spain

Dr. José Antonio Vázquez (Ourense, Galicia, Spain, 1973) has a BSc and a MSc in Biochemistry from University of Salamanca (1996), another BSc in Science and Environmental Technology (2007, University of Vigo) and a PhD in Chemical Engineering from University of Santiago de Compostela (2001). He has done his research career in laboratories of USalamanca, the Marine Research Institute (IIM-CSIC), UVigo, The University of Manchester (UK) and a biotech company (INNAVES S.A.). Since 2009, he is a Tenured Scientist of CSIC, leader of the Group of Recycling and Valorization of Waste Materials (REVAL) from 2013 and head of the Food Technology Department (IIM-CSIC) since 2020. As leader of REVAL, his main line of research focuses on the development, mathematical modeling, optimization, and scaling of sustainable processes (physicochemical) and bioprocesses (enzymatic and fermentative) with low environmental impact aimed at the depuration and valorization of effluents, discards and by-products generated by the food industry –mainly from fishing, canning and aquaculture activities– under the concept of Biorefinery. This approach has led to the production, recovery and isolation of a wide catalogue of high value added bio-compounds: microbial metabolites (lactic and hyaluronic acids, prebiotics, polysaccharides, enzymes, etc.), probiotic bacteria, antimicrobial effectors (bacteriocins), digestive enzymes, collagen and gelatins, chitin and chitosan, glycosaminoglycans (chondroitin sulfate, etc.), bioapatites, fish protein hydrolysates, fish oils, peptides and bioactive compounds (antioxidants and antihypertensives). These results have been published in 184 JCR-SCI articles (h-index: 46), 74 conference and congress contributions (proceedings, posters and oral communications), several book chapters and the participation in 47 research projects and R&D contracts (19 as PI). He has also taught various subjects at the Universities of Salamanca, Vigo and Manchester (UK) and has supervised and co-supervised several doctoral theses, dissertations and master's degree projects.

Optimal production and potential applications of biocompounds recovered from fish and seafood wastes

Food industry based on fishing, canning and aquaculture activities is one of the main productive sectors in Galicia, both by tradition and by the number of jobs and economic profits it produces. However, such activities generate anually miles of tons of discards, by-products (heads, viscera, skins, skeletons, etc.) and effluents (chemical and cooking wastewaters) that have to be efficiently managed to avoid events of environmental pollution and issues of health human in the places where they are produced. From the REVAL group (IIM-CSIC), we have developed, optimized and scaled-up a wide set of sustainable processes and bioprocesses aimed to depurate and valorize those residues obtaining different high-added value biocompounds, including biopolymers, bioactives, probiotics, hydrolysates, etc. Some of those biocompounds, many of them prepared with tailored chemical properties, were sucessfully used in the formulation of scaffolds for tissue regeneration, nutraceuticals, nanodevices for bioactive delivering, aquaculture feed, biotechnology ingredients and biocides.

KEYNOTE LECTURE

Gilson Khang
Dept PolymerNano Sci & Tech, Jeonbuk National Univ, Jeonju, 54896, South Korea

Dr. Gilson Khang was born in 1960 in South Korea, where he obtained his degrees at the Inha Univ. He was studying for Ph.D. degree at the Department of Biomedical Engineering, The Univ of Iowa (Iowa City, IA, USA) from 1991~1995. His academic career started at the Department of PolymerNano Science and Technology at Chonbuk National University (CBNU). Dr. Khang was the one of Founder Members of TERMIS-AP Chapter. Prof. Khang was General Secretary and Treasurer for 2005~2009 of TERMIS-AP Chapter and served as a council member for TERMIS-AP. He was TERMIS-AP Past-President, & Founding Fellow TERMIS.

He has co-authored or edited ~30 books. He has published ~700 original research papers, and ~200 editorials, reviews or chapters in books. His papers were cited 18,720 times. (h-index >71) His major scientific contribution has been the importance of natural/synthetic hybrid scaffold to reduce the host inflammation reaction as well as the commercialization for tissue engineered products as cartilage, bone, retinal pigment epithelium, cornea endothelium, etc. His international collaboration network is really worldwide and tight over 7 countries and 15 Universities. He is/was engaging the Visiting Professor of Tsinghua Univ, Peking Univ, Zhejiang Univ, China and Wake Forest Institute of Regenerative Medicine, USA.

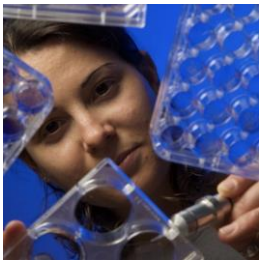
Biocompatibility Issues for the Tissue Engineered Products for Commercialization

Around 1992 as 20 years ago, Advance Tissue Science Co (USA), now merged to Smith & Nephew Co., USA, had been submitted to approve to USA FDA for first cartilage TEMPs as autologous chondrocyte/polyglycolic acid (PGA) nonwoven scaffold. At that time, no one had doubted to approve cartilage TEMPs since PGA was already approved by FDA in human clinical trial and chondrocyte was used autologous primary cell. At last, this product has been still retard up to approve FDA.

Main reason might be in terms of safety. Implanted TEMPs have been reported to induce sequential events of immunologic reactions in response to injury caused by implantation procedures and result in acute inflammation marked by a dense infiltration of inflammation-mediating cells at the materials-tissue interface. Prolonged irritations provoked by implanted biomaterials advance acute inflammation into chronic adverse tissue response characterized by the accumulation of dense fibrotic tissue encapsulating the implants.

In this lecture, we will discuss (1) recent advances for the commercialization trends for the tissue engineered products (TEMPS) including regenerative medicinal products, (2) scaffolds in terms of biocompatibility and safety issue, (3) smart scaffold for the application of clinical trial including improved biocompatibility and the reduction of host response, and (4) biocompatibility issue for the natural and synthetic polymers.

KEYNOTE LECTURE



Annalisia Tirella
Department of Industrial Engineering and BIOtech Center for Biomedical Technologies, University of Trento

Annalisa Tirella joined the University of Trento as Assistant Professor at the Department of Industrial Engineering and BIOtech research Center in November 2021 and is Honorary Senior Lecturer at The University of Manchester (UoM, UK). Annalisa was awarded a PhD in Materials for environment and energy from the University of Rome Tor Vergata in 2011. She has established expertise in emerging technologies for the manufacturing of biomedical technologies at micro- and nano-scale, such as 3D printing and microfluidics.

Annalisa's principal research interest focus on bioactive and functional biomaterials, delivery of therapeutics and cell-material interaction. The combination of these topics enables the manufacturing of biomedical products, such as 3D in vitro models for tissue regeneration, and nanotechnologies for controlled and/or sustained release of therapeutics. Moreover, the combination of 3D in vitro models and microfluidics is used to build organ-on-chip technologies as new tools for understanding biological processes (e.g. inflammation, tumour microenvironment), as well as test efficacy of therapies.

Annalisa has supervised 15 PhD students (10 to completion) and more than 40 UG/PG students. At UniTN she is involved in EU-projects, whereas at UoM she is sponsoring a NC3Rs fellowship and is co-I of MRC-DPFS project with highly translational impact. She has authored more than 40 research papers in peer-reviewed international journals and co-authored few book chapters, with > 1300 citations, and H-index 22. She has presented > 30 keynote/invited talks and oral presentations at international conferences, with > 20 published abstracts and conference proceedings. Annalisa has established a wide network of international collaborators across several disciplines, across academic, clinical and industrial partners.

Alginate-derived hydrogels to model ECM mechanics in vitro

Within the tumour microenvironment, several physico-chemical properties of the tissue extracellular matrix (ECM) are dysregulated. Traditional cell culture models and animal models, although being a valid alternative to study variations of tissues properties, do not provide sufficient control to further study cause-effect relationships between ECM composition and stiffness and cancer cells. Breast cancer is the most frequently diagnosed cancer in women, with mechanical properties of the ECM being recognised as regulators of tumour progression and eventually metastasis. Breast cancer stem cells (B-CSCs), a dynamic population within the tumour milieu, have been recently discovered as key players in breast tumour progression, metastasis and therapeutic resistance.
Alginate-based hydrogels are designed to model ECM variations within breast tumour, and used in engineered in vitro systems to transmit mechanical forces to cells. In this study we present for the first time how biomechanical and biophysical properties impact on B-CSC population using two human breast cancer cells (i.e. MCF-7, MDA-MB 231). The new 3D in vitro models enabled a fine control of ECM properties as tissue stiffness, extracellular pH and interstitial fluid flow and monitor cell response to the microenvironment monitoring clinically relevant biomarkers (ALDH, CD24, CD44, E-cadherin and vimentin). Results using these new in vitro models were found similar to in vivo observations, suggesting the urgency to develop new models to delineate and understand better interactions involved between ECM and cells, offering a precise control over ECM physical parameters.

KEYNOTE LECTURE

Subhas Kundu
3B's Research Group – University of Minho

Subhas C Kundu is a Research Coordinator at I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho, Portugal. Before this Research Professor position, he was the European Commission's European Research Area Chair (ERA Chair). He obtained his PhD in Genetics from Banaras Hindu University, India. Kundu received post-doctoral training at the Institute of Molecular Biology, Moscow; York University, Canada; Medical University, Lubeck, Germany and Brunel University, United Kingdom. He was the Founder, Head and Full Professor at the Department of Biotechnology, Indian Institute of Technology Kharagpur (IIT), India and a Distinguished Invited Professor at Dankook University, Korea. In his early teaching career, he was An Assistant to a Full Professor and Head of the Department of Life Sciences at Manipur University, Imphal. He taught genetics, cell and molecular biology, and recombinant DNA technology to undergraduates, post-graduates and pre-doctoral students. Kundu has published 225 major research articles in peer-reviewed high-impact factor journals such as Experimental Cell Research, Methods in Enzymology, J Biological Chemistry, Biotechnology and Bioengineering, Acta Biomaterialia, Biomaterials, Progress in Polymer Science, Biotechnology Advances, Advanced Drug Delivery Reviews, Journal of Controlled Release, Biomacromolecules, ACS Applied Materials and Interfaces, Advanced Functional Materials, Advanced Healthcare Materials, Advanced Materials and others. Kundu has an H-index of 73 and 22,915 citations. His interest is in 3D cancer modelling and drug screening, including naturally-derived silk biomaterial matrices.

Silk - a natural biomaterial for regenerative medicine

Different biomaterial scaffolds/matrices (synthetic, natural, and blends) are significantly interesting for biomedical applications. They promote the regeneration of various tissues based on the correlation of the extracellular matrix. Silk proteins fibroin and sericin from mulberry and nonmulberry silkworm species are well-established as natural biomaterials. Fibroin, the core and fibrous protein, is hydrophobic, and sericin is a globulus and hydrophilic glue-like protein. The essential characteristics are biocompatibility, tunable mechanical properties, controllable degradation rates, and noninflammatory. We can generate different material formats with the help of various technologies for obtaining regenerated silk proteins and silk gland proteins, like thin films, porous 3D scaffolds, hydrogels, bioink, nanofibers, nanoparticles, macro-patterns, sponges, microcapsules, conductive, coating reinforced with hydroxyapatite salts or carbon nanofiber and micro-beads. These silk-based materials are helpful for biomedical, tissue engineering, and regenerative medicine. The 2D/3D cell cultures and hard and soft tissue formations on silk-based biomaterials exhibit proper cell adhesion, proliferation, and differentiation of stem cells. They act as a substrate for tissue construction and repair or as a delivery vehicle to aid in developing damaged tissues/organs. Silk sericin and fibroin as surface coating materials on titanium facilitate osteoblast cell adherence and proliferation. We use silk fibroin matrices as 3D models for cancer investigation. Sericins are tested for their cytocompatibility and biomaterial potential, including delivery of bioactive molecules (growth hormones, drugs and antibiotics) and skin tissue engineering. Silk protein is proven to be a bioelectronic and optical material. The results of the silk proteins sericin and fibroin work will be presented.

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Modelling a dynamic printability window: a new take on printability assessment studies

Francesca Perin [1,2,4] and Eugenia Spessot [1,2] , Anna Famà [1], Alessio Bucciarelli [3], Carlos Mota [4], Antonella Motta [1,2], Devid Maniglio [1,2]

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Bioprinting is gaining increased attention in the field of regenerative medicine and tissue engineering thanks to its great potential. Among the various techniques currently available, the most widespread one is extrusion-based-bioprinting (EBB). Despite the great research effort on the topic, many challenges still need to be faced to bring this technology to the clinic. Due to the novelty of the field, standardized procedures to evaluate performance of the bioinks are still not defined, resulting in researchers relying to lengthy trial-and-error based optimization procedures. In the presented work, we propose a standardized procedure to evaluate the performance of polysaccharide blends bioinks by identifying a "dynamic printability window". The study was conducted on different compositions of a blend of alginic acid sodium salt and sodium hyaluronan, which were subjected to extensive rheological analysis and printing trials. To assess the printability of the inks, quantitative parameters were selected from literature. From the conducted analysis empirical models describing the rheology of the blends as a function of their composition and the geometrical accuracy as a function of the composition and the printing pressure were built. The dynamic printability window was identified by applying to the empirical model some constraints related to the geometrical accuracy of planar constructs. The proposed model was suitable to predict the printing quality of methacrylated versions the polysaccharide blends, to optimize the printing parameters by applying a desirability function.

Francesca Perin

Francesca holds a Bachelor in Industrial Engineering and a Master in Materials and Production Engineering, both from University of Trento. In her Bachelor thesis, she worked on 3D printing, while during the Master she discovered her interest for biomedical applications of materials engineering with a thesis on oxygen generating microparticles to prevent hypoxia in scaffolds.

In 2019, she began her PhD at BioTech (University of Trento) under the supervision of prof Antonella Motta and Devid Maniglio. In her PhD ject, she brings together her interest for 3D printing, tissue engineering and biopolymers, by working on the engineering of versatile bioinks. Currently, bioprinting is emerging as an ever-growin technology for biomedical research, however, it still presents many challenges regarding the engineering and testing of suitable materials. Francesca's project aims at developing versatile inks with multiple applications, with the hope to speed up translation from the lab to real life applications. In june 2022 she moved to MERLN Institute (Maastricht University) to finish her PhD under the cotutelle of Dr Carlos Mota and to join the BIRDIE project team.

Adhesive and biodegradable membranes from marine origin raw materials for soft tissue engineering applications

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Tissue engineering (TE) is 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. Different natural materials have been proposed for TE applications. An example is chitosan - a polysaccharide obtained by the deacetylation of chitin that is a structural element of the crustaceans exoskeleton. Chitosan is often combined with collagen to improve its mechanical properties and cytocompatibility. Collagen type I (Coll I) can be obtained from fish by-products, representing a sustainable strategy for marine by-products valorization with economic and ecological advantages[1]. Herein, we obtained new adhesives by conjugating marine Coll I with a catechol derivative 3,4-dihydroxybenzaldehyde (3,4-DB)[2]. The conjugate (Coll-Cat) was blended with chitosan (ChitColl-Cat) and evaluated as a material for regenerating soft tissues. Coll I was extracted from Atlantic cod (*Gadus morhua*) skins by supercritical fluids technology using CO_2 acidified water. Schiff's base reaction was performed by stirring 46 mg/mL of 3,4-DB dissolved in osmotized water at 60°C and 5 mg/mL of collagen dissolved in acetic acid (AcOH) for 2h at RT. The modification was confirmed by UV-spectrophotometry and ^{1}H-NMR. Membranes prepared from 2.5% w/v of deacetylated chitosan in AcOH (1.5% v/v) and glycerol (5% v/v) 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 in a Petri dish and then dried at 37°C.

The ChitColl-Cat membranes presented a dark brown color due to the presence of catechol groups, different from the chitosan (transparent) and ChitColl (opaque) membranes. The functionalization with catechol groups enhanced the adhesion strength (22.5 ± 4.9 kPa), which is similar to the natural adhesives used in the clinical context and higher than some synthetic adhesives in wet environment. Biological assessment was performed by seeding fibroblastic L929 cells in direct contact with the membranes. The results showed an improved cell attachment and viability for the ChitColl-Cat membranes. Our data demonstrated that the use of the underexploited marine-derived polymers is a sustainable approach to develop new adhesive membranes, which provide mechanical and structural support for an efficient soft tissue repair.

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Cátia Correia

Cátia Correia graduated in biochemistry from NOVA School of Science & Technology (FCT NOVA), NOVA University Lisbon, and received her MSc in neurosciences from Lisbon School of Medicine, University of Lisbon. Since 2020 she has been attending the doctoral Program on Tissue Engineering, Regenerative Medicine and Stem Cells (TERM&SC) from the University of Minho, affiliated to I3B's- Research Institute on Biomaterials, Biodegradables and Biomimetics (3B's Research Group), funded by Fundação para Ciência e Tecnologia (FCT).
Her research is mainly focused on the development of new bioadhesive materials based on natural biomaterials, including marine collagen, hyaluronic acid and chitosan, combined with catechol groups for spinal cord injury application. She participated as a research fellow in 4 projects, is the author of 3 published scientific papers, and has 1 book chapter.

Hyaluronic acid-based formulations for the treatment of neurological disorders

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High molecular weight hyaluronic acid (HMW-HA) is one of the main structural components of the brain extracellular matrix (ECM), playing a pivotal role in the central nervous system (CNS) homeostasis and repair.[1-2] Thus, it is very appealing the use of this natural polymer for the development of therapeutic approaches to treat neurological disorders. In this work, HMW-HA was selected for the development of different injectable formulations to treat multiple sclerosis (MS) and glioblastoma (GB), the leading cause of chronic neurologic disability in young adults and the deadliest brain cancer, respectively.

For the neurodegenerative disease treatment, the HMW-HA hydrogel physically crosslinked with large unilamellar liposomes (LUVs) was used to incorporate bone marrow mesenchymal stem cells (BMSCs). These cells possess important features for the treatment of MS (e.g., re-myelination, immunomodulation, neuroprotection and reduced gliosis).[3] *In vivo* assays demonstrated the distribution of the biocompatible hydrogel into the corpus callosum after its intracerebroventricular injection, without any evidence of tissue damage. This can be highly strategic for MS and other neurodegenerative diseases treatments, since damage of this white matter structure is responsible for important neuronal deficits. Moreover, the hydrogel, containing a lower number of cells than previously reported, significantly decreased disease severity and maximum clinical score. Importantly, it also eliminated the relapse in an experimental autoimmune encephalomyelitis (EAE) rat model.

For GB, the hydrogel was designed for the direct injection into the resection cavity, aiming to simultaneously promote the sustained release of an anticancer drug while avoiding stimulatory native ECM effects on tumour cells.

Thus, HMW-HA was functionalized with the fibronectin inhibitor peptide Arg-Gly-Asp-Ser (RGDS) and physically crosslinked with LUVs encapsulating doxorubicin (DOX).[4] Conversely to unmodified hydrogels, RGDS-functionalized HMW-HA hydrogels presented cytotoxicity even without DOX incorporation. Moreover, RGDS-functionalized HMW-HA hydrogels incorporating liposomes with DOX efficiently damaged GB cells without affecting the metabolism and viability of astrocytes, proving their safety.
Herein, it is demonstrated the potential of HMW-HA hydrogels in the engineering of different formulations for the healing and repair of diseases that are pressing health issues in the world.

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Helena Ferreira

Helena Ferreira graduated in Pharmaceutical Sciences (2001), had a post-graduation in Medical-legal Sciences (2001) and a PhD degree in Pharmaceutical Chemistry (2006) at Porto University (Portugal).
She was also Invited Assistant Prof. at CESPU (2006-2013) and a Doctoral fellow (2008-2016) at Minho University, CESPU, CIIMAR/CIMAR and 3B's Research Group of University of Minho. Since 2017, she is Assistant Researcher at 3B's Research Group, I3Bs – Research Institute on Biomaterials, Biodegradables and Biomimetics, University of Minho. She has demonstrated impact within a portfolio of research activities related to nanomedicine, biomaterials science, nanotechnology, immunology, pharmacology, microfluidics and tissue engineering and regenerative medicine fields. Helena Ferreira is author or co-author of 57 papers in peer-review journals, 2 book chapters with international circulation and 3 conference publications. Her work has 1468 citations and she has a index of 21. She was as lecturer in several National and International Conferences, Courses and Workshops (22 oral presentations and 78 posters). In 2017, she was awarded the IAAM Scientist Medal, due to her contribution in the field of "New Age Technology & Innovations". Her experience in IP rights and patent exploitation (improved by her selection for the EIT Jumpstarter Bootcamps) resulted in 6 international patents. In addition, Helena Ferreira has long and solid experience in the supervision of ungraduated (training period), master and PhD students. She is an expert reviewer for EU projects and of abstracts for international conferences. She is referee for several and high-ranked journals, reviewer board member of different journals and the Guest Editor of two Special Issues.

Methacrylate silk fibroin sponges as candidates for bone tissues substitutes

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Silk fibroin, extracted from silkworm cocoons, exhibits fascinating properties as biodegradability, low cost, ease of fabrication, tunable mechanical properties, and degradation kinetics, among others. It is widely used in tissue engineering and regenerative medicine fields[1]. Thus far, one of silk fibroin's extraordinary properties is its capability to promote MSCs differentiation toward osteogenic phenotype, making it a good candidate for bone tissue regeneration applications, both *in vitro* and *vivo*[2]. Silk fibroin's outstanding and versatile properties allow an easy control of the pore dimension, degradation rates, and mechanical properties while providing proper biological environment for cell differentiation, thus being a good candidate for bone applications[3]. Among the different crosslinking processes, photo-crosslinking allows the formation of covalent, stable bonds in a controlled and precise manner[1].
In this work, photo-crosslinked methacrylate silk fibroin sponges have been evaluated as bone substitute candidates. Specifically, the sponges were fabricated with different photo-initiator concentrations and in presence/absence of a porogen. The impact of the composition on the pore size, mechanical properties, and stem cells differentiation toward osteogenic phenotype has been investigated. The study demonstrated that the different sponges' compositions, thus the presence/absence of porogen and different photo-initiator concentrations did not affect silk fibroin secondary structure. Moreover, the lowest photo-initiator concentration in combination with the porogen led to widest pore distribution and enhanced osteogenic differentiation as confirmed by gene expression tests.

All the compositions supported cell differentiation toward osteogenic phenotype and that pore size can be controlled by tuning sponges' composition. A significant positive effect on pore dimension and osteogenic differentiation was obtained in presence of the porogen which might be due to stabilization of air bubbles inside silk fibroin sponges.

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Francesca Agostinacchio

Francesca holds a Bachelor in Medical and Pharmaceutical Biotechnology (University of Bari) and a Master in Cellular and Molecular Biotechnology (University of Trento). In her Bachelor thesis, she worked on the biomolecular Identification of Hepatitis E virus in pig's livers, while in the Master she discovered her interest in tissue engineering combining alginate scaffolds with neural stem cells for *in vitro brain* regeneration models. In January 2023, she successfully defended her PhD carried at BioTech Center (University of Trento) in collaboration with Tufts University, under the supervision of prof Antonella Motta, Sandra Dirè, and David Kaplan. In her PhD project, she worked on the development of silk fibroin-based tissue engineering approaches for the treatment of degenerated intervertebral disc. During her PhD she worked for 10 months at Tufts University, where she delved into silk manipulation and 3D printing techniques.

Microgels based on hyaluronic acid containing transforming growth factor-beta3 and bone marrow-derived mesenchymal stem cells for osteochondral regeneration

Tae Woong Kang[1], Ju Hwa Lee[1], and Gilson Khang[1]

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Osteochondral defects of the knee significantly alter the biomechanics of the joint. This can cause symptomatic and functional impairment as well as considerable risk of progressive joint degeneration. To solve this problem, we have prepared transforming growth factor-beta3 (TGF) and bone marrow-derived mesenchymal stem cells (BMMSC)-loaded microgels based on hyaluronic acid (HAm) crosslinked with 1,4-Butanediol di-glycidyl ether (BDDE) for effective osteochondral regeneration. Prepared HAm of the structural, physical, and chemical properties was characterized by proton NMR, FT-IR, SEM, and rheometer. The biocompatibility of HAm was confirmed by observing that NIH/3T3 cells survived more than 80% for 3 days in vitro. In order to determine the appropriate concentration of TGF to be used for osteochondral differentiation treatment, BMMSC cultured plates were treated with concentrations of 0 to 100 μg/ml, respectively, and the degree of proliferation and chondrogenesis were observed. Among them, chondrogenesis of BMMSC was most effective when treated with 20 μg/ml of TGF. Next, HAm, HAm-TGF, HAm-BMMSC, and HAm-TFG-BMMSC were treated for 4 weeks in an in vivo rat osteochondral defect model, respectively, and through histological analysis, the most effective osteochondral regeneration effect was shown when HAm-TGF-BMMSC was treated. Therefore, the prepared HAm-TGF-BMMSC can be used as a potential and promising alternate material for osteochondral regeneration.

Tae Woong Kang

Tae Woong Kang joined Jeonbuk National University (JBNU) in 2022 as a postdoctoral researcher under Prof. Gilson Khang. His research interest is broadly in the area of synthesis and characterization of functional organic biomaterials. His current research focuses on the microneedle patch containing exosomes for applications of tissue engineering. He received his Bachelor, Master, and PhD degrees in Kongju National University, Ajou University, and Tokyo Medical and Dental University.

Metal ions mediate silk fibroin gelation: Mechanism insight and potential biomedical applications

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Gelation of a regenerated silk fibroin solution can be accelerated by using a metal ion to induce crosslinking reaction. Gelation of the silk fibroin solution, which typically takes approximately 3 to 7 days, can be achieved within a day with the addition of gold (III) chloride. The mechanisms and feasibility of the gold salt as a crosslinker of silk fibroin for hydrogel formation were investigated. It was proposed that the gold ions were reduced by amine groups and tyrosine residues, leading to the formation of dityrosine and the reduction of gold (III) to gold nanoparticles. The color of the obtained hydrogels changed from yellow to purple-red, which is the characteristic color of gold nanoparticles. When the amine groups of silk fibroin were replaced with thiols, there was no change in appearance after gelation. Bonding between gold (I) ions and thiol groups as well as the disulfide bond formation were proposed, which prevents further reduction of gold ions to form the nanoparticles.

Cytocompatibility of gold (III) ion-mediated silk fibroin-based hydrogels was confirmed *in vitro* using a mouse fibroblast cell line. There was no difference between the hydrogels made of regenerated and thiolated silk fibroin. The viability and growth rate of the cells cultured on the hydrogels were normal even with the highest testing amount of gold salts. The results showed the potential of metal ion-mediated silk fibroin hydrogels as biomaterials for biomedical purposes.

Chavee Laomeephol

Chavee Laomeephol graduated from biomedical engineering program, faculty of engineering, Chulalongkorn University. The PhD works were focusing on the silk fibroin gelation and the applications of the hydrogels in tissue engineering and drug delivery. In 2018, Chavee received the REMIX scholarship for an exchange as a research intern at 3B's research group, University of Minho, Portugal under the supervision of Dr. Helena Ferreira and Prof. Nuno M. Neves. Currently, Chavee is a post-doctoral researcher at the department of pharmaceutics and industrial pharmacy, faculty of pharmaceutical sciences, Chulalongkorn University, Thailand. The current works focus on the development of nanoparticles, such as virus-like particles or polymeric nanogels, for *in vivo* targeting delivery of genetic materials.

An evaluation of the effects of different harvesting sources of demineralized bone particles-Gellan Gum scaffolds on bone regeneration

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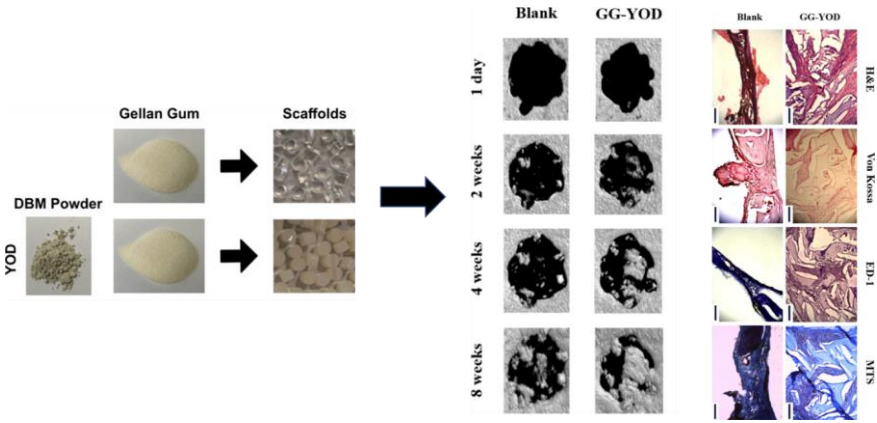
Bones are the hard tissues that make up the skeleton. Generally, when these tissues are damaged, they have some self-healing potential, but if the ability is limited, the process of transplanting bone scaffold entails surgery. Various natural and synthetic polymers are used as the material of Bone scaffolds. Above all, demineralized bone particles (DBP) are processed derivatives of homologous grafts that are immunologically harmless, biocompatible, and contain factors such as collagen, non-collagen protein, and bone-forming protein (BMP). In this study, we exploited the effect of the use of different sources of demineralized bone powder (DBP) in combination with gellan gum (GG) to form a GG–DBP hydrogel scaffold with the purpose of regenerating the bone tissue. we screened poultry that was cost-effective and simple manufacturing process with DBP. DBP was extracted from the femurs of two typologies of Gallus gallus domesticus (Yeonsan Ogye, a traditional and rare black chicken from Korea, and the Cornish cross, the most common breeds for industrial meat production) and the Pekin duck. The composite scaffold has been studied both in vitro and in vivo. Using rat bone marrow-derived mesenchymal stem cells (rBMSC), in vitro studies confirmed that bone-specific genes were expressed in seeded GG-DBP scaffolds, that differentiation was possible, and that there was significant upregulation. When compared to scaffolds with DBP obtained from other sources, the scaffold containing DBP derived from Yeonsan Ogye (YO) bone showed higher levels of cell proliferation and osteogenic differentiation. According to the study, YO DBPs have higher melanin levels than Cornish cross and Pekin ducks, as analyzed by the fluorescence intensity of melanin. Overall, this study clearly shows the use of YO DBP as a promising material in bone tissue regeneration.


Gellan Gum
Scaffolds
DBM Powder
YOD
Blank
GG-YOD
1 day
2 weeks
4 weeks
8 weeks
Blank
GG-YOD
H&E
Von Kossa
ED-1
MTS

Sunjae Park

Sunjae Park graduated from Kyung-Hee University (KHU) with a degree in medical science. From 2019 to 2021, he earned a master's degree in pharmaceutics from Chung-Ang University (CAU). During his master's degree, he conducted research on the solubilization of poorly soluble drugs using mesoporous silica nanoparticles. And he joined Jeonbuk National University (JBNU) in 2021 as Ph.D. course under Prof. Gilson Khang. He is currently focusing on research on drug or exosome delivery systems through hydrogels formulation using natural polymers such as silk fibroin and gellan gum.

Development of natural products in the pharmaceutical industry

Supamas Napavichayanun, Pornanong Aramwit
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Natural products are used in the treatment of various diseases for a long time. They are developed to be medical devices, cosmetics, food supplements, and herbal medicines. However, the acceptance of physicians and medical professionals for treatment with natural products, especially herbal medicines, is lower than that of chemical drugs. The key problem of natural products is the control of natural substances to ensure high quality and consistency in every production batch. In addition, evidence of product research and development including clinical study must fully support the products. There are a few natural products that have been successfully developed and registered as herbal medicines in Thailand. Therefore, the aims of our teams are to develop natural products to be therapeutic products in the market. Our product development process has collaboration with farmers, researchers, veterinarians, physicians, and manufacturers. We developed products in whole process including *in vitro*, *in vivo*, clinical studies, and product registration. The natural substances are extracted and analyzed using technologies that are possible to process in industry. Cost effectiveness is always concerned. Many natural products of our team's such as scar cream and calcium supplement were successfully researched and developed into therapeutic products in the market. Herbal medicines including herbal artificial saliva and psoriasis cream are also developing in the process.

Supamas Napavichayanun

Dr. Supamas Napavichayanun is a researcher at Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand. She received Ph.D from the Department of Pharmacy practice, Faculty of Pharmaceutical Sciences, Chulalongkorn University, in 2017. Her expertise is in the development of natural products and biomaterials including herbal medicine, supplements, and medical devices. She also conducts clinical trials on medical products.

ORAL PRESENTATION – OP9

Tuning physico-chemical properties of alginate hydrogels to culture and characterize cancer spheroids

Lekha Shah, Ayşe Latif, Kaye J. Williams, Annalisa Tirella

Within the tumour microenvironment, several physico-chemical properties such as extracellular matrix (ECM) stiffness, extracellular pH and interstitial fluid flow are dysregulated during tumour progression. Traditional preclinical models, although useful to study biological processes, do not provide sufficient control over these physico-chemical properties, hence limiting the understanding of cause-effect relationships between the TME and cancer cells. Breast cancer stem cells (B-CSCs), a dynamic population within the tumour, are known to affect tumour progression, metastasis and therapeutic resistance. With their emerging importance in disease physiology, it is essential to study the interplay between above-mentioned TME physico-chemical variables and B-CSCs.

In this work, 3D in vitro models with controlled physico-chemical properties (hydrogel stiffness and composition, perfusion, pH) were used to mimic normal and tumour breast tissue to study changes in proliferation, morphology and B-CSC population in two separate breast cancer cell lines (MCF-7 and MDA-MB 231). Cells encapsulated in alginate-gelatin hydrogels varying in stiffness (2-10 kPa), density and adhesion ligand (gelatin) were perfused (500 µL/min) for up to 14 days. Physiological (pH 7.4) and tumorigenic (pH 6.5) media were also used to mimic changes in extracellular pH within the TME.

We found that both cell lines have distinct responses to changes in physico-chemical factors in terms of cellular proliferation, spheroid size and morphology. Most importantly, stiff and dense hydrogels (10 kPa) and acidic pH (6.5) play a key role in B-CSCs dynamics, increasing both epithelial (E-CSCs) and mesenchymal cancer stem cell (M-CSCs) marker expression, supporting direct impact of the physico-chemical microenvironment on disease onset and progression.

Lekha Shah

Lekha Shah is a biologist by training and currently works in the field of tissue engineering, with a focus on effect of biomaterials on cellular phenotypes. She completed her Bachelor's and Master's degree (BS-MS) in Biological sciences from Indian Institute of Science education and Research (IISER), India. As a part of her Master thesis, she worked on intracellular trafficking of proteases in breast cancer cells and their implication in extracellular matrix degradation. She received her Doctoral degree from the University of Manchester, UK (April 2022) under the supervision of Dr Annalisa Tirella and Prof Kaye Williams. Her thesis explored effects of mechanical properties of alginate hydrogels on breast cancer phenotypes (stemness and EMT markers, invasion and bone metastatic potential). Currently, she is working as a post-doctoral research associate at BIOtech, University of Trento, Italy where she is standardizing use of enzymatically crosslinked Silk fibroin and hyaluronic acid based hydrogels for artificial corneal implants.

Development of biomedical silk: optimization and standardization of the production

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In 1400, since the annexation of Trentino to the Republic of Venice, and thus to the Silk Road, silk fibres are entangled in this territory. Venice started to share knowledge on mulberry and silkworm farming, creating fertile soil for the silk industry in Trentino. During the XIX century, Trento and Rovereto, the two main cities of Trentino, were at the frontline of the production and commercialization of silk fabrics and fibres.
In the following century, the use of silk broadened in other fields than fabrics and arrived in the biomedical research world. Indeed, the silk's protein fibroin is extremely versatile, biocompatible, and biodegradable, characteristics that define silk as an interesting candidate for tissue regeneration, drug release systems, and sensors. However, the lack of standards in the production and characterization of silk often causes the loss of reproducibility in research.
The project "Development of biomedical silk" aims to bridge the gap between the sericulture and research world, creating a local high-quality production of silk. High quality will be pursued through the standardization of the whole production process, from the mulberries to the wellness of silkworms, to the silk. An overview of the project will be presented, showing the results of the market research, the pilot silkworm rearing and the one scheduled in April 2023, and the Standard Operative Procedures (SOPs) developed during the first year of the project and in collaboration with the Chulalongkorn University.

Ilaria Corridori

Ilaria Corridori has a Master's of Biomedical Engineering and a Ph.D. in Civil, Environmental, and Mechanical Engineering. She is currently a Postdoctoral Fellow at BIOtech Research Center, Department of Industrial Engineering (University of Trento, Italy). During her Ph.D., she focused on two possible solutions for the regeneration of the spinal cord after injuries: an intraspinal medical device and microstructured fibroin hydrogels. Ilaria is now working on the project "Development of Biomedical Silk", intending to produce high-quality silk for biomedical applications. The project is funded by the Municipality of Rovereto and in collaboration with a local farm, and it is willing to introduce Rovereto (Trento, Italy) to a new production and use of this traditional material.

Extraction and characterization of collagen from fish by-products as promising building-block for Biomedicine

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Collagen is a naturally occurring structural protein present in animals, comprising about 30% of the total protein content of vertebrates. Several industries, including the biomedical, cosmetics, food, and pharmaceutical industries, employ collagen and derivatives, being extracted mostly from by-products resulting from mammal slaughter, with current ethical standards claiming for alternatives. The sustainable exploitation of aquatic resources, namely the use of fish by-products, to obtain valuable compounds for different biotechnological applications is receiving increasing attention under the Circular Economy concept. Because of its special qualities, which include the lack of religious limitations, no risk of disease transmission, biocompatibility, biodegradability, functionality, and low antigenicity, the usage of aquatic-based collagen as a substitute source of biopolymer suitable for human applications is quickly growing. The main objective of the present study was the valorization of selected fish by-products resulting from the processing of this organism for food purposes, particularly studying the properties of collagen that are influenced by the source material and the conditions of extraction, which then dictate its use.

Three main collagen extraction procedures – acetic acid, pepsin, and CO_2 acidified water – were employed to extract collagen from the skin and swim bladders of salt-cured Atlantic codfish (*Gadus morhua*) and/or Asian sea bass scales). The extraction of collagen was possible by all methods, with higher yields being obtained for the codfish skin collagen extracted using CO_2 acidified water (AWCs). All extracts were characterized by the SDS-PAGE and the obtained profiles were compatible with type I collagen. The results of FTIR-ATR and CD spectroscopy have shown that the pepsin soluble collagen from codfish swim bladders (PSCsb) structure underwent a slight denaturation, while the other extracts structure remained intact, with preserved triple helix. All extracts exhibited a typical shear thinning behaviour – non-Newtonian fluid, like hyaluronic acid –, an interesting property particularly for injectable materials or processing by 3D printing. Furthermore, the thermal analyses suggested that the extracts from Asian sea bass scales presented a higher thermal stability when compared with the extracts from codfish by-products. Overall, the results showed that it is possible to produce aquatic collagen with promising qualities for biomedical applications using a quick, low-cost, environmentally friendly technique.

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Rita Sousa

Rita O. Sousa is a PhD student (since 2019) in Tissue Engineering, Regenerative Medicine and Stem Cells at 3B's Research Group, I3Bs - Research Institute on Biomaterials, Biodegradables and Biomimetics (3B's Research Group) of University of Minho. She has a BSc degree in Marine Biology and Biotechnology (2012) and a MSc degree in Biotechnology of Marine Resources (2017) from the Escola Superior de Turismo e Tecnologia do Mar (ESTM) of the Instituto Politécnico de Leiria (IPL), Peniche, Portugal. She developed her knowledge in the valorization of marine biological resources and derived by-products, focusing on the areas of green and blue biotechnology, through the extraction and characterization of compounds of interest (biopolymers), aiming the development of marine inspired biomaterials for regenerative medicine strategies. In this perspective, she is developing a PhD thesis on the production of fish collagen and its use on biomaterials for wound healing and skin regeneration. She is the author or co-author of 6 experimental scientific publications, 1 book chapter, and 1 patent application.

Chitosan/hyaluronic-acid nanoparticles biofunctionalized with neutralizing antibodies as a new therapeutic strategy for osteoarthritis

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Osteoarthritis (OA) is a progressive multifactorial degenerative joint disease that affects millions of people worldwide [1]. Pro-inflammatory cytokines, especially tumour necrosis factor-a (TNF-a) and interleukin-6 (IL-6) play an important role in synovial inflammation and articular cartilage destruction. Although antibody (Ab) therapy has beneficial effects, it also presents deleterious side effects and insufficient efficacy due to its short half-life [2]. In this work, anti-IL-6 and anti-TNF-a Abs were immobilized at the surface of chitosan/hyaluronic acid (Ch/HA) nanoparticles (NPs) to selectively capture and neutralize those pro-inflammatory cytokines. Our system intends to extend and increase the Abs therapeutic efficacy, owing to the protection from degradation that the NPs provide, and to reduce the systemic side effects using intra-articular (IA) administration.

Ch/HA NPs were prepared by polyelectrolyte complexation as a stable monodisperse population [3,4]. The maximum Ab immobilization ability was 10 µg/mL for anti-TNF-a Ab and 15 µg/mL for anti-IL-6 Ab. Biological assays demonstrate the NPs cytocompatibility with human articular chondrocytes (hACs) and human macrophages. A co-culture model of inflammation was used to validate the biological properties as well as their synergistic effects. Indeed, the biofunctionalized NPs with both Abs exhibited a prolonged action and stronger efficacy than the free Abs.

The in vivo therapeutic effect was assessed in a carrageenan-induced inflammatory arthritis model of OA by measuring different clinical parameters, nociceptive behaviour, and histological analyses. Biofunctionalized NPs exhibited a safe profile, a prolonged action, and a stronger efficacy than the soluble Abs. Hence, as this strategy is able to increase the therapeutic efficacy of the currently available treatments, biofunctionalized NPs can lead to a revolution in OA treatment.

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Ana Cláudia Lima

Dr. Ana Cláudia Lima is an FCT Individual Fellow (Junior researcher grant from the Stimulus of Scientific Employment, Individual Support (CEECIND) – 5th Edition), at the 3B's Research Group, University of Minho. She holds a Ph.D. in Tissue Engineering, Regenerative Medicine and Stem Cells from the University of Minho (2020) and a master's degree in Pharmaceutical Sciences from the Faculty of Pharmacy of the University of Porto (2013). During her doctoral studies, she developed 3 novel nanomedicines to treat autoimmun /inflammatory conditions, which significantly contributed to the state-of-art of the nanotechnology field. Currently, her work focuses on the development of an immune on-a-chip platform for effective personalized medicine. Dr. Ana Lima has broad scientific interdisciplinary expertise in the fields of nanotechnology, immunology, cellular/molecular biology, pharmacology, drug delivery, bioengineering, material science, biomaterials, tissue engineering, microfluidic , and microfabrication. As a result of her research work, she is the (co-)author of 12 full papers in Q1 score International journals, 2 reviews, and 2 patents. Her work has been cited around 183 times and has an h-index of 7 (Google Scholar). Dr. Ana Lima attended several important national and international scientific conferences, including 5 oral presentations. In recognition of her work, she was awarded several grants/fellowships. Moreover, she is/was a team member in several funded projects, has an active role in student supervision/mentoring, and organized many conferences. To foster fruitful collaborations with different universities, she enrolled in 2 secondments at the Strathclyde Institute of Pharmacy and Biomedical Sciences in Glasgow, United Kingdom, and at the Faculty of Engineering in Chulalongkorn University, Bangkok, Thailand (REMIX project).

Hydrogels for biomedical applications

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This work was focused on the preparation and characterization of naturally crosslinked sodium alginate/ gelatine (SA/G) hydrogels with various concentrations of SA and G. In an attempt to overcome the cytotoxicity problem of the chemically crosslinked hydrogels, genipin (GP) was used to obtain a biocompatible wound dressing material. The effects of SA/ G ratio (20/80 to 80/20) on the morphology and chemical structure were examined via Scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR), respectively. Swelling properties were determined gravimetrically.

FTIR spectra of SA/G-GP hydrogels revealed an increase in amide I and II absorbencies indicating the formation of heterocyclic compound of GP linked to the G and also the formation of the secondary amide group as a result of the reaction between G and GP. With increasing GP concentration, the swelling degree markedly reduced and the thermal stability enhanced. Since GP shows low toxicity, crosslinked hydrogels could be a promising candidate for biomedical applications, such as wound dressings.

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POSTER 1

Integrating Microengineered Materials and Biophysics for Enhanced Trapping of Metastatic Cancer Cells

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Current cancer treatments, including chemotherapy, radiotherapy, immunotherapy, and surgery, are generally effective for treating non-disseminated tumors, but they often prove to be ineffective against metastatic tumors. To improve treatment efficacy, bio-engineered implantable cancer traps based on chemoattractant-loaded (bio-)materials have been developed to prevent the uncontrolled spread of infiltrating cancer cells and potentially enable their eradication. However, these traps can have adverse effects on other cells and generate unpredictable gradients, which may perturb tissue homeostasis. In this work, we introduce a novel trap concept based on mechanical ratchet-based structures to capture metastatic cancer cells [1-4]. The traps use an array of asymmetric local features to mechanically polarize cancer cells and direct their migration over prolonged periods. Our results show that the trapping efficiency of these structures is higher than that of isotropic or inverse anisotropic ratchet structures on disseminating cancer cells and tumor spheroids. This approach may have potential clinical implications for fighting cancer and could also be used to control cell motility in other biological processes.

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POSTER 2

Hyaluronan-based 3D extracellular matrix model to study glioblastoma progression

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain cancer, with a five-year relative survival rate (after diagnosis) of only 36%.[1] During GBM onset/progression its microenvironment, in particular, the extracellular matrix (ECM), is altered, triggering the invasive character of cell subclones, namely, glioma stem cells. The brain's ECM is composed by different fibrous proteins (i.e., collagens, fibronectin, etc.), as well as, glycosaminoglycans, proteoglycans and glycoproteins. Importantly, the glycosaminoglycan Hyaluronan (HA), that comprises 30-50% of GBM's ECM [2], can promote different cancer cell behaviors, namely: its low molecular weight (Mw) fractions induces cancer invasion, while long HA chains promote cancer latency.[3] Herein, we developed a 3D ECM model based on an hydrogel combining alginate and HA, and tested its ability to copycat the role of HA Mw (i.e., 5.6kDa and 1450kDa) on the migration and invasive character of U-87 glioma cells. We encapsulated U-87 spheroids in the hydrogels and followed cellular motility by live imaging. The analysis of the time-lapse images showed a correlation between the Mw of HA in the hydrogel and the invasive character of U-87 cells: HA of 5.6 kDa promoted the migration of individual U-87 cells from the spheroids to the surrounding hydrogel; in the case of the spheroids cultured in the presence of HA of 1450 kDa, no morphological alterations or changes in motility were observed. We then evaluated the role of CD44 (the main receptor for HA) and cortactin (a key player in the modulation of cellular motility) in this process. Immunostaining experiments showed an increased expression of CD44 on migratory U-87 cells cultured in the presence of HA of 5.6kDa, which co-localizes with cortactin.

These results clearly show that HA of low Mw promotes the migration and invasive character of glioma cells in a CD44/cortactin-dependent mechanism. Overall, the proposed GBM 3D microenvironment model is able to mimic the bioactivity of HA, in particular the ability of its low Mw to induce the migration of glioma cells.

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POSTER 3

Engineered CHT/VCO-based emulsion structures doped with photosensitive capsules: a functional carrier device

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Drug carriers are the most effective vehicles for protecting and preserving therapeutic molecules during their release pathway. The design of carriers with stimulus-sensitive moieties would lead to the generation of ''smart'' delivery systems, allowing a temporal control of the drug release. As well ensuring the sustainable drug delivery, promoting low toxicity, high stability through cargo protection, and targeting efficiency. Those systems will allow the addressment of different biomedical and pharmaceutical applications, particularly in the treatment of local skin and systemic diseases [1-3].
This work proposes the employment of the Layer-by-layer (LbL) methodology to prepare hollow capsules loaded with curcumin (CUR). LbL assembly is a simple and highly versatile methodology to fabricate robust and highly-ordered nanostructured coatings over almost any substrate and employing a wide range of substances.
A polyoxometalate (POM) ([NaP5W30O110]14-) combined with a natural origin polymer, chitosan (CHT) was used to build up the multilayer system, being the POM responsible for a spatially controlled disruption of the assembled layers due to the weakening of the electrostatic interactions between them [4]. Thereby promoting capsule disruption and consequent content release [4]. The selection of the natural bioactive compound CUR relies on its antioxidant and wound healing properties [5], according to the envisioned application.
The capsules, with different sizes between 2-5µm (SEM), were further dispersed into CHT/VCO emulsion solutions that were casted into molds and dried at 37°C for 48h.

The swelling, release profile, and antioxidant performance of the designed structures were further studied, revealing a synergistic beneficial effect resulting from CUR and VCO individual bioactivities.
In summary, the rational design and characterization of tailored, stimuli-responsive carriers and their subsequent stepwise screening for delivery efficiency and anti-oxidant activity opens routes for its potential application as a top-down approach yielding the best carrier system.

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POSTER 4

Vescalagin and Castalagin Prevent a-Synuclein Aggregation and Cytotoxicity

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Neurodegenerative disorders, such as Parkinson's (PD) or Alzheimer's disease (AD), are usually characterized by the massive neuron loss caused by the pathological supramolecular assembly of specific proteins/peptides and their deposition in the brain. The type of protein/peptide deposition is disorder specific, *i.e.*, alpha-synuclein (aS) in the case of PD and amyloid β (Aβ42) in the case of AD.[1] In both cases, most of the current pharmacological strategies target the relief of symptoms, and no solution is currently available that is able to halt or reverse the neurodegenerative process.

In the case of PD, the protein aS (*i.e.*, 140 amino acid residues with a hydrophilic tail) has been shown to generate amyloid-like cytotoxic fibrils in the form of intracellular Lewis bodies (LBs) and neurites. These aggregated species are associated with the onset and progression of neurodegeneration in specific brain areas (i.e. basal ganglia and the substantia nigra), and are at the basis of the impairment of the neuronal activity.

Polyphenols present a variety of significant biofunctional activities, *e.g.* anti-microbial, anti-oxidant or anti-amyloidogenic.[2] Herein, we extracted/isolated two cork-based polyphenols (i.e. vescalagin and castalagin) and evaluated their ability to interact with cytotoxic preformed fibrils (PFFs) of aS and alter their aggregation state into non-cytotoxic forms.

Our preliminary results from CD spectroscopy show that both vescalagin and castalagin can modulate the conformation of PFFs. Fluorescence spectroscopy data (i.e. using Thioflavin-T) and STEM images corroborate these findings, indicating that both polyphenols disrupt the PFFs and generate smaller aS species. We further tested the cytotoxicity of PFFs in SH-5YSY culture. Immunocytochemistry results confirm that cells pre-treated with vescalagin/castalagin present a lower amount of PFFs in the cellular environment. Furthermore, the polyphenol-modulated PFF species are non-cytotoxic to SH-5YSY cells during 5 days of culture. Our data confirms that both vescalagin and castalagin are able to modulate the aggregation pathway of aS and induce the formation of non-cytotoxic forms, demonstrating a relevant potential for the treatment of PD.

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POSTER 5

Self-Assembling glycopeptide hydrogels induce the differentiation of stem cells into neural lineages

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Proteoglycans are one of the constituents of the extracellular matrix (ECM), presenting significant and diverse biological roles, e.g. acting as storage depots of proteins, activation of growth factors, and co-receptors at the cell surface. In particular, heparan sulfate (HS) proteoglycans are present in the ECM of the central nervous system and are essential in neurogenesis.1 Herein, we designed a minimalistic HS-mimicking glycopeptide amphiphile, i.e. Fmoc-diphenylalanine-glucosamine-6-sulfate (Fmoc-FF-GlcN6S), that self-assembles into nanofibers (similar to its nanomorphological presentation in the ECM) and gel under physiological conditions.2 Its supramolecular organization and subsequent gelation were induced using temperature (T) or solvent (S) switch methods. In the T method, the glycopeptide was dissolved in water at a temperature of 90°C and gelation was promoted by cooling the solution to room temperature. In the S method, the glycopeptide was dissolved in DMSO, followed by its dilution into water, which also induced spontaneous gelation.

AFM images revealed that both methods resulted in an entangled network of nanofibers, while CD and FTIR showed that glycosylation of the peptide backbone promotes a shift of the supramolecular arrangement from β-sheets (Fmoc-FF) into α-helixes (Fmoc-FF-GlcN6S). The preparation method influenced the stiffness of the hydrogels: G' (T) = 2.4kPa > G' (S) = 0.5kPa (within the range of neural tissues, i.e., between 0.5-3.0kPa).3 The Fmoc-FF-GlcN6S hydrogels showed no cytotoxicity towards human adipose-derived stem cells (hASC).

Moreover, T-hydrogels promoted and evenly distributed adhesion and spreading of hASC throughout their surface; while S-hydrogels promoted the formation of cellular clusters. qPCR and immunostaining demonstrated that both hydrogels promoted the overexpression GFAP and Nestin by hASCs at day 3 of cell culture and MAP2 and βIII-tubulin at day 9.
Overall, hydrogels generated with the proposed HS-mimicking glycopeptides recapitulated the mechanical properties of the neural microenvironment, as well as its chemical composition. They were also able to induce the differentiation of hASC into different neural lineages, making them as promising supports for the regeneration of neural tissues.

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POSTER 6

Electroactive hydrogel as a drug delivery system for ureteral stent application

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The increasing interest in personalized pharmacotherapy has prompted the development of sophisticated stimuli-responsive drug delivery systems. Using an external trigger, as an electrical field, enables the spatial-, temporal-, and dosage-controlled release of drugs.[1] Electrically responsive drug delivery systems represent an attractive alternative to treat ureteral stent-associated pain, a frequent side effect of stent placement.[2] A hybrid approach incorporating a conducting polymer, such as polyaniline, within a polymeric scaffold, such as gelatin, may present advantages for drug delivery application, including electrochemical activity, excellent biocompatibility, and tissue-like mechanical properties.[3] Here, we show an electroactive hydrogel capable of programmed drug delivery for ureteral applications.

Electroactive hydrogels composed of gelatin and polyaniline were chemically crosslinked with genipin at 37°C. These hydrogels were loaded with ropivacaine, a local anesthetic with proven intravesical effectiveness.[4] To characterize hydrogels, conductivity analysis, *in vitro* cytotoxicity tests (ISO10993), swelling behavior, and degradation assays were performed. In an *ex vivo* porcine assay, hydrogels were placed below a kidney and an abdominal section, and drug release was analyzed in response to different electrical stimuli (2 and 12 V).

It was confirmed that hydrogels are semiconductor materials whose conductivity varies proportionally with the amount of polyaniline in the material.

The hydrogel composed of 10% gelatin, 3% polyaniline, and 0.25% genipin showed a conductivity of 4.19E-04 ± 6.30384E-05 S/cm. Due to the appropriate properties of swelling, degradation, and cytotoxicity, the beforementioned formulation was chosen to undergo *ex vivo* assays. Results showed that drug release from hydrogels with polyaniline was higher when electrical stimuli were applied, compared with no electrical stimulation, which can lead to a more effective therapeutic effect.

We proved that the developed hydrogel system is capable of programmed drug delivery, in response to electrical stimuli. Loaded with an anesthetic with local action, as ropivacaine, these electroactive hydrogels may represent a novel suitable strategy for efficient ureteral stent-pain management.

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POSTER 7

Development and Characterization of Sustainable Alkali Lignin-Methacrylated Gelatin Hydrogels with Potential Use for 3D Bioprinting

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The conventional methods for producing biodegradable hydrogels involves complex chemical processes to attain both high elasticity and fatigue resistance [1]. However, this often requires the use of cytotoxic agents, which may compromise the biocompatibility of the material. Herein, we describe an innovative range of sustainable hydrogels and bioinks based on alkali lignin and methacrylated gelatin that are exceptionally elastic. These materials exhibit tunable mechanical properties, comparable to those found in soft tissues. While methacrylated gelatin is a well-known biomaterial [2], alkali lignin is a byproduct of the paper and pulp industry that has been underutilized [3]. Nevertheless, it is easily obtainable and economical biomaterial. The unique chemical structure of alkali lignin, which contains several reactive functional groups, such as phenolic hydroxyls and carboxylic acids, allows for easy chemical modification [4], making it a biomaterial with enormous potential for high-value applications. The alkali lignin-methacrylated gelatin hydrogel possesses several advantageous properties, including the ability to customize its mechanical properties and processing temperature according to its formulation. The material also has adjustable degradation kinetics that can be modified through different processes, and it can be cast-molded or 3D printed. Moreover, these high-performance hydrogels display high elasticity beyond that of natural hydrogels.

As a result, this versatile material holds great promise as a foundation for developing a range of new biomaterials that could be useful in a variety of biomedical and industrial applications, while simultaneously revalorizing industrial waste.

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POSTER 8

Magnetically-assisted cell sheet approaches to modulate tendon inflammation

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Persistent inflammatory cues have been associated to the development of tendon pathologies, and to changes in tissue oxygen levels contributing to tissue low oxygen tension (hypoxia). In turn, hypoxic environments regulate inflammatory and fibrotic pathways through oxygen-sensitive pathways with impact in the quality of the tissue. Recently works reported a potential therapeutic role of pulsed electromagnetic field (PEMF) over inflammatory cues expressed by human tendon cells (hTDCs)[1], and on hypoxia-induced inflammation by decreasing TNFα,IL-6,IL-8 in neuron-like and microglial cells[2].

We investigated the effect of hypoxia (1%,2% of oxygen tension-OT) using magnetic cell sheet (IL-1β-magCSs) constructs made of hTDCs primed with pro-inflammatory IL-1β, and magnetic nanoparticles [3]. IL-1β-magCSs were exposed to 1h, 4h,6h of low OT in a hypoxic chamber and the inflammatory profile assessed by qPCR, immunochemistry and ELISA assays. To confirm the role of PEMF (5Hz, 4mT, 50% duty cycle) on hypoxia modulation, IL-1β-magCSs, previously exposed to OT, were 1h-stimulated with PEMF.

Our results show a significant increase in *HIF-1α, HIF-2α* expression on IL-1β-magCSs after exposure to 2%-OT in all time points, compared to 1%-OT and to normoxia. The *TNFα, IL-6, IL-8* expression also increased in a 6h exposure to 1%-OT. IL-1β-magCSs exposed to hypoxia are PEMF-stimulated, there is a decrease in pro-inflammatory genes and an increase in anti-inflammatory, *IL-4, IL-10* expression compared to unstimulated-magCSs. The release of IFN γ, TNFα, IL-6 was increased after 6h-exposure, regardless of %-OT. In contrast, IL-10 levels tend to rise after PEMF-stimulation at 2%-OT in comparison to unstimulated-magCSs.

We also observed an increment in *NFkB* expression on IL-1β-magCSs exposed to 4h and 6h of 2%-OT suggesting a link between NFkB and the production of pro-inflammatory factors. Moreover, PEMF stimulation showed a significantly decrease of the NFkB levels on IL-1β-magCSs.
Overall, low OT enhances expression of hypoxia-associated genes and inflammatory markers in IL-1β-magCSs with the involvement of NFkB. PEMF modulates the response of magCSs, previously conditioned to hypoxia and to inflammatory triggers, favouring expression of anti-inflammatory genes and proteins, supporting PEMF impact in pro-regenerative tendon strategies.

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POSTER 9

Engineering melt-based and solvent-free polymeric foamed architectures for biomedical applications

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Materials are an important component of current regenerative medicine strategies since the material can mimic the native extracellular matrix (ECM), contributing to the structure and function of a new tissue [1]. In addition, porous scaffolds allow proliferation and differentiation of the cells, being the porosity and pore size important factors for any scaffold applied in tissue engineering and regenerative medicine (TERM). The work provides the recent progress on melt-based foaming technologies, preferably using solvent-free methods, for scaffolds production, i.e., technological melt-based methods that leads to porous architectures without use of solvents, as advantageous solutions for the processing of biomaterials. Each foaming technique has its advantages; however, none can be considered the ideal scaffold manufacturing route to be employed in all functional tissues. The selection of a foaming technique is dependent on the requirements of the specific clinical application. In the last decade, a number of foaming technologies have been used to produce highly interconnected, porous scaffolds with appropriated mechanical properties for TERM, that includes additive manufacturing by fused deposition modeling, extrusion or injection molding with gas foaming, and supercritical carbon dioxide (CO_2)-assisted foaming strategies [2]. Those technological approaches are reproducible, scalable, and cost-effective. However, to process biopolymers with reinforcing agents or additives, it is necessary to use temperatures above the glass transition or melting temperature of the biopolymer.

The major limitation is the high processing temperature required, which may degrade some drugs and growth factors used in the scaffold formulations, limiting the incorporation of bioactive agents during the melt process, that are essential to cell viability [3]. Thus, additional steps after the production of the porous scaffold are required to promote the spreading, differentiation, growth, and proliferation of cells. We hope that the advances and challenges in the design of cellular materials for TERM will inspire materials scientists, chemists, biologists, and medical doctors to translate these laboratory results to a clinical context.

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POSTER 10

Exploring cork in natural based biocomposite materials for potential use in patient recovery

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The immobilization of a limb is a common practice after an injure or a surgery procedure and usually splints or casts are used to decrease the swelling, reduce the pain, maintain alignment, and enable the healing[1]. The current used materials in these immobilization devices for long periods can cause dermatitis and skin breakdown[2], and after used generates high amount of wastes. Therefore, it is needed new sustainable and safer materials to increase patient comfort, while contributing to the rehabilitation process. In this study, we developed biocomposite materials that allow the correct immobilization of the limb, are less rigid and decreases the waste amount produced by the conventional casts, contributing to a greener solution. The concept has arisen from our advancing knowledge on materials from renewable sources. We selected cork as a basis for the biocomposites, due to its low density, chemical stability, low permeability to liquids, high elasticity, thermal insulation and anti-vibration properties[3]. Extrusion process was used to produce a biocomposite profile that combined a bio-based TPE with cork containing different particle sizes. The biocomposites were evaluated in terms of density, mechanical, morphology, water uptake, and antibacterial activity. It was successfully produced lightweight biocomposites, containing appropriated mechanical behaviour, low water uptake and antibacterial activity. Moreover, biocomposites with higher cork particle size showed a significant decrease in the density and stiffness, however, increase the water uptake content.

These changes in the properties resulted by the presence of close cells in the biocomposite structure, as observed by scanning electron microscopy. The cork biocomposites showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. This strategy showed that cork biocomposites can be considered as promising lightweight materials with antibacterial activity, reduced water absorption and lower stiffness, good appearance, as well as thermal and physical comfort, to be applied as medical assisting devices, namely to support in rehabilitation stage of patients recovering from broken bones, tendon healing and other injuries.

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POSTER 11

Adhesive Polyelectrolyte Complex Membranes for Bone Tissue Engineering

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Mussels produce a unique amino acid – L-3,4-dihydroxyphenylalanine (DOPA) – which expresses catechol groups that contribute greatly to their outstanding adhesive properties. DOPA allows them to tether to several surfaces in wet environments, including rocks, ships, and other animals. We hypothesized that catechol-modified polysaccharides could be used as key ingredients for biomedical membranes with improved adhesiveness in the wet physiological environment [1].

Herein we used a compact polyelectrolyte complexation (CoPEC) method to produce membranes. It is a straightforward method that relies on the synthesis and sedimentation of polyelectrolyte complexes which, after drying, coalesce as though membrane constructs. The natural polymers chitosan (CHI) and hyaluronic acid (HA) – unmodified or modified by catechol groups, CHI-cat and HA-cat – were used as polycation and polyanion, respectively. Non-polymeric components are also building blocks compatible with the CoPEC method: we embedded ternary bioactive glass nanoparticles (BGNPs) to stimulate mineralization alongside adhesiveness, thus enabling the proposed system for bone tissue regeneration.

CoPEC membranes with BGNPs (15%) and all modified and non-modified polymers (17.5% CHI, 17.5% CHI-cat, 25% HA, and 25% HA-cat) showed good resistance to degradation: they lost as little as 30% in weight after 31 days in lysozyme.

The use of catechol-modified polymers increased the adhesiveness of the CoPEC membranes when compared to unmodified polymers, with lap shear strengths between 3.9 kPa and 6.9 kPa. These values are within the ranges found for other commercial adhesives. BGNPs also improved the Young's modulus about two-fold compared to purely polymeric membranes, thus foreseeing a better mechanotransduction to maintain the functionality of bone cells. Hydroxyapatite formed on these membranes with a Ca/P ratio of 1.9, usually associated with the improved adhesion of osteoblasts. In fact, we observed that SaOs-2 osteoblast-like cells adhered well to CoPEC membranes made of catechol-modified polymers and BGNPs and retained high viability. The use of BGNPs is aligned with the current trends of supplementing orthopedic devices with bioceramics and makes such membranes potentially suitable for guided bone regeneration.

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POSTER 12

Design and technological approaches for the detection and isolation of Circulating Tumor Cells

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Metastasis, the process by which tumor cells spread throughout the body via the bloodstream, is the leading cause of cancer-related mortality[1]. Circulating tumor cells (CTCs) are shed tumor cells that have entered the bloodstream from the primary tumor. The functional and molecular properties of CTCs may provide in-depth knowledge of cancers with a high mortality rate. Given the extremely low quantity of CTCs in the blood of cancer patients, it remains a substantial problem to consistently isolate CTCs from the innumerable blood cells and, in particular, to develop technologies that can efficiently detect live CTCs for in-depth analysis. The exponential growth of CTC-related technologies during the past two years are analyzed herein. A conceptual roadmap to CTC-related technologies is provided, with a particular emphasis on the most creative techniques employing nanomaterials or unique microfluidic chips. In general, CTCs technologies apply three core methods[2]: i) separation and enrichment; ii) detection and characterisation; and iii) release. The first method requires a specific interface between CTCs and materials, either through physical processes or antibody-antigen interaction. The second method, recognizing CTCs, involves a number of technologies (*i.e.* fluorescence spectrophotometry/microscopy; flow cytometry). In the final strategy, CTCs are employed mostly for downstream analysis (*i.e.* genomes, -omics, CTCs culture).

In brief, emerging technologies for the detection of CTCs have profoundly contributed to cancer monitoring, efficacy evaluation and the design of targeted cancer treatment approaches, and will become an essential component of cancer management in the future.

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POSTER 13

3D Writing of Multicellular Human Tendon Microphysiological Systems

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Tendinopathies are debilitating and poorly understood diseases for which current treatments have poor recovery outcomes. Therefore, relevant *in vitro* models to study tendinopathies and develop better treatments are highly needed. Recently, we proposed a new strategy that allows the automated 3D writing of microphysiological systems (MPS) embedded into its own biomimetic fibrillar support platform based on the self-assembling of cellulose nanocrystals (CNCs)[1]. Bioinks based on decellularized extracellular matrix (dECM) are able to closely recapitulate the tendon rich microenvironment, whose extracellular matrix (ECM) is responsible for the activation and regulation of several signaling pathways controlling cell behavior. Here, we explored this CNC platform as support for writing humanized *in vitro* tendon models using tendon dECM-based bioinks to closely recapitulate the biophysical and biochemical cues of tendon cell niche and self-induce the tenogenic differentiation of stem cells.

Porcine flexor tendons were decellularized to produce the dECM bioink hydrogel and the easily accessible 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 and their *in vitro* maturation. To evaluate the effects of cellular crosstalk with endothelial cells, tendon constructs were co-printed with compartmentalized microvascular structures. The CNC embedded 3D structures showed high cell viability and proliferation during culture up to 21 days. The synergy between dECM cues and printed patterns induced anisotropic cell organization similar to tendon tissues.

Gene and protein analysis showed upregulation of the most important tendon related markers on tendon constructs, demonstrating that the biophysical and biochemical cues of dECM induced hASCs commitment toward tenogenic phenotype. In co-culture system, chemotaxis induced endothelial cells migration toward the tendon compartment, but without showing significant infiltration. Gene and protein expression results suggest that the cellular crosstalk established in MPS with endothelial cells boosted hASCs tenogenesis, emulating tendon development stages.
Overall, 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, pathology, or the effect of drugs for the treatment of tendinopathy.

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POSTER 14

A microreactor for the maintenance and conditioning of multilayer tissues or multi-tissue structures

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Dynamic culturing systems can overcome challenges of in-vitro fabrication and maintenance of complex 3D tissues, however, the unique physiological conditions to which each tissue is subjected has been hampering noteworthy developments for many engineered tissues. Here we report the development (design, manufacturing, and testing) of a microbioreactor for the preparation, maintenance, and/or conditioning of human multilayer tissues or multi-tissue structures, providing evidence for vascularized skin tissue-engineered analogs and ex-vivo human skin.The autoclavable bioreactor comprises a sandwich modular structure of hard undeformable layers of 3D printed medical grade polycarbonate intercalated with soft deformable layers of silicone. When compressed the soft layers expand laterally against the sample sealing the layers between fluid streams avoiding their intermixing. The bioreactor is modular, each module being an independent fluid circuit for one tissue, and more modules can be assembled to enable multi-tissue structures to be cultured in dynamic conditions. The bioreactor is capable of holding skin tissue samples of 8mm in diameter and of providing, without mixing, different culture media corresponding to the three layers of the tissue, the outmost epidermis, the underneath dermis, and the innermost adipose tissue(Fig 1C). By changing the thickness of the soft layers, it can easily be adapted to accommodate samples from different anatomical regions and with varied thicknesses. The bioreactor allows nourishing each cell type/tissue layer with a specific cell culture medium with a continuous or pulsatile flow of controlled flow rate, increasing the maintenance time of the native structure in the ex-vivo skin.

Furthermore, the bioreactor permits establishing an air-liquid interface for the epidermis turnover in the skin explant by automatically switching the epidermal medium with air, while still maintaining the separation of the culture media underneath. This dynamic culture system contributes to diminishing the time of preparation of complex tissues or multi-tissues and prolonging the viability and use of in vitro and ex-vivo tissues being, therefore, a valuable tool for drug discovery, personalized medicine, and cancer development studies.

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POSTER 15

Immobilizated bioengineered spider silk on a nanofibrous mesh reduces bacterial adhesion while improving abdominal muscle tissue repair

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The loss of the abdominal wall biomechanical properties together with the occurrence of surgical site infections (SSI) after abdominal surgery represents a health problem that must be addressed. Current materials solutions (i.e. implantation of surgical meshes) restore the abdominal wall but also induce significant clinical complications that need to be tackled. Proteins derived from bioengineered spider silk have tremendous potential as drug-free biomaterials [1]. Moreover, the fusion with human-derived antimicrobial peptides (AMP) is an innovative method that confers antimicrobial activity on the materials [1]. In this study, we investigate the antimicrobial potential of immobilized bioengineered spider silk proteins with AMP on nanofibrous meshes (NFM) aiming to simultaneously prevent infections and restore abdominal wall biomechanics simultaneously.

To achieve this goal, a facile functionalization method comprising the immobilization of bioengineered spider silk protein with AMP (6mer-HNP1), as well as bioengineered spider silk protein alone (6mer), on electrospun polycaprolactone (PCL) NFM was employed [2]. Both sides of PCL NFM were activated by exposing them to ultraviolet ozone for two minutes each. The capacity for protein immobilization on the activated NFM was evaluated, and the functionalized mesh was further characterized in terms of antibacterial activity and cytocompatibility [2].

The maximum immobilization capacity of the bioengineered proteins 6mer and 6mer-HNP1 were 200 μg mL−1 and 250 μg mL−1, respectively. The formation of beta-sheets by the spider silk domain was unaffected by protein immobilization on the NFM. Functionalized meshes with 6mer-HNP1 significantly inhibited the adherence and biofilm formation of Methicillin-Resistant Staphylococcus aureus (MRSA) and Escherichia coli (E. coli), demonstrating their antimicrobial potential. In vitro cell studies using a human umbilical vein cell line (EA.hy926) validated the cytocompatibility of the functionalized meshes and the proliferation of muscle-related cells (C2C12 mouse myoblasts cell line) further demonstrate their biological potential. Overall, the use of functionalized meshes with bioengineered spider silk proteins can be a safe and effective alternative to the development of high-performance surgical meshes for challenging abdominal wall repair surgeries.

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POSTER 16

Magnetic Cell Sheet Patches: Prospects for Tendon Regeneration

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Tissue engineering and regenerative medicine (TERM) aims to provide therapies for injured tendons despite the challenging cues of tendon niche and of specific factors to guide regeneration. The emerging potential of magnetic nanoparticles (MNPs) functionalities offers new perspectives to tackle TERM challenges.

Pulsed electromagnetic field (PEMF) is an FDA approved therapy for orthopaedics, holding relevant contributions for the re-establishment of local cell functions and inflammation control upon injury. To further understand the potential of magnetically assisted living patches [1, 2], we conducted *in vivo* studies using a rat patellar defect model.

After labeling human adipose stem cells with MNPs, magCSs were cultured up to 3d in a-MEM medium. The magCSs effect in ameliorating healing was assessed in window defects created in the patellar tendon of rats. PEMF was externally applied (3mT, 70Hz, 1h) 3d/week. After 4 and 8w, tendons were histologically characterized for immune-detection of tendon and inflammatory markers, and for Perls van Gieson and HE stains. Blood and detoxification organs were screened for inflammatory mediators and biodistribution of MNPs.

In vitro results suggest that PEMF stimulates cellular metabolic activity, influences protein synthesis and the deposition of collagen and non-collagenous proteins in magnetic conditions. No infection or swelling were observed after surgery or during follow-up.

After 8w, magCSs remained at the implantation site and no MNPs were detected on detoxification organs. Plasma levels of IL1a, β, IL6 and TNFa assessed by multiplex assay were below detectable values.

The combination of cells and magnetic technologies prospects engineered living tendon substitutes that can be also used as *in vitro* models.

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POSTER 17

Engineering Scaffolds for Orthopedic Tissues

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The use of tissue engineering scaffolds to treat lesions of orthopedic tissues has been studied preclinically and clinically since the last few decades. Orthopedic lesions are common, and the use of biomaterials is supported by the clinical need [1,2]. Two of the critical advances in the field are the 3D-printing of scaffolds and bioprinting of alive scaffolds/hydrogels using bioinks containing cells; where both enable patient-specific health care thanks to advances in the use of medical images [3,4]. It is known that modification of properties of a scaffold will affect the cell function and the outcome. The current outstanding challenges include the mismatch between the neotissue and the native tissue in terms of composition and biomechanics [5]. The eventual goal is to address the current challenges and limitations and provide functional healthcare services to the patients.

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POSTER 18

Soft enzymatic-crosslinked silk fibroin hydrogel lab-on-a-chip: a proof-of-concept

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Based on our group's patented "methods of production of eSF hydrogel microfluidic platform"[1] , we used UV-photolithography and double replica molding to obtain a soft silk microfluidic platform. The design is very simple, containing channels of 200 µm in width, mimicking the tortuous morphology of the tumor microvasculature. Hydrogels were prepared as reported previously in our group [2]. After a range of physicochemical characterization, HCT-116 Colorectal cells were encapsulated in the silk and endothelial colonic cells seeded in the serpentine microchannel. ATP quantification and Alamar blue were performed before and after the perfusion of 5 µL of Gemcitabine.
We found that 14% eSF works better in terms of flexibility and transparency. SEM images demonstrate the eSF hydrogel maintained the microfluidic features with excellent fidelity after the entire fabrication process and crosslinking. By performing tensile tests on the 14% eSF, a mean tensile strain of 103.96 % was found. Alamar blue and ATP quantification of cells without any drug perfusion showed HCT-116 cells are viable overall, and the endothelial cells did form a lumen-like structure in the serpentine microchannel. On the other side, perfusion with GEM showed a limited cell viability after day 3.
Overall, this work shows how the combination of enzymatically-crosslinked silk fibroin and microfluidics can be employed for developing soft lab-on-a-chip platforms with superior performance.

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POSTER 19

Application of cell sheet engineering to model blood-brain barrier in a glioblastoma scenario

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Glioblastoma is the most aggressive and common type of brain cancer. The efficient therapeutic delivery to the brain is hindered by the protective blood-brain barrier (BBB) - a dynamic interface between the central nervous system and the circulating blood that restricts the transport of toxic or harmful molecules from the blood to the brain, but allows the transfer of nutrients to the brain and the removal of metabolites. The important role of BBB has motivated the development of different *in vitro* models that aid the understanding of this barrier and validate the efficacy of new therapeutic solutions. The majority of these models are oversimplified in terms of composition and structure leading to bias in the generated data.

To overcome this drawback, we applied cell-sheet engineering (CSE) to develop a complex 3D multicellular model that better mimics the native BBB. We first obtained homocellular sheets from each cell type: namely astrocytes, pericytes, and endothelial cells. These sheets were characterized for the expression of specific markers (immunocytochemistry and qPCR) and assembled in a specific order (astrocytes, followed by pericytes, and finally the endothelial cells) to obtain the 3D construct. The generated construct was stable upon detachment, handleable, and maintained the expression of specific markers (Claudin5, SMA, GFAP). Histological analysis of the stratified construct showed different layers of tightly packed cells in a collagen matrix.

The construct was placed in direct contact with glioblastoma (GBM) spheroids. The interaction of GBM spheroids with the 3D construct was evaluated by light sheet microscopy and revealed reorganization of the astrocytes around the GBM spheroid. . In the following step, the model will be validated with well-established GBM chemotherapeutics.
Because in a glioblastoma scenario, the BBB plays a crucial role in the therapeutics delivery, we expect that the developed 3D construct can foster the development and validation of new and efficient glioblastoma therapeutics.

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POSTER 20

Hybrid nanovehicles for combined drug delivery and cellular reprogramming

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Hybrid nanoarchitectures constitute a promising tool for modulating cellular responses in complex processes such as resolving inflammation or tissue regeneration. Magnetic polymeric micelles (MPMs) present a hydrophobic core and hydrophilic shell enabling distinct cargo and incorporate magnetic nanoparticles which provide an external magnetic control for intracellular delivery and real-time imaging. The MPM's unique features offer great possibilities in RNA-based theranostics for immunotherapy and tissue regeneration, overcoming the lack of efficient, multifunctional, and safe RNA carriers for successful cell reprogramming and precision guidance of healing events.

In this work, we propose the assembly of MPMs from palmitic acid-*grafted*-chitosan (PA-*g*-CS) and commercial superparamagnetic iron oxide nanoparticles (SPIONs), aiming at the modulation of macrophage functions via miR regulation.

MPMs were produced by ultrasonication and two SPIONs:PA-*g*-CS mass ratios of 1:2 and 1:5 were investigated. Nile Red (NR) and Fluorescein Sodium (FL) were incorporated to evaluate the carrier-loading potential for hydrophobic (NR) and hydrophilic (FL) drugs. As a proof-of-concept, a miR antagonist (miR-155) was loaded in the CS segment (shell) to stimulate pro-regenerative (M2) cues in macrophages. With the actuation of a static magnetic field (MagnefectNano,350mT,20min), MPMs were internalized in THP1-derived macrophages (naïve and M1-primed) via magnetofection. Various MPMs concentrations at each mass ratio were investigated for viability, uptake, and functional assessments by immunochemistry and flow cytometry.

NMR and FTIR revealed the successful synthesis of PA-*g*-CS that self-assembles at >254±6μg/mL. STEM showed that SPIONs were incorporated into the core of MPMs. The size of the MPMs was 118±8nm(1:2) and 115±18nm(1:5)(dry) and 354±6nm(1:2) and 353±35 nm(1:5)(hydrated). MPMs show positive surface charge +23.5±0.2mV(1:2), and +21.1±0.8mV(1:5) due to protonated amine in CS. SPIONs loading efficiency (LE) was determined by ICP: 67%(1:2) and 72%(1:5). A LE of 40±2%(1:2) and 63±5%(1:5) was found for NR while for FL was 5.2±0.3%(1:2) and 24±2%(1:5). MPMs did not compromise cell viability and are well-tolerated by macrophages. The intracellular accumulation of NR-loaded-MPMs for at least 7 days suggests MPMs' feasibility to deliver sensitive nanotherapeutics. Varying the miR:polymer mass ratio it is possible to bind miR to MPMs, whose impact is being addressed on macrophage polarization mechanisms. MPMs were successfully produced and show potential as contactless and high-precision multi-load delivery RNA-based nanoplatforms targeting immune cells.

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POSTER 21

In vivo anti-inflammatory potential of *Echinacea purpurea* root extracts-loaded liposomes

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According to WHO, chronic inflammatory diseases are correlated with more than half of global deaths. The severe side effects observed with the constant administration of anti-inflammatory drugs limit their use. New entities derived from plants have shown their potential for the development of safe and effective therapies. Particularly, *Echinacea purpurea* extracts have demonstrated strong anti-inflammatory properties. In this work, dichloromethanolic extracts of *E. purpurea* roots (DE-R), rich in alkylamides, were used to produce a new anti-inflammatory formulation. To overcome the compounds' poor water solubility and increase the therapeutic index, DE-R were loaded in large unilamellar liposomes (LUVs). Besides taking advantage of the passive targeting, liposomes were also engineered to actively target key players of the inflammatory process, namely M1 macrophages, by their functionalization with folic acid (FLUVs). The developed formulations were homogeneous (polydispersity index of ≈0.13), presented a mean particle size of ≈114 nm and a zeta potential of ≈-3 mV. The liposomes were cytocompatible at all tested concentrations and strongly reduced the interleukin (IL)-6 production by lipopolysaccharide-stimulated macrophages. As expected, DE-R-loaded FLUVs were 6.3 and 9.7 times better than LUVs with DE-R, and free DE-R, respectively. The anti-inflammatory activity of DE-R-loaded FLUVs was assessed in an experimental rat model of inflammation.

A single injection of the FLUVs with DE-R promoted a significant reduction of edema, inflammatory pain, and immune cell infiltration, particularly of $CD68^+$ macrophages, as well as IL-6 expression. This formulation reduced synovial inflammation more efficiently than free DE-R. We also demonstrated that the developed formulation is safe, as no changes or harmful toxicity in major organs were observed. Moreover, free DE-R and DE-R loaded FLUVs showed more robust anti-inflammatory activity than a conventional anti-inflammatory drug widely used in the clinic. Therefore, extracts of *E. purpurea* roots loaded in liposomes can be used as natural, green, innovative, and powerful new formulations to treat chronic inflammatory diseases.

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POSTER 22

Interrogation of the Multicellular Crosstalk in Healthy and Diseased Tendons Using a 3D Tendon-on-Chip Model

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Tendon injuries are common and can drastically impair patient mobility and productivity, resulting in a reduced quality of life. To date, no treatment has proven to adequately improve tendon healing process, mainly due to current limited knowledge on the contribution of various cell types to tendon physiology and on how they modulate tendinopathy. Therefore, a better understanding of the complex cellular environment during tendon healing is critical to develop therapies that can prevent progression of degenerative conditions. Both intrinsic and extrinsic cell populations of tendon tissue contribute to its healing process. The initial phase of process starts with increased vascular permeability and influx of inflammatory cells at healing site, which leads to increased production of growth factors and cytokines that summons circulating immune cells. The aim of this work is to establish a compartmentalized multicellular ex-vivo model to recreate hallmarks of inflammatory phase in tendon healing. To achieve that, we propose a 3D compartmentalized tendon-on-chip model that recapitulates the cellular patterns and microstructural features of tendon matrix to understand key biological events. A three-channel chip is used to recapitulate the tendon tissue compartmentalization, where the central channel is loaded with the hydrogel and tendon cells, while vascular cells are cultured in the side channel.

In the central channel for recreating the anisotropic architecture of tendon matrix on-chip, we present a hybrid system based on superparamagnetic nanoparticles (SPIONPs) incorporated in the structure of short electrospun polycaprolactone (PCL) microfibers integrated within platelet lysate hydrogels. External magnetic field is applied to control SPIONPs@PCL microfiber alignment within hydrogel matrix, and thus the topographic cues controlling cell alignment and de novo matrix deposition. Cell organization embedded in hydrogels is assessed by measuring cytoskeletal angle and aspect ratio. Changes on the expression of genes related with extracellular matrix components and inflammatory signaling pathways are being evaluated. This model provides the basis, and it is envisioned to incorporate immune cells to provide insights between immune system and tendon cells interplay and access the immunomodulatory potential for these biomaterial/scaffolds, not only laying groundwork for better understanding tissue crosstalk in tendon disease, but also for tendon tissue regeneration and repair.

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POSTER 23

Mussel-inspired chitosan hydrogels for bone tissue engineering

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Hydrogels are described as three-dimensional polymer networks that can absorb large amounts of water, but not disintegrate nor dissolve in water. Regarding its high water content, the hydrogel can stimulate biological tissue being compatible with most living tissues while minimizing damage after being implanted in the host tissue. A good candidate for the production of hydrogels is chitosan, a positive polymer obtained from deacetylated derivated chitin, the second most abundant natural polysaccharide in nature. Chitosan has been used in different fields, such as tissue engineering, wound dressings, drug release, due to its biocompatibility, biodegradability, low allergenicity, non-toxicity and anti-bacterial properties. Chitosan hydrogel can be produced through electrostatic interactions, using b-GP, an organic compound found in the body, already approved by FDA for intravenous administration. The main challenge of these hydrogels is the lack of bioadhesion between the hydrogel and tissue i.e. the capacity to bind the target tissue and the low cell affinity/adhesion. Mussel-inspired polymers are rising as novel adhesives due to the excellent wet adhesion properties of the DOPA (3,4-dihydroxyphenylalanine) found in mussel foot proteins. The catechol unit provides strong single-molecule adhesion to both inorganic and organic surfaces in the aqueous environment. In vitro and in vivo studies have demonstrated that bioactive glasses induced the differentiation of osteo/odontoblasts and enhanced calcified tissue formation. This work aims the production of a new class of adhesive hydrogels based on Chitosan/β-GP hydrogels combined with bioactive glass nanoparticles (BG-NPs).

These hydrogel structures will provide a highly hydrated environment with an adequate porosity that promotes the cell-cell and cell-extracellular matrix interactions necessary for bone regeneration, whereas the presence of BG-NPs will act both as a reinforcing agent and a promoter of osteoconductivity and the presence of the adhesive catechol groups will significantly enhance the biological response.

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POSTER 24

Development of a 3D *in vitro* Breast Cancer Model based on Silk Fibroin Hydrogels

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Breast cancer is one of the most common types of tumors found in women. In a consistent number of cases patients develop resistance to standard treatments, due to differences in therapeutic response based on the genetic background of the different tumor subtypes (1). To unveil novel therapeutic strategies for anti-cancer formation and development, in latest years there has been an increased interest in the development of *in vitro* tumor models (2, 3). In this work we aimed to generate a 3D *in vitro* breast cancer model, based on horseradish peroxidase (HRP)-crosslinked silk fibroin (SF) hydrogels. Human MCF7 breast cancer cells and mammary fibroblasts (HMF) were encapsulated into SF hydrogels, and their viability and behavior were evaluated up to two weeks of culture. We observed an increased proliferation, clusters formation and matrix stiffness when MCF7 cells are co-cultured with HMF, as compared to MCF7 monoculture. In addition, cells in co-culture exhibited an upregulated gene expression of markers related with the remodeling of the extracellular matrix (collagens, matrix metalloproteinases) and to the activation into cancer associated fibroblasts (vimentin). Overall, our results suggest that SF hydrogels can adequately support the development of a heterotypic breast cancer *in vitro* model, to be used in the future for drug discovery and finding novel molecular targets for therapy.

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POSTER 25

Engineering natural-based hydrogels for precise laser ablation in TE applications

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The development of functional vasculature that support tissue growth and function is a significant challenge in tissue engineering. Without a functional vasculature, thick and metabolically demanding tissues cannot survive for long after implantation. 3D culture of endothelial cells to create prevascular networks has received significant attention as a tool to potentially solve this problem and develop more physiologically relevant tissue constructs. Hydrogels have emerged as promising scaffolds for this type of 3D culture since they present similar properties to native extracellular matrix (ECM). Laser ablation has been proposed as a subtractive technique to fabricate intricate microstructures in 3D matrices such as hydrogels, namely channels and cavities that mimic the complex architecture of capillary beds. However, the efficiency of the ablation process is conditioned by the material features such as optical properties and molecular complexity.

In the present work, the compliance of natural-based hydrogels with the laser ablation process was studied. For that, several formulations of gelatin, collagen and collagen-gelatin hydrogels were characterized in terms of optical and rheological properties. Then, the same materials were subjected to different laser ablation protocols. Data shows that materials allowing a higher degree of light transmittance are ablated more effectively.

Although there was no direct correlation between rheological properties and laser ablation behaviour, a certain degree of stiffness was needed for the hydrogels to maintain the ablated 3D structures. Crosslinked gelatin hydrogels were shown to be the easiest to ablate, demanding less laser intensity to create hollow structures within the 3D matrix. This study provides important data to outline hydrogel formulations for efficient laser ablation.

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POSTER 26

Extracellular Matrix of cultured Adipose-Derived Stromal Vascular Fraction Cells as Raw Material to produce Highly Angiogenic Hydrogels

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Tissue engineering is an interdisciplinary field that creates biologic substitutes for damaged tissues or organs. The vascularization of these constructs is essential for their survival and functionality. Using 3D matrices with angiogenic cues may be a solution to this challenge. The stromal vascular fraction (SVF) of adipose tissue has been shown to be a promising tool for pre-vascularization due to its spontaneous vasculogenesis when cultured in vitro [1]. Indeed, the extracellular matrix (ECM) produced by these cells is a crucial component in this process. Recent reports highlight the use of ECM-derived hydrogels as scaffolds for tissue engineering due to their similarity to native tissue's ECM. In this study, we present the development of an angiogenic hydrogel produced from the ECM of SVF cell sheets.

SVF cell sheets were subjected to a decellularization protocol and then freeze-dried and digested with an acidic pepsin solution. The resulting ECM-solution was then incubated at 37°C to polymerize and form ECM-derived hydrogels. Decellularization effectiveness was confirmed by DNA quantification and Hematoxylin and Eosin (H&E) staining. SDS-PAGE, Western blot and Sirius Red/Fast Green staining revealed high protein complexity within the ECM extract, with type I collagen being the predominant one. Through Circular Dichroism technique, it was possible to detect the triple helix conformational structure typical of collagen, confirming conservation of protein structure after the extraction protocol.

These properties allowed the production of stable hydrogels, which were used to successfully culture Human Dermal Microvascular Endothelial Cells (HDMECs).
Overall, these results show that the ECM from SVF cell sheets was successfully isolated and used to create hydrogels with angiogenic properties. Ongoing studies are focused on the hydrogel's proteomic characterization and on preclinical studies to verify its angiogenic potential compared to commercially available hydrogels. If confirmed, the development of regenerative strategies based on angiogenic ECM-like hydrogels could lead to promising advances in the field of tissue engineering and regenerative medicine.

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Acknowledgements: EU Horizon 2020 research and innovation program under the ERC grant CapBed (805411)

POSTER 27

Platelet-derived Extracellular Vesicles Promote Stem Cells Tenogenic Commitment in a Bioengineered Tendon 3D Model

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Worldwide, tendon disorders are one of the main causes of disability that decrease the quality of life of individuals and represent a substantial economic burden on society. Currently, the main therapies used for tendon injuries are not able to restore tendon functionality. In order to discover new therapies, extracellular vesicles (EVs), key players in cell-cell communication, have been widely explored for tissue engineering and regenerative medicine applications. Thus, the aim of this study is to assess the role of EVs derived from platelets in stem cell tenogenic commitment using a bioengineered tendon *in vitro* model for potential use as tendon therapeutic agents.

Biomimetic platelet-derived EVs were produced by freeze-thaw cycles of platelets and isolation at different centrifugation speed (1). To recreate the architecture of tendons, a 3D system consisting of electrospun anisotropic nanofiber scaffolds coated with collagen encapsulating human adipose stem cells (hASCs) and different types of platelet-derived EVs, were produced. Then, the influence of the tendon-mimetic constructs and the distinct EVs populations in the hASCs tenogenic differentiation were assessed over culture time.

We observed that the hASCs on the nanofibrous tendon scaffolds, show high cytoskeleton anisotropic organization that is characteristic of tenocytes. Moreover, acting as biological cues, platelet-derived EVs boosted hASCs tenogenic commitment, supported by the increased gene expression of tendon-related markers (*SCX* and *TNMD*).

Additionally, EVs enhanced the deposition of tendon-like extracellular matrix (ECM), as evidenced by the increased gene expression of ECM-related markers such as *COL1*, *COL3*, *DCN*, *TNC*, and *MMP-3*, which are fundamental for ECM synthesis and degradation balance. Moreover, EVs induced lower collagen matrix contraction on hASCs, which has been related with lower myofibroblast differentiation.
Overall, the results revealed that EVs are capable of modulating stem cells' behavior boosting their tenogenic commitment, through the increased expression of healthy tendon cell markers, potentiating ECM deposition and decreasing cell contractility. Therefore, platelet EVs are a promising biochemical tool, worthy to be further explored, as paracrine signaling that might potentiate tendon repair and regeneration.

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POSTER 28

Collagen/CaP scaffolds produced with marine origin materials promoting bone regeneration in a New Zealand rabbit model

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Every year millions of people are affected by severe bone damage. Natural origin materials, including marine origin compounds, have been successfully obtained and processed to develop biomaterials for bone therapies. Collagen-based 3D engineered structures stand out as promising biomaterials since they offer attractive biological cues that encourage cell and tissue integration *in vivo* with reduced inflammatory response. The present work proposes the use of skin and teeth of *Prionace glauca*, to extract collagen[1] and bioapatite[2], respectively, to produce collagen-apatite matrices (mColl:BAp) by freeze-drying, as potential templates for bone regeneration[3]. Collagen extracted from bovine hides was combined with synthetic hydroxyapatite (bColl:Ap) to produce 3D composite scaffolds serving as a comparative biomaterial. Scaffolds were characterized regarding physicochemical features, and the *in vivo* performance regarding bone tissue regeneration was evaluated upon implantation in critical-size femoral condyle defects created in New Zealand rabbits, 12 weeks post-surgery. Collagens showed a similar profile, while the ceramic particles differed in their composition, being bioapatite a fluoride-enriched ceramic.

Both scaffolds' formulations presented high porosities, being the marine ones more porous. Bioapatite reinforced fish collagen scaffolds displayed a higher compression modulus when compared with synthetic apatite reinforced bovine collagen. Micro-CT analysis of the retrieved condyles showed that the implanted mColl:BAp scaffolds promoted an higher tissue formation (17.9±6.9 %), when compared with bColl:Ap scaffolds (12.9±7.6 %). The histomorphometry analysis corroborated the observed tendency, determining 13.1±7.9 % and 10.4±3.2 % of new tissue formation for mColl: Bap and bColl: Ap composites. Overall, the present findings revealed that both composite structures promoted bone-like tissue formation, suggesting an improvement when compared with the control group (9.6±6.3 % by micro-CT and 6.8±4.3 % by histomorphometry). As bovine collagen and synthetic hydroxyapatite are well-established materials in bone tissue engineering, the observed outcomes highlight the potential use of marine-origin biomaterials for bone regeneration.

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POSTER 29

Engineering Complex 3D Tissue Constructs: Integration of Microfluidics and Computational Fluid Dynamics

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Biological tissues are complex hierarchical structures that rely on their unique 3D morphologies and properties to execute their critical physiological functions. The present research aims to develop in vitro tissue models with intricate 3D architectures that mimic the complexity of biological tissues for biomedical applications. To this end, microfluidic approaches were utilized to attain reproducibility, rapid processing, and high efficiency; crucial factors for achieving successful results. Coupled with that, Computational Fluid Dynamics (CFD), a powerful and cost-effective method for examining and predicting fluid flow phenomena [1], was used to overcome the challenges, forecast outcomes, and optimize initial conditions.

To synthesize hydrogel fibers with diverse morphologies experimentally, different mixtures of natural-based polymers, *i.e.* Gellan Gum and Alginate, were used. Additionally, multi-phase constructs were generated employing Oleic Acid as the dispersed component. The primary settings and boundary parameters were adjusted based on the optimal values calculated by the software. This study underscores the potential of Computational Fluid Dynamics (CFD) simulations in optimizing microfluidic applications and provides evidence for the feasibility of obtaining a diverse range of shapes and morphologies of polymer-based structures derived from natural sources. These novel and multifaceted fiber structures hold immense promise and applicability in the fields of tissue regeneration and biomedical research.

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Introduction
3D
2D
3D


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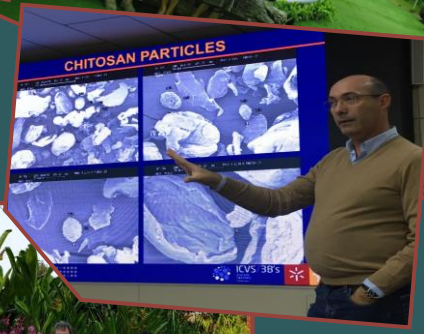
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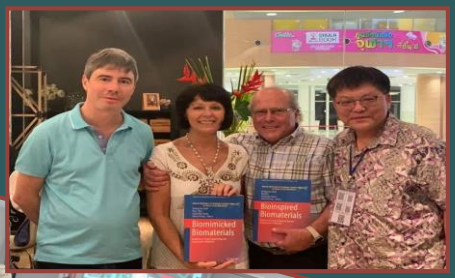
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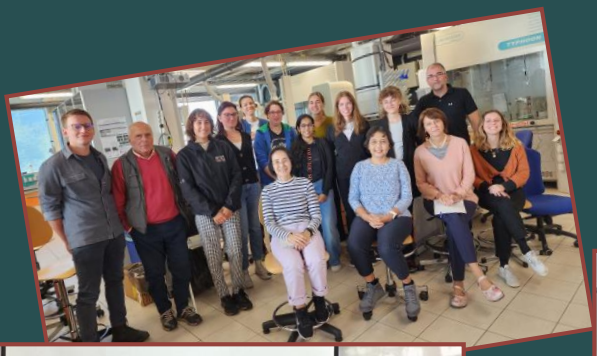


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