III Jornada IBEROS+

(Braga, 29 novembro 2024)

Comunicaciones flash

Micelle into gel thermosensitive intra-articular hydrogels loaded with natural products for Osteoarthritis management
Persona que presenta: Helena Rouco.
Autores: Helena Rouco, Maria Permuy, Fernando Muñoz, José Antonio Vázquez, Jose Ramon Caeiro-Rey, Patricia Diaz-Rodriguez, Mariana Landin.

Exploring new biobased material sources as platforms to advance skin wound healing and regeneration
Persona que presenta: Ana L. Oliveira
Autores: Viviana Ribeiro, Beatriz G. Bernardes, Marta Duarte, Marta Rosadas, Teresa Sousa, Alda Sousa, Julia Serra, Carlos García-Gonzaléz, Ana L. Oliveira

Targeting Molecular Signatures in Pancreatic Cancer: Novel Therapeutic Strategies and Experimental Validation in Xenograft and Organoid Models
Persona que presenta: Immacolata Maietta
Autores: Immacolata Maietta, Rosana Simón Vázquez, África González Fernández

Engineered naturally-derived bioinks for the extrusion bioprinting of skin models
Persona que presenta: Rúben Pereira
Autores: Luís B. Bebiano, Rafaela Presa, Rúben F Pereira

Comunicación en formato poster

1. **A peripheral-central nervous system on-chip to study targeted delivery of peripherally administrated nucleic acids**
   Autores: Miguel Xavier, Ana P. Spencer, Adriana Vilaça, Sofia C. Guimarães Rafael Santos, Ariel Ionescue, Yael Leichtmann-Bardoog, María Lázaro, Victoria Leiro, Eran Perlson, Ben M. Maoz, and Ana P. Pêgo.
2. **Single cell-derived breast cancer spheroids for real-time growth and secretomic studies**
   Autores: Margarida Esteves, Arantxa Ibáñez, Alexandra Teixeira, Beatriz Martín, Carolina Rodrigues, Sara Abalde-Cela, Miguel Xavier.
3. **Engineering Magnetic Lipid Nanovehicles for Macrophage Repolarization in Triple Negative Breast Cancer.**
   Autores: Andreia Patricia Magalhães#, Lara Diego-González#, Ana Cristina Ribeiro, Manuel Bañobre-López, Juan Gallo. (#Equal contribution)

4. **Fabrication of GelMA derived from fish by-products for tissue regeneration applications**
   Autores: Sara Pérez Davila, Jesús Valcarcel, José Antonio Vázquez, Patricia Díaz-Rodríguez, Mariana Landín, Miriam López-Álvarez, Julia Serra, Pío González.
5. **Breast cancer micro-aggregates for tumorigenesis studies**
   Autores: Ana Sofia Martins, David Caballero, Roberto Piñero; Lorena Diéguez
6. **A Novel Microfluidic Platform for the Rapid Detection of Inflammatory Biomarkers in Cancer**
   Autores: Rui Gomes, B-Blow, Felismina Moreira
7. **A Biomimetic Microfluidic Device for the Detection of Serum Albumin**
   Autores: Daniela Oliveira, B-Flow, Gabriela Martins e Felismina Moreira
8. **Development of a Plastic Antibody for Cardiac Biomarker Detection with Microfluidic Integration**
   Autores: Inês Vinagre, Stefano Schuissi e Felismina Moreira
9. **A Potentiometric Biomimetic Sensor for Cholesterol Detection Using Chiral Monomers**
   Autores: Cristina Sousa, Lorenzo Gomes e Felismina Moreira
10. **From 3D back to 2D: can organoid-derived monolayers match the physiological significance of organoid cultures?**
    Autores: Mafalda D. Neto, I. Marques, S.F. Martins, M. Xavier, C. Gonçalves.
11. **Breaking (blood-brain) barriers: shaping lung-to-brain metastasis with microfluidics**
    Autores: Tamagno Pesqueira, Sara Abalde-Cela, Elena Martínez, Lorena Diéguez
12. **Adenosine-Loaded Silk Fibroin: A Promising Approach for Chronic Wound Healing and Regeneration**
    Autores: Beatriz G. Bernardes, Raquel Costa, Carlos A. García-Gonzaléz, Ana Leite Oliveira
13. **Development of coaxial-printing of scaffolds for meniscus replacement**
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14. **3D printed Bioactive interference Screw and PCL Bio-Filler for ligament fixation**
    Autores: Mafalda Rodrigues,Rui Moreira, Inês V. Silva, Marta M. Duarte, Viviana P. Ribeiro, Ana L. Oliveira and João B. Costa
15. ***Porphyridium cruentum*: a factory for a new polysaccharide-based biomaterial Platform in tissue regeneration**
    Autores: Marta M. Duarte, Artem Suprinovych, Oscar L. Ramos, Joana R. Costa, Luis Rojo, Ana L. Oliveira
16. **I-CARE - Implantable decellularized-based cardiac patch for Cardiac tissue Repair**
    Autores: Ana L. Oliveira and Sara Amorim

17. **Unlocking the Potential of Supercritical CO_2 for Efficient Pancreatic Tissue Decellularization Towards a Platform for Cell Therapies**
Autores: Simone C. Sá, Carlos Pazmino, Joana Sá, Sara Amorim, Viviana P. Ribeiro, Raquel Costa, Ana L. Oliveira.

18. **Del paciente a la Investigación: Flujo de Trabajo para la Obtención de Organoides en un Biobanco.**
Autores: Dominguez-Aristegui P., Maietta I.,Gomez-de Maria C., Liste L., Fernandez-Costas A., Ortiz-Rey J.A., Sanchez-Espinel C., Sanchez-Santos R., Garcia-Fontan E.M., Gonzalez-Piñeiro A., Fernandez-Victoria R., Abdulkader S., Duran A., Gonzalez-Fernandez A., Teijeira, S.

19. **3D Culture Model for CTC clusters: Understanding the crosstalk between cancer cells and immune cells.**
Autores: Adriana Carneiro, Paulina Piairo, Tamagno Pesqueira, Rodrigo Mariño, Luis Lima, Lorena Diéguez

20. **Functionalization of Chitosan with Norbornene Groups for Improved Hydrogel Systems in Drug Delivery and Tissue Repair**
Autores: Caio Souza, Pedro L Granja

21. **Bioprinting of a vascularised cell-laden spinal cord graft: an anatomically relevant approach**
Autores: Filipa Leal, Ana P Pêgo, Pedro L Granja

22. **Bioengineered Mechanically Tunable Thiol-Ene Bioinks for Bioprinting of 3D Skin Models**
Autores: Luís Bebiano, Rafaela Presa, Francisca Vieira, Bianca N. Lourenço, Rúben F. Pereira

23. **Exploring subsets of CD44+ intervertebral disc cells as new therapeutic targets for discogenic pain**
Autores: Sofia Pilão, Luzia Teixeira, Raquel M Gonçalves

24. **Bioengineering Study Models for Annulus Fibrosus Research**
Autores: Luísa Castro, Raquel M Gonçalves

25. **Recovery of Collagen and derivates from Codfish skin using CO_2 acidified water**
Autores: Rita O. Sousa, Ana C. Carvalho, Alexandra Brito, Alexandra P. Marques, Rui L. Reis, Carlos A. García-González, Tiago H. Silva.

26. **Estudio de la eficacia antibacteriana de sistemas nanoparticulados cargados con rifabutina**
Autores: Carolina Menéndez Rodríguez, Helena Rouco Taboada, Irene Vieitez González, Myriam Castro Visos, Mariana Landín Pérez, Patricia Díaz Rodríguez y Carmen Potel Alvarellos.

Exploring new biobased material sources as platforms to advance skin wound healing and regeneration

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Chronic wounds are one of the major therapeutic and healthcare challenges affecting the population globally. One of the research interests of the Biomaterials and Biomedical Technology Lab (BBT Lab) is to explore the potential of biobased material platforms to advance skin wound healing and regeneration solutions. From the use of natural based biopolymers such as silk fibroin (SF) or sulfated exopolysaccharides (EPS), to the processing of more complex matrices such as the extracellular matrices, the group has been collaborating with some strategic partners in IBEROS+ to process, functionalize and characterize the materials for their physicochemical properties, structural adaptability, biocompatibility and bioactivity.

SF microparticulate aerogels loaded with adenosine have been developed via supercritical fluid technology in collaboration with the University of Santiago de Compostela. These particles exhibit a high porosity, biocompatibility, and positive interactions with skin cells towards regeneration, highlighting their promise in wound healing. A new Exopolysaccharide (EPS) produced by *Porphyridium cruentum* microalgae was developed as a novel biomaterial platform, offering bioactive properties, high molecular weight, thermal stability, and cytocompatibility for complex wound healing. An extensive characterization is ongoing, with contribution of the University of Vigo.

For extensive burn wounds, where autologous grafts are impractical, skin xenografts may provide a viable alternative, mostly if depleted from its immunogenic load. To achieve this, our group has developed and optimized methods for obtaining highly-preserved animal-origin decellularized tissues for human skin healing and regeneration. An important example is the valorization of rabbit skin, a valuable agro-food by-product that exceeds 5000 skins/day only in Europe. Our group has recently developed decellularized rabbit dermal matrices with preserved microarchitecture and human-like biochemical properties and expects to continue further developments in collaboration with the IBEROS+ consortium.

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Adenosine-Loaded Silk Fibroin: A Promising Approach for Chronic Wound Healing and Regeneration

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Chronic wound healing is frequently impeded by excessive exudate, which prolongs inflammation and increases the risk of infection. Traditional wound dressings often fail to manage exudate effectively, leading to tissue maceration and discomfort for patients. Aerogels, with their high porosity and extensive surface area, present promising solutions for wound care. This study developed silk fibroin (SF) microparticulate aerogels loaded with adenosine (ADO) using supercritical fluid technology to create a targeted drug delivery system for chronic wound healing. SF concentrations of 3%, 5%, and 7% (w/v) were utilized, producing particles with high porosity (93%-94%), appropriate densities, and surface areas ranging from 191 to 306 m^2/g. Drug release testing demonstrated rapid adenosine release, with 80% being released within 30 minutes.

In vitro studies involving keratinocytes, fibroblasts, and endothelial cells confirmed the biocompatibility of the developed particles, with fibroblasts showing particularly favorable interactions. The findings indicate that SF aerogels loaded with adenosine possess advantageous properties for wound healing, supporting cell proliferation. Future research will focus on optimizing the formulations to enhance therapeutic efficacy and evaluating clinical applications for chronic wound management and tissue regeneration.

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DEVELOPMENT OF COAXIAL-PRINTING OF SCAFFOLDS FOR MENISCUS REPLACEMENT

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Knee meniscus lesions are a common injury that can significantly impact an individual's quality of life. Current treatments for total meniscus replacement are unavailable, and the partial replacement alternatives provide only short-term relief, increasing the risk of knee arthritis in the long run. However, advancements in knee meniscus implants and 3D printing technology have the potential to revolutionize the treatment of knee meniscus lesions.

This project uses a dual coaxial printing approach which allows for the simultaneous extrusion of two or more materials through a single nozzle. The materials are extruded in a coaxial manner, meaning that they are aligned along the same axis and flow out of the nozzle together. This allows for the choice of two different materials, one to provide good mechanical properties and the other to support cell attachment. In this work, we aimed to use polycaprolactone (PCL), for the mechanical properties, and silk fibroin (SF) for the biological properties, once these materials are already approved by Food and Drug Administration (FDA) and European Medicines Agency (EMA). However, various studies have highlighted the enhanced mechanical properties of SF when in the β-sheet, which can be achieved through different treatments, like the addition of organic solvents. Having this in mind, we developed a method capable printing PCL and SF at the same time, while changing the SF conformation instantly upon contact with acetone used to dissolve PCL. This innovative approach led to the formation of scaffolds with enhanced mechanical properties while maintaining the biological properties provided by the SF.

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3D printed Bioactive interference Screw and PCL Bio-Filler for ligament fixation

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Musculoskeletal injuries, widespread across all ages, genders and sociodemographic groups [1], are prevalent in the knee joint and require a range of treatments - from conservative methods to surgical interventions, such as meniscal resection, repair, reconstruction or tissue engineering (TE) approaches [2]. To address one of the most significant challenges in orthopedic procedures – long-term implant fixation – an innovative solution is being developed for knee ligaments and meniscus fixation. PLA screws are being developed through the combination of 3D printing, supercritical CO2 (scCO2) foaming and impregnation technologies, aiming to achieve a biodegradable and bioactive screw with improve bone integration ability. Additionally, to improve the anchor and fixation of the ligament treatments, PCL Bio-Fillers are being developed through the use of 3D printing, electrospinning and dipping methods with the final goal to induce the bone cells to reproduce itself (osteogenesis) and create a better grip between the ligaments and the bone.

PLA screws were manufactured by 3D printing and further process to induce porosity by scCO2 foaming, followed by EPS impregnation through scCO2. Scanning electron microscopy (SEM) was used to evaluate microporosity and the EPS impregnation. The CO2 concentration, density and expansion ratio of the PLA screws were evaluated. FTIR (Fourier-transform infrared spectroscopy) was performed to evaluate chemical composition changes of the samples. DSC (Differential scanning calorimetry) was applied to analyze thermal stability both before and after treatment. PCL Bio-Fillers were produced by 3D printing, coated with PCL using electrospinning and dipped with bruxite. Then, to study the PCL fibers and bruxite dispersion, we have used the scanning electron microscopy (SEM) method. Results: Several conditions of foaming were tested (pressure, time, temperature and controlled expansion measures) and then, analyzed through SEM imaging. Samples with greater porosity were selected for further testing and analysis. The CO2 concentration results revealed that the saturation increase is proportional to the increase in pressure and inversely proportional to the increase in infill density. The expansion ratio results demonstrated that it typically decreases with increasing infill density and batch pressure. To optimize the 3D printed Bio-Fillers coated with PCL several parameters were adjusted (PCL concentration, flow rate, distance, potential difference, and nozzle size). Firstly, samples with apparent macroscopy uneven coating were remove with further analysis being performed via SEM analysis. The SEM analysis showed that increasing the potential difference and decreasing the flow rate produced more dispersed and thinner fibers. It also revealed that increase PCL concentration led to higher fiber density and size. In the end, the parameters that resulted in the better PCL fibers dispersion were with a concentration of 7,5% w/v of PCL, 20 µL/min flow rate, 10 cm of distance between the nozzle and the Bio-Filler, 23 kV of potential difference and 20 G of nozzle size. Further work is being performed to optimize the dipping process with bruxite and achieve an even coating. Conclusion: The sCO_2 methodologies implemented were efficient in terms of generating porosity and EPS impregnation. EPS-induced bioactivity will be studied in the future.

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Porphyridium cruentum: a factory for a new polysaccharide-based biomaterial Platform in tissue regeneration

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Marine algae and their metabolites have been widely recognized for their bioactive properties with applications in various industries, such as nutraceutical, cosmetical, and pharmaceutical. In this study, the best strategy for the extraction and processing of the exopolysaccharides (EPS) from *Poryphydium cruentum* for a laboratory scale was discussed, and the collected, purified EPS fraction was extensively characterized. The results show that impurities and other co-precipitants can be significantly reduced via trichloroacetic acid (TCA) treatment, followed by dialysis, resulting in a more purified EPS fraction with a higher carbohydrate content and solubility ability, at the cost of lower mass yield. The obtained EPS fraction was of a high molecular weight, presented a high crystallinity index, was thermally stable and cytocompatible within the range of tested concentrations. These results show its potential to be used as a new platform for healing and regeneration of chronic wounds.

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I-CARE - **I**mplantable decellularized-based cardiac patch for **Ca**rdiac tissue **Re**pair

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Cardiovascular diseases account for 32% of the global deaths, comprising different pathologies related to heart failure. [1] The heart self-regeneration is limited, which is associated to the highest mortality and morbidity rates of cardiovascular diseases. The developed strategies to guide the regeneration of cardiac tissue lacks on biocompatibility, cell homing and engraftment. Thus, the incapacity of endogenous regeneration reduces therapeutic approaches to the relief of symptoms rather than restoring the proper function of the heart. The I-CARE project intends to develop an ECM-based implantable cardiac patch to promote *in situ* the self-regeneration of myocardial tissue. Supercritical carbon dioxide ($scCO_2$) is a well-known technique, particularly interesting as a solvent-free alternative for the decellularisation of tissues. Here, we propose the use of $scCO_2$ to decellularise cardiac tissue. The decellularized matrix will be further used to develop a biocompatible patch with chemical and physical features able to support the recellularization with cardiomyocytes. The decellularized cardiac patch will stimulate *in-situ* cardiomyocyte contractility and support the regeneration of myocardial tissue.

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Unlocking the Potential of Supercritical CO_2 for Efficient Pancreatic Tissue Decellularization Towards a Platform for Cell Therapies

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Decellularization, the process of removing cells from a tissue or organ while preserving the extracellular matrix (ECM), often employs harsh chemical solvents, compromising ECM integrity. This is particularly damaging in case of soft tissues. Supercritical carbon dioxide ($scCO_2$), achieved at 31°C and 74Bar, is an alternative solvent which presents high transfer rates and diffusivity while being chemically inert and non-toxic [1][2][3].

This study investigates the potential use of $scCO_2$ for decellularizing pancreas, a highly sensitive tissue, aiming to preserve as effectively as possible, its native architecture and biochemical cues, while ensuring its immune tolerance. This approach seeks to establish a platform for pancreatic cell therapy applications.

Porcine pancreatic tissue was decellularized by traditional and $scCO_2$ decellularization methods. Our first results indicate that the use of $scCO_2$ contributes to a more efficient cell removal protocol, while it allowed to considerably reduce the use of chemicals and proved to be less time-consuming. This innovative approach highlights $scCO_2$ as a viable alternative for pancreatic tissue decellularization, opening new opportunities for advancing current cell therapies that lack proper mimetic environments to maintain cell viability and post-implantation functionality.

Keywords: Decellularization; Pancreatic Tissue; cell therapies; supercritical CO_2

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