StemNet

Third International StemNet Meeting

Bologna, Italy • 12-13 March 2026

Abstracts Book

https://STEMNET2026.azuleon.org

Welcome

Welcome to the third **International StemNet Meeting** that will take place at the Zanhotel Europa, Bologna (Italy) on March 12-13, 2026.

StemNet is a federation of the four main associations of stem cell research in Italy (**FIRST**, **GISM**, **IPLASS**, **SCRI**) that share and synergize experiences to enhance both the quality and the impact of research in this advancing field. This meeting marks the constructive relationship among our associations and aims to improve the exchange of relevant and up-to-date information in the field of advanced cell therapy.

The six sessions of the meeting will spotlight relevant progress in basic and translational research. The meeting also includes discussions on critical aspects of biomedical communication and research valorization and will include a "next generation session" organized by young scientists. A faculty of national and international experts and renowned speakers will foster exciting and inspiring debates of the challenging scientific program.

The best three posters will be awarded with the "Young Investigator Award" (500 € / each) to encourage the attendance of the youngest members of our community.

Sincerely,

The StemNet President and the local organising committee

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Programme

9:00-10:30 **Registration**

10:30-11:00 **Welcome and opening of the meeting**

9:15-12:30 **Innovative Trends in Science Communication *(in Italian)***

Chairs: Augusto Pessina (Milan, Italy), Umberto Galderisi (Naples, Italy)

9:45-10:00 **Emanuele Frontoni** *(Macerata, Italy)*

Lo storytelling della scienza nell'epoca della intelligenza artificialet

11:20-11:40 **Samuele Negro** *(Padua, Italy)*

Alla scoperta dei meccanismi della rigenerazione nervosa: la mia ricerca raccontata attraverso i Social Media

11:40-12:30 **Round table and discussion**

13:00-14:00 ***Lunch***

14:00-16:00 **"StemNet Next Generation"**

Chairs: Pasquale Marrazzo (Urbino, Italy), Federico Divincenzo (Turin, Italy)

14:00-14:20 **Valentina Bugani** *(Meldola, FC, Italy)*

Training and development strategies in healthcare research organizations

14:20-14:40 **Filippo Piccinini** *(Bologna, Italy)*

Scientific medical writing with Artificial Intelligence

14:40-15:00 **Ákos Diósdi** *(Szeged, Hungary)*

Patenting microscopy technologies: a case study

15:00-15:15 **"StemNet Next Generation – Contest 2026" - 3rd Place**
Domitilla Mandatori *(Chieti, Italy)*

Effects of mesenchymal stromal cells and human recombinant Nerve Growth Factor delivered by bioengineered human corneal lenticule on an innovative model of diabetic retinopathy

15:15-15:30 **"StemNet Next Generation – Contest 2026" - 2nd Place**
Angelo Canciello *(Teramo, Italy)*

Graphene oxide accelerates TGFβ-mediated epithelial-mesenchymal transition and stimulates pro-inflammatory immune response in amniotic epithelial cells

15:30-15:45 **"StemNet Next Generation – Contest 2026" - 1st Place**
Domenico Aprile *(Naples, Italy)*

Lineage specification into GABAergic, glutamatergic, dopaminergic, and astrocytic phenotypes using MUSE stem cells: a novel approach for modeling neurodegenerative and psychiatric disorders

15:45-16:00 **Award Ceremony**
"StemNet Next Generation – Contest 2026"

16:00-16:30 *Coffee break*

16:30-18:00 **Innovation, Sustainability and Market Access for ATMPs**
Chairs: Laura Calzà (Bologna, Italy), Lorenza Lazzari (Milan, Italy)

16:30-16:50 **Simona Guidi** *(Leiden, The Netherlands)*
Innovative technologies, decentralized model versus centralized model in ATMPs manufacturing, the state of the art

16:50-17:10 **Claudio Jommi** *(Novara, Italy)*
Sustainability and market access for medicines: general issue and focus on ATMPs

17:10-17:20 **Cristina Zanini** *(IWT PHARMA Casale Litta, Varese, Italy)*
Ludovica Filippini *(Cellex, Rome, Italy)*
Bone tissue GMP production in a closed system: using a closed-circuit between a bioreactor and an incubator integrated in a Grade A isolator for sustainable Advanced Therapy Medicinal Products (ATMPs) manufacturing

17:20-17:30 **Vincenzo Raffo** *(Bari, Italy)*
Inflammatory priming activates the healing ability of adipose-derived stem cells in osteoarthritis

17:30-18:00 **Discussion**

18:00-19:00 **General Assembly**

19:30 *Standing aperitif-style light dinner (Apericena)*

21:00 *Social Event and Dancing, the event will include a bachata lesson with DJ Set and group dances*

9:00-10:30 **Biofabrication and Bioengineering Tools for Tissue Repair**
Chairs: Maria Letizia Focarete (Bologna, Italy), Laura Mercatali (Bologna, Italy)

9:00-9:20 **Gianluca Cidonio** *(Rome, Italy)*
Skeletal stem cell 3D bioprinting for the functional engineering of bone tissue

9:20-9:40 **Dario Carugo** *(Oxford, United Kingdom)*
Ultrasound-mediated stimulation of biological systems for enhanced drug delivery and tissue repair

9:40-9:50 **Andrea Lolli** *(Rotterdam, The Netherlands)*
Making a long story short: tissue engineering bone with stromal cells subjected to brief chondrogenic priming

9:50-10:00 **Mattia Dessena** *(Parma, Italy)*
Mesenchymal stem cell fate across monoclonal gammopathies: from single-cell profiling to 3D niche modeling

10:00-10:30 **Discussion**

10:30-11:00 *Coffee break*

11:00-12:30 **Stem Cells in Cartilage (Tissue) Repair**
Chairs: Gina Lisignoli (Bologna, Italy), Eleonora Iacono (Bologna, Italy)

11:00-11:20 **Serena Duchi** *(Melbourne, Australia)*
Stem cell cartilage tissue engineering for post-traumatic osteoarthritis prevention: advancing equity in regenerative medicine

11:20-11:40 **Leonardo Ricotti** *(Pisa, Italy)*
Stimulation of cartilage healing with ultrasound stimulation of stem cells

11:40-11:50 **Nicola Mondanelli** *(Azienda ospedaliero-universitaria Senese - Ospedale Santa Maria alle Scotte)*
Multimodal approach in the surgical management of a complex case of hypertrophic femoral pseudoarthrosis

11:50-12:00 **Maira Bacchiega** *(Schaefer SEE Srl, Italy)*
EV purification & characterisation: where precision meets potential

12:00-12:30 **Discussion**

12:30-13:30 *Lunch*

13:30-14:30 **Poster Session**

14:30-16:00 **Omic Studies for Stem Cell Biology**
Chairs: Krisztina Buzas (Szeged, Hungary), Roberta Piva (Ferrara, Italy)

14:30-14:50 **Giada Pietrosi** *(Palermo, Italy)*
The application of perinatal stem cells in advanced liver disease: results and future perspectives

14:50-15:10 **Nereo Kalebic** *(Milan, Italy)*
Glioblastoma stem cell morphotypes convey distinct cell states and clinically relevant functions

15:10-15:20 **Arianna Minoia** *(Verona, Italy)*
Modelling CLCN7-associated osteopetrosis: iPSC/iMSC-based approach and PBMC-derived osteoclast differentiation in patient and control

15:20-15:30 **Alice Zaramella** *(Padua, Italy)*
MicroRNA-31- engineered extracellular vesicles as a target therapy for inflammatory bowel disease

15:30-16:00 **Discussion**

16:00-17:00 **Cancer Stem Cells**
Chairs: Francesco Alviano (Bologna, Italy), Gianandrea Pasquinelli (Bologna, Italy)

16:00-16:20 **Ilio Vitale** *(Turin, Italy)*
Immune escape by cancer stem cells: mechanisms and therapeutic opportunities

16:20-16:30 **Silvia Pontara** *(Genoa, Italy)*
A humanized 3D bioprinted model for studying breast cancer bone metastasis

16:30-16:40 **Annalisa Astolfi** *(Bologna, Italy)*
Smooth muscle-committed TP53-knockout pluripotent stem cells as a novel leiomyosarcoma cell model for preclinical target discovery

16:40-17:00 **Discussion**

17:00-17:30 **Poster Award**

18:00 **Conclusions**

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Equine osteoarthritis-related microRNA analysis in extracellular vesicles from horse mesenchymal stromal cells: a preliminary investigation

P.2 Emilia Attolini *(Ozzano dell'Emilia)*
Effect of equine mesenchymal stromal/stem cell secretome on equine oocyte nuclear maturation

P.3 Elia Bari *(Novara)*
Decellularized and lyophilized dermal scaffolds loaded with the lyosecretome of mesenchymal stem cells for skin regeneration

P.4 Maria Bernazeaud *(Naples)*
Adaptive mitochondrial quality control supports oxidative stress resistance in Muse cells

P.5 Edoardo Bertania *(Novara)*
Design of silk fibroin-based hydrogels functionalized with extracellular matrix and mesenchymal stem cell secretome for skin bioprinting

P.6 Edoardo Bertania *(Novara)*
Cell-specific targeting via a gmp-ready microfluidic carrier-in-carrier system of silk fibroin nanoparticles encapsulated in extracellular vesicles

P.7 Paola Bisaccia *(Padua)*
Mesenchymal stromal cells - derived extracellular vesicles inhibit pyroptosis and protect tissue integrity in models of bronchopulmonary dysplasia

P.8 Angelo Canciello *(Teramo)*
KLHL14 and its X1 isoform act as central regulators of epithelial plasticity by controlling epithelial-mesenchymal transition in amniotic epithelial stem cells and hepatocarcinoma cells

P.9 Angelita Capone *(Ozzano dell'Emilia)*
Hypoxia-driven functional modulation of canine umbilical cord–derived mesenchymal stromal cells: implications for pulmonary vascular regeneration

P.10 Melania Carniato *(Bologna)*
Moda project: a randomized controlled trial to evaluate the decellularized human dermis in combination with orthobiologic stimuli for the arthroscopic augmentation of massive rotator cuff tears

P.11 Ludovica Carpinelli *(Rome)*
Patient iPSC-derived cerebral organoid as a multicellular model to mimic the complexity of the central nervous system

P.12 Gaia Cendron *(Genoa)*
A three-dimensional bioprinted colorectal cancer model based on tumor-derived extracellular matrix, apelin-13 and type I collagen to study tumor–stroma interactions

P.13 Camilla Bruna Cerchier *(Bologna)*
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P.14 Carmen Ciavarella *(Bologna)*
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P.15 Giorgia Codispoti *(Bologna)*
In vivo evaluation of an ultrasound-stimulated smart piezoelectric nanocomposite hydrogel enriched with adipose-derived mesenchymal stromal cells for osteoarthritis treatment

P.16 Roberta Costa *(Bologna)*
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P.17 Daniele D'Arrigo *(Pieve Emanuele)*
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P.18 Giovanni D'Atri *(Milan)*
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Mesenchymal stem cell fate across monoclonal gammopathies: from single-cell profiling to 3D niche modeling

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P.21 Ivana Ferrero *(Turin)*
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Modelling CLCN7-associated osteopetrosis: iPSC/iMSC-based approach and PBMC-derived osteoclast differentiation in patient and control

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P.51 Vincenzo Raffo *(Bari / Milan)*
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P.58 Mariachiara Stellato *(Bologna)*
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P.60 Alice Zaramella *(Padua)*
MicroRNA-31- engineered extracellular vesicles as a target therapy for inflammatory bowel disease

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Oral Presentations

In chronological order

Lo storytelling della scienza nell'epoca dell'intelligenza artificiale
Science Storytelling in the Age of Artificial Intelligence

Emanuele Frontoni
Professore Ordinario di Informatica all'Università di Macerata e co-director del VRAI Vision Robotics & Artificial Intelligence Lab
Full Professor of computer science at the University of Macerata and the Co-Director of the VRAI Vision Robotics & Artificial Intelligence Lab

La comunicazione della ricerca scientifica è entrata in un'era di trasformazione con l'avvento dell'intelligenza artificiale. Questa presentazione esplorerà come l'IA stia ridefinendo il panorama dello storytelling scientifico, esaminando sia le opportunità che le sfide che emergono quando la tecnologia d'avanguardia si interseca con la comunicazione biomedica. Mentre la ricerca sulle cellule staminali e le terapie cellulari avanzate continuano a progredire, la capacità di comunicare efficacemente concetti scientifici complessi a pubblici diversi—dalla comunità scientifica al grande pubblico—diventa sempre più cruciale. Questo intervento affronterà come gli strumenti di IA possano migliorare la valorizzazione della ricerca, potenziare il coinvolgimento del pubblico e favorire narrazioni più accessibili e incisive intorno alle scoperte scientifiche. La discussione considererà anche le implicazioni etiche e l'importanza di mantenere l'accuratezza scientifica e la prospettiva umana in un ambiente di comunicazione potenziato dall'IA, particolarmente rilevante per il campo della medicina rigenerativa e della ricerca sulle cellule staminali.

The communication of scientific research has entered a transformative era with the advent of artificial intelligence. This presentation will explore how AI is reshaping the landscape of science storytelling, examining both the opportunities and challenges that emerge when cutting-edge technology intersects with biomedical communication. As stem cell research and advanced cell therapies continue to advance, the ability to effectively communicate complex scientific concepts to diverse audiences—from the scientific community to the general public—becomes increasingly critical. This talk will address how AI tools can enhance research valorization, improve public engagement, and foster more accessible and impactful narratives around scientific discoveries. The discussion will also consider the ethical implications and the importance of maintaining scientific accuracy and human perspective in an AI-augmented communication environment, particularly relevant to the field of regenerative medicine and stem cell research.

Unlocking nerve regeneration: my research journey through the lens of social media

Samuele Negro[1], Chiara Baggio[1] Giorgia D'Este[1], Giulia Zanetti[1], Federico Fabris[1], Aram Megighian[1,2], Alessandro Bertoli[1], Marilina Massimino[1,3], Manuela Basso[4], Valentina Bonetto[5], Roberta Schellino[6,7], Marina Boido[6,7], Cesare Montecucco[3], Marco Pirazzini[1], Michela Rigoni[1]
[1]Dept of Biomedical Sciences, University of Padua, Padua, Italy
[2]Padua Neuroscience Center, University of Padua, Padua, Italy
[3]CNR Neuroscience Institute, Padua, Italy
[4]Dept of Cellular, Computational and Integrative Biology - CIBIO, University of Trento, Trento, Italy
[5]Dept of Molecular Biochemistry and Pharmacology, IRCCS-Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy
[6]Dept of Neuroscience Rita Levi-Montalcini, University of Turin, Italy
[7]Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Orbassano, Italy

The peripheral nervous system (PNS) has retained, through evolution, a remarkable ability to regenerate after certain types of damage. My research focuses on uncovering the mechanisms behind this extraordinary capacity. Understanding these processes is crucial for developing therapeutic strategies for neurodegenerative diseases, where these regenerative mechanisms are either impaired or only partially activated. Using a neurotoxin derived from the venom of the black widow spider, we have identified a molecular axis involving the chemokine CXCL12 and its receptor CXCR4 as a pivotal driver of axonal regeneration in response to various types of peripheral nervous system injuries. Currently, we are extending this research to explore how this axis functions in chronic conditions like Amyotrophic Lateral Sclerosis (ALS), which affect the PNS. But, forget the boring charts, figures and graph! I'm bringing science to life in a way that's actually fun! I will share my work through funny videos I post on social media (find me at @Samuscientist). These clips capture not just the science but also the everyday realities of life as a researcher, offering a fresh perspective on what drives us and the challenges we face.

Training and development strategies in healthcare research organizations

Valentina Bugani
IRCCS Istituto Romagnolo per lo Studio dei Tumori (IRST) "Dino Amadori", Meldola, Italy

Objective
This contribution aims to present a strategic, conceptual, and practice-based framework for training and competency development in healthcare research organizations. Drawing on institutional experience within an Italian IRCCS, the oral presentation explores how moving beyond a compliance-driven view of training toward a competency-oriented approach can support professional growth, staff engagement, organizational performance, and long-term sustainability in complex research and care environments.
Materials and Methods
The contribution is grounded in an institutional, practice-informed analysis of training and competency development strategies implemented within a healthcare research organization. It integrates organizational training models, competency mapping and assessment tools, leadership development practices, and continuous learning pathways. A qualitative and descriptive approach is adopted, consistent with professional, policy, and organizational development contexts rather than experimental research designs.
Results
The analysis highlights that positioning training as a continuous and competency-based learning pathway enhances professional identity, motivation, and engagement. Systematic competency assessment supports personalized development trajectories, strengthens interdisciplinary collaboration, and improves organizational adaptability. Leadership involvement emerges as a key enabling factor in embedding training within onboarding, performance, and career development processes. Furthermore, integrating training with well-being and resilience initiatives contributes to sustainable professional performance in high-pressure healthcare research environments.
Conclusions
This contribution suggests that training and competency development should be understood as strategic investments rather than operational costs for healthcare research organizations. A competency-oriented and people-centred approach supports alignment between individual development and institutional missions, fostering innovation, engagement, and organizational sustainability. Embedding training within leadership and well-being strategies is essential to support resilient professionals and ensure the long-term quality of research and patient care.

Scientific medical writing with artificial intelligence

Filippo Piccinini
IRCCS Istituto Romagnolo per lo Studio dei Tumori "Dino Amadori" (IRST) & Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Italy

In the rapidly evolving landscape of scientific research, Artificial Intelligence (AI) platforms can definitively boost scientific writing. The seminar titled "Scientific Medical Writing with Artificial Intelligence" aims to help researchers with skills and insights needed to exploit AI to enhance their scientific writing and publication success. Participants will explore the latest AI-driven tools and techniques that can streamline the scientific writing process and improve the clarity and impact of manuscripts. Topics covered will include AI-powered literature reviews, automated data analysis, and language processing for drafting and editing. In addition, the seminar will address best practices, limitations, and ethical considerations associated with the use of AI in scientific communication, including transparency, reproducibility, and authorship issues. By the end of the session, participants will be better prepared to integrate AI tools thoughtfully and responsibly into their writing workflows, ultimately enhancing the impact and dissemination of their research.

Patenting microscopy technologies: a case study

Akos Diosdi[1,2], Peter Horvath[1,2,3,4]
[1]Synthetic and Systems Biology Unit, HUN-REN Biological Research Centre (HUN-REN BRC), Szeged, Hungary
[2]Single-Cell Technologies Ltd, Szeged, Hungary
[3]Institute of AI for Health, Helmholtz Zentrum München, Neuherberg, Germany
[4]Institute for Molecular Medicine Finland, University of Helsinki, Helsinki, Finland

Objective
This study examines the patenting pathway of the HCS-3DX project as a representative example of how early-stage innovations for biotechnology applications can be protected and strategically positioned. The primary aim is to show the practical steps, decision points, and responsibilities associated with securing intellectual property (IP), with a particular emphasis on why young scientists should understand the implications of patenting in research-driven environments.
Materials and Methods
The analysis is based on internal project documentation from the HCS-3DX development process, including technical descriptions, prototype evaluations, and correspondence related to IP strategy. The study reviews the criteria used to determine suitability for patent application, and outlines the procedural steps taken during prior-art assessment, claims formulation, and submission. This reflection, which incorporates personal experiences, acknowledges errors and lessons learned that have led to a more developed comprehension of the duties associated with protecting scientific innovation.
Results
The HCS-3DX project demonstrated that IP protection can be justified for even small but truly innovative advancements in microscopy. The work proceeded in a methodical manner, first obtaining utility model protection for the fundamental mechanical innovations, then moving on to a complete patent application, and finally going into the PCT stage to obtain more extensive international coverage. Only after these steps were completed did we publish the technology in a prestigious journal, ensuring that novelty was preserved while still enabling scientific dissemination. The resulting protection unlocked new partnerships, visibility, and development pathways, demonstrating the transformative effect that a well-timed patent strategy can have on a technology's trajectory.
Conclusions
This case study demonstrates that IP protection is a strategic element of scientific innovation rather than just an administrative procedure. For young researchers, understanding the responsibilities and consequences of patenting (e.g. timing, disclosure, inventorship, and long-term commercial implications) is essential for safeguarding their work and maximizing its impact. The HCS 3DX experience underscores the value of early education in IP management and demonstrates how thoughtful protection strategies can support both scientific dissemination and technological translation.

Innovative technologies, decentralized model versus centralized model in ATMPs manufacturing, the state of the art

Simona Guidi
ProPharma, Leiden, The Netherlands

Cell and gene therapies have the potential to address significant therapeutic unmet needs; however, patient access remains a major challenge. Developing a decentralized production model directly at the patient's bedside could unlock their full potential. This approach would support clinical protocols and strengthen the distribution of authorized products, ensuring timely and equitable access to advanced treatments. Ultimately, it would reinforce a patient-centered approach to care.

Sustainability and market access for medicines: general issue and focus on ATMPs

Claudio Jommi
Dept of Pharmaceutical Sciences, Università del Piemonte Orientale, Novara, Italy

The speech will discuss the assessment and appraisal process for ATMPs, ATMPs price and reimbursement in the major European Countries (France, Germany, Italy, Spain, UK) and solutions to make ATMPs sustainable from a financial and organizational viewpoints.

Bone tissue GMP production in a closed system: using a closed-circuit between a bioreactor and an incubator integrated in a Grade A isolator for sustainable Advanced Therapy Medicinal Products (ATMPs) manufacturing

Cristina Zanini[1], Ludovica Filippini[2], Pietro Bosi[1], Giuseppe Falvo D'Urso Labate[2]
[1]IWT PHARMA Casale Litta (VA), Italy
[2]Cellex, Rome (RM), Italy

Osteoporosis is a prevalent condition among the elderly, characterised by reduced bone density and deterioration of bone microstructure, which increases the risk of fractures. By 2034, the incidence of fractures is projected to rise by 28.4%.
This fragility poses a significant societal and economic burden worldwide, as the costs associated with osteoporosis exceed 3.5% of total health expenditure in Europe. Therefore, developing innovative and technologically advanced therapeutic approaches to produce bone tissue under Good Manufacturing Practice (GMP) conditions, while minimising costs, is crucial.
This study aims to evaluate a synergistic strategy for the GMP production of bone tissue substitutes in dynamic three-dimensional (3D) conditions, using a bioreactor named BioAxFlow within a grade A isolator (ISOCELL) installed in a grade D environment. The BioAxFlow bioreactor facilitates the dynamic culture of poly lactic acid-made scaffolds seeded with SAOS-2 cells, a commercially available human osteosarcoma cell line, all incubated within an ISOCELL.
To ensure biocontainment under Good Manufacturing Practice (GMP) conditions, a closed-circuit system was developed for the aseptic transfer of biological fluids between the bioreactor and the incubator, which is integrated within a grade A isolator. This system uses validated devices known as Aseptic Transfer Port (AT). This port allows for changing media, adding trypsin and transfer reagents, all while maintaining a completely closed circuit to prevent microbiological contamination.
Indeed, preliminary results in standard laboratory conditions indicated that SAOS-2 cells proliferated within the 3D-dynamic culture device, which promotes cell penetration along the z-axis of the scaffolds and ensures homogeneous colonisation of the surfaces (2). Additionally, the closed-circuit that will be established between the bioreactor and the isolator will prove advantageous for microbiological safety, as it reduces the risk of cross-contamination, improves operating conditions, and lowers costs.
Using grade A Isolators within a background grade D environment, in other words, by means of closed systems as defined by Eudralex guidelines (vol. 4, part IV and Annex 1), significantly lessens environmental and economic impacts compared to traditional grade B clean rooms (open systems).

Inflammatory priming activates the healing ability of adipose-derived stem cells in osteoarthritis

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Objective
Osteoarthritis (OA) is a chronic joint disease characterized by cartilage degradation and inflammation, where macrophages play a pivotal role in pathogenesis. Intra-articular injection of adipose-derived stem cells (ASCs) is a promising therapy, but the therapeutic efficacy may require improvement. This study aims to evaluate whether priming strategies (inflammatory or hypoxic) can "unlock" the immunomodulatory and regenerative potential of ASCs to better counteract the OA environment.
Materials and Methods
We isolated human ASCs and co-cultured them with macrophages from healthy donors to test their effect on macrophage polarization. To identify the most effective activation stimulus for enhancing their therapeutic efficacy in the OA environment, ASCs were exposed to three different priming conditions: hypoxia (1% O_2), interferon γ (IFNγ), or interleukin-1β (IL-1β). The molecular changes were characterized by using a multi-omics approach: mass spectrometry for the secretome, TaqMan Arrays for exosomal miRNAs, and Next-Generation Sequencing (NGS) for gene expression.
Results
Under basal conditions, ASCs did not induce an anti-inflammatory phenotype in macrophages. However, IL-1β priming, unlike hypoxia or IFNγ, triggered a massive proteomic and transcriptomic remodeling. Specifically, IL-1β- upregulated proteins related to immune regulation (chemotaxis) and cartilage matrix organization. The exosome cargo is changed by this priming, enriching specific classes of miRNAs. These molecules are predicted to target and downregulate inflammatory signals, while supporting pathways involved in matrix remodeling.
Conclusions
ASCs require specific signals to become active because they are not inherently anti-inflammatory. IL-1β priming promotes both inflammation resolution and tissue repair. This study suggests that priming could be a key strategy to develop more effective, tailored treatments for OA.

Advancing with 3D bioprinting beyond the horizon of current clinical practice for skeletal regeneration and modelling

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Nowadays, clinical interventions in skeletal regeneration is still limited by the scarce bone tissue availability, stability and functional response following implantation. The need for a viable skeletal substitute has given input to a new class of approaches for bone repair, with 3D bioprinting currently advancing at the forefront of bone tissue engineering and regenerative medicine. The possibility to fabricate three-dimensional tissue that might resemble the ultimate copy of the native tissue is appealing, promising to deliver a personalised therapy for patients in need for skeletal tissue implantation. Nevertheless, the lack of resolution and the inability to deposit multiple cell types and drive the hierarchical organization, is currently impeding 3D bioprinting approaches to reach the most needed clinical translation. Novel 3D bioprinting approaches are on the verge of driving
In particular, the use of microfluidic-assisted 3D bioprinting technology has been found capable of delivering in high resolution cellular biomaterials for the active repair of bone tissue. Microfluidic-based printhead can be used to compartmentalize skeletal stem cells for the functional fabrication of bone-like constructs, that might resemble mineralisation and biocompatible features of the native bone. This approach is now advancing towards pre-clinical practice, facilitating the assembly of hierarchical biomimetic bone tissue constructs that might resemble the functional architecture of gradient-like structure of patient-specific needs.

Ultrasound-mediated stimulation of biological systems for enhanced drug delivery and tissue repair

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Ultrasound (US) has demonstrated potential for inducing therapeutic effects in a wide range of clinical applications. Gas-filled microbubbles (MBs) stabilised by a coating layer are often employed in conjunction with ultrasound stimulation. The MB coating can be employed as a scaffold for the attachment of biologically active compounds, which has paved the way for the use of MBs as vehicles in therapeutic applications such as drug delivery and/or gene therapy. Their responsiveness to US facilitates triggered release of the therapeutic material, and the interaction between MBs and living cells in an US field has been observed to increase cell membrane permeability. This presentation will first provide an introduction to gas microbubbles, their physical response to US waves, and the effects of this response on biological cells. In this context, the use of miniaturised fluidic systems integrated with US stimulation (i.e. 'sonofluidic' devices) in mechanistic research will be briefly discussed. Subsequently, two research case studies will be presented: the first study will illustrate integration between microfluidic, bioprinting and ultrasound technology for the manufacturing of functional tissue-engineeering scaffolds. The second study will instead demonstrate development of custom devices for the application of controlled physical stimuli onto complex *in vitro* models of urothelial tissue, and their application in the evaluation of US-mediated antibiotic delivery for the treatment of urinary tract infections.

Making a long story short: tissue engineering bone with stromal cells subjected to brief chondrogenic priming

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Tissue engineering bone via endochondral ossification requires the implantation of cartilage which remodels into bone. It is a promising avenue to repair large bone defects, but the lengthy phase of *in vitro* priming to generate cartilage from progenitor cells (3-6 weeks) severely hinders translation. While new strategies with brief cell priming may provide a solution, little attention has yet been paid to the extent to which the priming may be shortened and how bone formation is affected. We set out to investigate the effect of chondrogenic priming duration on human mesenchymal stromal cell(hMSC)-mediated endochondral ossification, and characterise the transcriptomic landscape of implanted cells linked to successful bone formation.

hMSC pellets were cultured in the presence of TGF-β3 for 1, 3, 5, 7 or 21 days (N=4 donors). The pellets were subjected to bulk and single-cell RNA-sequencing, or implanted subcutaneously in athymic mice up to 12 weeks. *In vivo* mineralisation was analysed by μCT. The implants were retrieved at several time-points post-implantation (d3, d7, d14, d28, d56, d84) and subjected to (immuno)histological characterisation or flow cytometry to monitor the dynamics of bone formation.

After implantation, 7d- and 21d-primed pellets consistently led to bone formation. While priming the cells for less than 7 days caused variable outcomes, local bone formation occurred even when hMSCs were primed for as little as 1 day, indicating that osteoinductive signals arise far earlier than assumed. *In vivo* longitudinal studies showed that shortening the priming from 21 to 7 days did not impact the fate of implanted cells and led to accelerated tissue remodelling. We next analysed the full transcriptome of 7d-primed pellets via bulk (mi)RNA-sequencing and single-cell RNA-sequencing, reconstructing regulatory networks linked to bone formation. miRNA-140 and the actin-regulating protein scinderin were found to be associated with bone formation potential, and their knockdown in hMSCs caused accelerated chondrogenesis within 7 days.

We provide proof-of-concept that a brief chondrogenic priming leads to bone formation without impacting the dynamics of endochondral ossification. We identified the cell and gene signature associated with bone formation capacity, providing new gene targets to speed-up cell priming and bone formation. Our data raise exciting opportunities for next-generation therapies for bone defect repair requiring minimal cell manipulation.

Mesenchymal stem cell fate across monoclonal gammopathies: from single-cell profiling to 3D niche modeling

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Objective
In multiple myeloma (MM) and its precursor conditions (monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM)) malignant plasma cells remodel the bone microenvironment (BME) cells, mesenchymal (MSCs) and osteoblastic cells (OBs), but mechanisms remain incompletely defined. We profiled non-hematopoietic BME cells at single-cell level across disease stages and investigated WISP2, a modulator of Wnt and TGF-β signaling.
Materials and Methods
Rare non-hematopoietic BME cells from 16 bone biopsies (MGUS, SMM, newly diagnosed MM) were analyzed by single-cell RNA sequencing (10x Genomics Chromium). Differential expression was assessed with Scanpy. Pathway/biological process enrichment was inferred by ORA and GSVA. For *in vitro* validation, hTERT-MSCs and primary MSCs were differentiated to OBs ±conditioned media (CM) from human myeloma cell lines (HMCLs); osteogenic markers and *WISP2* expression were quantified by qPCR. Additionally, 3D hydrogel and bioprinted models of MM niche were developed by embedding MM cells (RegenHu 3D Discovery) and culturing constructs for up to 10 days.
Results
Across 42,823 BME cells, we identified 14 clusters that recapitulate stromal to osteoblast differentiation trajectories. Two pre-osteoblastic states emerged with opposite programs: (i) dysfunctional, immunosuppressive *pre-OBs* and (ii) *pre-OBs WISP2+* with a pro-osteogenic signature. Across disease stages, we observed a significant, progressive depletion of *pre-OBs WISP2+ cluster*, inversely correlated with tumor burden, and a reduction of *WISP2* expression in MM. In primary MSCs, *WISP2* was significantly upregulated in SMM-derived but not in MM-derived OBs. Exposure to HMCL-CMs suppressed *WISP2* in hTERT-derived and SMM-derived OBs, whereas MM-derived OBs displayed limited transcriptional responsiveness. Preliminary *WISP2* overexpression experiments partially attenuates CM-driven inhibition, increasing functional OB marker expression. 3D hydrogel models preserved cell viability and morphology, showing low cytotoxicity and sustained proliferation over 10 days.
Conclusions
Our single cell analysis on bone biopsies deciphers the OB populations complexity highlighting their altered dynamics through the progression from precursors diseases to MM. By integrating with our 3D niche models, we can perform a physiologically relevant platform for mechanistic studies and preclinical testing, potentially informing therapeutic strategies and early intervention.

Stem cell cartilage tissue engineering for post traumatic osteoarthritis prevention: advancing equity in regenerative medicine

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Post-traumatic osteoarthritis of the knee is a major public health burden that arises following joint injury and is driven by chronic inflammation and cartilage degeneration. Autologous therapies using stem cells hold immense promise, but their success is often limited by differences in regenerative potential between individual, raising critical challenges for consistent clinical outcomes and equitable access to effective care.

Our work centres on a cartilage repair strategy that harnesses stem cells isolated from the infrapatellar fat pad (IFP), a metabolically active knee joint tissue with strong cartilage regenerative capacity. These cells are embedded in a biodegradable GelMA hydrogel and reimplanted into cartilage defects. While we have demonstrated effective cartilage repair with this approach in preclinical animal models, variability in the biological quality of stem cells isolated from the IFP introduces uncertainty in therapeutic performance as the approach moves toward clinical translation.

To promote greater consistency and equity in treatment outcomes, we have developed an in vitro screening platform to evaluate the chondrogenic potential of stem cells isolated from the IFP prior to implantation. This platform was rigorously optimized, testing combinations of hydrogel composition, photoinitiator concentration, crosslinking conditions, and growth factor delivery (TGF-β3 and BMP6), to support robust, reproducible chondrogenesis. Validation across several patient-derived cell lines revealed consistent immunophenotypes but wide variability in proliferation and matrix production. Nevertheless, 80% of the cell lines achieved substantial cartilage formation.

To further reduce disparities in therapeutic response, we are integrating mass spectrometry-based proteomics to identify predictive biomarkers in patient-specific preparations of stem cells isolated from the IFP. These biomarkers will enable pre-implantation stratification and pave the way for a precision medicine approach, where each patient's cells are evaluated and matched to the most effective therapy strategy.

Altogether, our platform supports the development of accessible, reproducible, and personalized therapies that address the needs of diverse patient populations and reduce outcome variability in musculoskeletal care.

Programming stem cell fate with sound and electricity: piezoelectric nanomaterials for cartilage regeneration and beyond

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Stem cells offer significant promise due to their ability to differentiate into various mature cell types. However, directing this differentiation in a controlled and efficient manner remains a major bottleneck. Critical cues - mechanical, chemical, and electrical - are often missing or insufficiently understood.
This talk will explore an intriguing paradigm based on the synergy between controlled ultrasound stimulation and piezoelectric nanomaterials as a means to enhance stem cell differentiation. Nanoscale particles with piezoelectric properties can be synthesized and functionalized to be safely internalized by cells. When exposed to ultrasound - mechanical waves - they undergo deformation, generating localized electrical charges. Acting as intracellular nano-transducers, these particles provide localized stimuli that activate specific intracellular pathways, modulating cellular behavior with therapeutic potential.
We developed a piezoelectric hydrogel embedding barium titanate nanoparticles and human adipose-derived mesenchymal stromal cells. When stimulated with finely tuned ultrasound parameters, the system significantly enhanced chondrogenic differentiation and exerted strong anti-inflammatory effects. These findings were validated in two preclinical models of osteoarthritis (rabbit and sheep), supporting the technology's promise for cartilage regeneration.
A key issue in the current landscape of ultrasonic stimulation is the lack of precise control over the delivered mechanical energy. Conventional setups often fail to account for attenuation, reflection, and scattering of waves, resulting in poorly characterized stimulation conditions. This talk will emphasize the importance of accurately controlling ultrasound dosage, both in vitro via advanced setups, and in vivo, through predictive computational models of acoustic wave propagation.
Beyond cartilage, this strategy can be extended to other tissues such as skeletal muscle. Indeed, when combined with biofabrication techniques, ultrasound and piezoelectric nanoparticles enhance the expression of myogenic markers. Recent preclinical studies suggest that this platform can be harnessed not only for regenerative purposes (e.g., treating volumetric muscle loss), but also for developing regenerative peripheral nerve interfaces. By facilitating the integration of tissue-engineered muscle grafts with peripheral nerves, this approach supports advanced neural control of robotic prosthetics.

Immunomodulatory effects of human amnion-derived mesenchymal stromal cells in cirrhosis associated spontaneous bacterial peritonitis: a novel approach to antibiotic resistance

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Spontaneous bacterial peritonitis (SBP) is a severe complication in patients with decompensated cirrhosis and ascites, typically treated with broad-spectrum antibiotics. The increasing prevalence of antibiotic resistance, particularly among carbapenem-resistant Enterobacterales, limits the effectiveness of conventional therapies and underscores the need for alternative approaches. Human amnion-derived mesenchymal stromal cells (hA-MSCs) exhibit immunomodulatory and anti-inflammatory properties, suggesting potential therapeutic utility in SBP and other resistant infections.

Objective

To evaluate the effects of hA-MSCs on bacterial clearance and immune modulation in ascitic fluid from cirrhotic patients infected with carbapenem-resistant *Enterobacterales*.

Materials and Methods

In vitro experiments were conducted using ascitic fluid obtained from cirrhotic patients with refractory ascites. The impact of hA-MSCs was assessed using omics and targeted immune profiling approaches: *Next-generation sequencing* was used to analyze transcriptomic changes in hA-MSCs exposed to LPS-stimulated ascitic fluid, providing insights into immune modulation and inflammation; *Quantitative PCR (qRT-PCR)* validated these findings by quantifying key immune-related genes, such as CXCL5, CCL20, and MAPK13, involved in immune responses and macrophage polarization; *Flow cytometry* was used to assess macrophage polarization (M1/M2) and phagocytic activity of macrophages and NK cells; Complement activation was evaluated by *Luminex quantification* of C3a and ficolin-3.

Results

Treatment with hA-MSCs significantly reduced bacterial proliferation in ascitic fluid infected with carbapenem-resistant *Enterobacterales* at 24 hours. hA-MSCs promoted M2 macrophage polarization, associated with anti-inflammatory responses, while preserving phagocytic activity of macrophages and NK cells, essential for bacterial clearance. Additionally, hA-MSCs modulated immune mediators, including CXCL5, CCL20, and MAPK13, and increased C3a and ficolin-3 levels, indicating enhanced complement activation. These effects were strain-dependent.

Conclusions

hA-MSCs effectively modulate both immune responses and bacterial clearance, supporting their potential as an alternative or adjunctive strategy to antibiotics in the context of antimicrobial resistance. Further in vivo studies are required to validate these results and assess their clinical applicability in cirrhotic patients.

Glioblastoma stem cell morphotypes convey distinct cell states and clinically relevant functions

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Glioblastoma (GBM) is an aggressive brain tumor and an unmet clinical need due to its invasiveness and therapy-resistance. These features are driven by glioblastoma stem cells (GSCs), which exhibit remarkable functional heterogeneity. However, GSC transcriptional profiling alone cannot predict clinically relevant behaviors.
Here, we developed CellShape-seq, a spatial transcriptomics platform that integrates cell morphology with transcriptome.
This identified three GSC morphoclasses corresponding to distinct transcriptomic states and functions: (1) nonpolar cells show differentiation and therapy sensitivity, (2) elongated cells are invasive, and (3) multipolar cells form intercellular networks. Importantly, chemoresistance is morphoclass-specific: elongated GSCs depend on YAP/TEAD1 signaling, while multipolar GSCs rely on gap junction–mediated networks. Targeting these vulnerabilities with specific inhibitors sensitized resistant GSC morphoclasses to temozolomide (TMZ) in patient-derived organoids.
Our findings demonstrate that morphology provides critical insights into GSC behavior and establish a rationale for morphology-informed therapies to overcome resistance and improve outcomes in GBM.

Modelling CLCN7-associated osteopetrosis: iPSC/iMSC-based approach and PBMC-derived osteoclast differentiation in patient and control

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Objective
Osteopetrosis is a rare group of genetic skeletal disorders characterized by the accumulation of abnormally dense and brittle bone. The CLCN7 gene encodes ClC-7 protein, a chloride/proton antiporter, that localizes to the ruffled border of osteoclasts, fundamental in lysosomal acidification and osteoclast-mediated bone resorption.
The study aimed to develop patient-specific cellular models to investigate the molecular mechanisms of CLCN7 mutations responsible for osteopetrosis.
Materiald and Methods
We established patient-specific cellular models to investigate the impact of CLCN7 mutations and to elucidate the molecular basis of CLCN7-related osteopetrosis. Osteoclasts were generated from PBMCs, and in parallel PBMCs were reprogrammed into iPSCs, that subsequently differentiate into iMSCs.
Results
PBMCs carrying *CLCN7* mutations exhibited significantly higher expression of *RANKL, RANK,* and *CTSK* compared to controls. We obtained fully mature osteoclasts derived from PBMCs for CTRL line while the CLCN7-mutated line presents fused osteoclasts aggregates. We validated iPSCs for both populations, and iMSCs were derived from them. iMSC-derived from iPSCs exhibited the characteristic mesenchymal surface markers and retained the ability to differentiate into adipocytes, chondrocytes, and osteoblasts confirming their multipotent differentiation capacity. We analysed the master gene of each specific differentiation commitment and observed a significant reduction of PPARG in CLCN7 line while SOX9 was highly expressed, instead of its protein levels were reduced. RUNX2, SP7 and COL1A1 showed an increase in protein level, while TGBF1 levels, which is associated with an impairment of lysosomal integrity, leading to the dysregulation of the autophagic pathway, was lower.
Conclusions
In our study we generated stable cellular systems for *in vitro* modelling of osteopetrosis. CLCN7 mutations lead to a general impairment of final cellular maturation. The dysregulation cause by CLCN7-mutations is confirmed by an altered commitment of the mesenchymal stem, toward adipocytic, chondrocytes and osteogenic lineages. Our work suggests new ways to investigate *in vitro* rare diseases, without the involvement of *in vivo* models.

MicroRNA-31- engineered extracellular vesicles as a target therapy for inflammatory bowel disease

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Objective
The aim of this project is to demonstrate the role of microRNA-31-carrying extracellular vesicles (EVs) in inflammatory pathological conditions, such as Inflammatory Bowel Disease (IBD). IBD is a chronic gastrointestinal disorder characterised by impaired epithelial regeneration and barrier dysfunction. Current treatments primarily target inflammation and do not directly promote mucosal healing. MicroRNA-31 (miR31) regulates epithelial regeneration and inflammatory pathways, but its therapeutic potential in IBD remains insufficiently explored. Bone marrow mesenchymal stromal cell (BM-MSC) -derived EVs represent promising delivery vehicles due to their low immunogenicity and barrier-crossing ability.
Materials and Methods
Murine colon organoids were obtained from an already established protocol. EVs were isolated from BM-MSCs and loaded with miR31 using electroporation or passive loading. EVs were characterised by tunable resistive pulse sensing and flow cytometry. EV uptake was confirmed by confocal microscopy. Therapeutic effects were assessed *ex vivo* in an IBD-like mouse colon organoid model. Outcomes included organoid viability, metabolic activity evaluation, and surface area measurements. RT-qPCR was used to analyse the expression of inflammatory (*Tnfa, Il6st*) and regenerative (*Ccnb1, Lats2*) genes.
Results
Both loading methods successfully incorporated miR31 into EVs, with passive loading better preserving EV surface markers. miR31-loaded EVs enhanced viability, metabolic activity, and growth of IBD-like organoids. Gene expression analysis showed downregulation of inflammatory markers (*Tnfa, Il6st*) and *Lats2*, together with the upregulation of *Ccnb1*, indicating suppression of inflammation and activation of Wnt/β-catenin signalling. Although electroporation induced stronger gene modulation, passive loading achieved comparable functional outcomes with minimal impact on EV phenotype.
Conclusion
miR31-loaded BM-MSC EVs exhibit regenerative and anti-inflammatory effects in *ex vivo* IBD models, supporting their potential as a cell-free therapeutic strategy. Passive loading is the preferred approach for translational development due to its superior preservation of EV integrity.

Immune escape by cancer stem cells: mechanisms and therapeutic opportunities

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Objective
Cancer stem cells (CSCs) are a highly adaptable subpopulation of malignant cells that drive disease initiation, progression, therapy resistance, and relapse. Increasing evidence suggests that CSCs possess enhanced immune-evasive properties, representing a major barrier to durable responses to immunotherapy. While multiple mechanisms of CSC-mediated immune escape have been described, whether these mechanisms operate in the human setting and how they are coordinated and can be therapeutically targeted remain incompletely understood.
Materials and Methods
Ploidy, mutational burden, and microsatellite instability (MSI) were assessed by cytogenetic, flow-cytometric, and targeted sequencing approaches. Antigen presentation was analyzed by flow cytometry and RT-PCR. Clones with a duplicated genome were obtained using optimized approaches based on forced mitotic entry and/or limiting-dilution separation of distinct ploidy fractions. Immune interactions were evaluated using microfluidic co-cultures and humanized NSG mice reconstituted with HLA-matched PBMCs. Proteomic profiling and apoptosis were assessed by reverse-phase protein arrays and membrane premeabilization assays.
Results
CSC immune evasion was influenced by ploidy and microsatellite stability status. Near-to-diploid (D) CSCs with MSI displayed low HLA class I expression despite a high mutational burden, enabling immune escape through antigenic camouflage. Independently of microsatellite status, D CSCs were broadly resistant to perforin–granzyme B–mediated cytotoxicity, revealing a mechanism of cytoprotection against T cell-mediated killing. In contrast, induction of whole-genome duplication increased chromosomal instability, enhanced immune recognition, and restored sensitivity to cytotoxic lymphocytes. Despite resistance to T cell-mediated killing, D CSCs exhibited high apoptotic priming and a selective dependence on anti-apoptotic BCL-2 family proteins. Pharmacological targeting of these proteins selectively sensitized these subset of CSCs to immune-mediated elimination. both *in vitro* and *in vivo*.
Conclusions
These findings indicate that CSC immune evasion is sustained by coordinated mechanisms of immune camouflage and cytoprotection. Targeting apoptotic dependencies represents a rational strategy for CSC-oriented combination therapies aimed at overcoming immune resistance and preventing tumor relapse.

A humanized 3D bioprinted model for studying breast cancer bone metastasis

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Objective
Breast cancer shows a marked propensity to metastasize to bone, where tumor–stroma interactions contribute to osteolytic lesions and disease progression. This study aimed to develop a humanized, physiomimetic *in vitro* platform capable of reproducing key biological features of breast-to-bone metastasis, while enabling future vascularization and advanced microenvironmental modeling.
Materials and Methods
Breast tumor constructs were generated by 3D bioprinting Laminink 111 bioink enriched with metastatic breast cancer cells (MDA-MB-231), non-tumorigenic mammary epithelial cells (MCF-10A), and decellularized human mammary extracellular matrix to better recapitulate biochemical and mechanical tumor cues. Bone tissue was modeled using two complementary approaches: a 3D bioprinted Cellink Bone scaffold containing human mesenchymal stem cells, and a β-tricalcium phosphate/hydroxyapatite ceramic scaffold combined with fibrin, both supporting osteogenic differentiation and matrix mineralization. Tumor and bone constructs were integrated in a Transwell co-culture system to allow paracrine signaling and directional tumor cell migration.
Results
The integrated system successfully reproduced breast cancer osteotropism, as evidenced by the migration of GFP-labeled MDA-MB-231 cells toward bone constructs and the activation of tumor–bone crosstalk. Both bone models maintained osteogenic features in co-culture conditions. To further enhance model complexity, *in ovo* chorioallantoic membrane (CAM) assays were initiated to promote pre-vascularization of bone scaffolds, supporting vessel ingrowth and improved nutrient diffusion. In parallel, decellularization of neoplastic mammary tissue was performed to generate tumor-specific ECM-based bioinks.
Conclusions
This humanized and modular 3D bioprinted platform provides a reproducible tool for modeling breast cancer bone metastasis. The integration of vascularization strategies and tumor-derived extracellular matrices further enhances physiological relevance, supporting applications in metastasis research, drug screening, and translational oncology while reducing reliance on animal models.

Smooth muscle-committed *TP53*-knockout pluripotent stem cells as a novel leiomyosarcoma cell model for preclinical target discovery

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Objective: Leiomyosarcoma (LMS) is a mesenchymal tumor of smooth muscle origin and one of the most common types of soft tissue sarcoma. LMS genetic landscape is driven by *TP53* mutation, that recurs in more than 60% of patients. Leiomyosarcoma prognosis is still disappointing, since up to now no drug combination has shown relevant clinical activity in the advanced or metastatic setting. Therefore, the discovery of novel therapeutic options for LMS is an area of high unmet medical need. The aim of this project was to derive a novel leiomyosarcoma cell model recapitulating LMS molecular and lineage phenotypes to identify novel actionable pathways.
Materials and Methods: We engineered donor-derived induced pluripotent stem cells (iPSC) at the *TP53* locus using the Crispr/Cas9 system. The model was then differentiated towards smooth muscle and characterized by gene expression profiling, protein and transcript biomarker expression, *in vitro* 2D and 3D cell growth and drug response testing.
Results: *TP53*-edited cell pool was subcloned to select lines carrying either homozygous or compound heterozygous frameshift mutations (*TP53* KO clones). Lineage commitment upregulated specific mesoderm markers in both parental (iPSC WT) and *TP53* KO lines, while only *TP53* KO cells significantly downregulated p53-target genes. Differentiation towards smooth muscle showed upregulation of terminal smooth muscle markers at the transcript and protein level in iPSC WT and *TP53* KO clones. *TP53* loss promoted cell growth at early and late stages of smooth muscle differentiation ($p<0.001$), and significantly induced 3D spheroid cell growth (3.6-fold). Gene expression profiling uncovered the malignant phenotype driven by *TP53* knockout, shown by the upregulation of the molecular signature associated with sarcoma aggressiveness (CINSARC). Moreover, it identified *FBXW7* as the most significantly silenced gene after *TP53*. Analysis of the TCGA Firehose Legacy dataset confirmed that *FBXW7* is significantly downregulated in *TP53*-mutant or deleted sarcomas with respect to WT tumors. Since it is known that *FBXW7*-deficient cells are more sensitive to Tigecycline, we tested this drug in *TP53* KO LMS model showing that smooth muscle-committed *TP53* KO cells are three times more sensitive to this compound than wild-type cells.
Conclusions: This study shows that *TP53* KO LMS iPSC model is an effective tool for target discovery and that *FBXW7* pathway could be an amenable target in *TP53*-mutant LMS.

Posters

P.1

Equine osteoarthritis-related microRNA analysis in extracellular vesicles from horse mesenchymal stromal cells: a preliminary investigation

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Objective
Osteoarthritis (OA) represents the most common cause of retirement from athletic activities in horses. As current pharmacological treatments can alleviate clinical symptoms, but lack regenerative effects on damaged cartilages, extracellular vesicles (EVs) derived from mesenchymal stromal cells (MSCs) have emerged as a promising alternative. A relevant EVs' cargo is represented by microRNAs (miRNAs), significant post-transcriptional gene regulators that can fully or partially regulate mRNAs, and whose gene expression can be modulated by the surrounding extracellular microenvironment. As further research is needed to explore their role in EVs and how MSC preconditioning influences them, the aim of this study was to establish optimized protocols for miRNA extraction and RT-qPCR analysis for characterizing EVs secreted by horse MSCs, allowing the future assessment of miRNA expression changes under different extracellular environment conditions in the context of equine OA.
Materials and Methods
Previously obtained MSCs derived from bone marrow and fat tissue (3 healthy horses), were cultured under standard conditions. EVs were isolated via ultracentrifugation and characterized for size distribution and concentration through Nanoparticle Tracking Analysis. In parallel, a literature analysis was performed to identify a panel of miRNAs linked to the inflammatory response of OA. MiRNAs were therefore extracted from isolated EVs and amplified through RT-qPCR, and the expression of miRNAs selected from literature was evaluated.
Results
MSC-derived EVs were successfully isolated, with a concentration mean of 2,57 x 10^{10} particles/mL and a size mean of 139,40 nm. Extracted miRNAs showed a concentration ranging from 0,705 to 4,48 ng/µL. Moreover, nine out of the 23 miRNAs linked to OA from literature were detected through RT-qPCR.
Conclusions
This preliminary investigation demonstrates the presence of miRNAs associated with the inflammatory process of OA within extracellular vesicles derived from equine MSCs under standard culture conditions. Indeed, miRNAs were extracted and detected through RT-qPCR in all samples, thus allowing for testing the miRNA-panel across different culture conditions with the future aim of better understanding the role of EVs as mediators in the crosstalk between MSCs and target cells.

P.2

Effect of equine mesenchymal stromal/stem cell secretome on equine oocyte nuclear maturation

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This study aimed to assess the effect of conditioned medium derived from equine Wharton's jelly mesenchymal stromal/stem cells (eWJ-MSC-CM) on equine oocyte nuclear maturation. eWJ was collected from umbilical cords obtained after physiological parturition from three healthy mares. MSCs were isolated by enzymatic digestion, expanded, characterized and cryopreserved. For CM production, eWJ-MSCs were thawed, plated and expanded in DMEM/F12 with 10% FBS until 90% confluence. Cells were then washed three times with DPBS, and the culture medium was replaced with serum-free DMEM/F-12. After 6 h of incubation, the CM was collected and centrifuged to remove cellular debris. The resulting supernatant was collected as eWJ-MSC-CM. Equine cumulus-oocyte complexes (COCs) were collected by aspiration from follicles of abattoir-derived ovaries. Compact COCs were selected and randomly allocated to two IVM groups: FBS (n = 105) or CM (n = 102), using a basal DMEM/F12-based IVM medium supplemented with 10% FBS or CM, respectively. After 20 h or 26 h, COCs were denuded, stained with 10 μg/mL bisbenzimide (Hoechst 33342), and examined under an epifluorescence microscope to assess the nuclear maturation. The experiment was done in 5 replicates. Data were analysed using a binomial generalized linear model (GLM) with logit link and Wald pairwise tests (IBM SPSS Statistics 29), with significance set at $P<0.05$. Cells from all samples were adherent to plastic and exhibited a fibroblast-like morphology. Tri-lineage in vitro differentiation was successful, and RT-PCR confirmed the expression of specific surface markers (positive for CD73, CD90, and MHC-I; negative for CD45, CD34, and MHC-II).
In all samples, eWJ-MSCs remained viable and adherent after 6 h of conditioning in serum-free medium, with only a few detached cells and no evident signs of cellular distress. Both the FBS and CM groups showed significantly higher ($P < 0.05$) maturation rates after 26 h of IVM (35% and 54.9%, respectively) compared with 20 h (14.8% and 26.7%, respectively), with only a trend ($P = 0.069$) toward an improved maturation rate in the CM group at the same time point. Degeneration rates did not differ between experimental groups or across incubation times. eWJ-MSC-CM appears to be a promising alternative to FBS, although further studies are needed to evaluate its effects on embryo development.

P.3

Decellularized and lyophilized dermal scaffolds loaded with the lyosecretome of mesenchymal stem cells for skin regeneration,

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Objective
This study aimed to develop and evaluate decellularized dermal scaffolds loaded with an optimized mesenchymal stem cell (MSC) secretome to enhance wound healing by promoting cell migration, angiogenesis, and regeneration.
Materials and Methods
Decellularized dermal scaffolds were produced using detergent-based protocols and characterized for structure, composition, swelling, and cytocompatibility. An optimized MSC-secretome – obtained under serum starvation with pro-inflammatory or hypoxic preconditioning – was concentrated, lyophilized, and adsorbed onto the scaffolds. Secretome release was evaluated up to 48 h, and regenerative efficacy was assessed in vitro using fibroblast and endothelial wound-healing assays combined with automated image analysis and proteomic profiling.
Results
All decellularization protocols preserved dermal extracellular matrix architecture and high swelling capacity, while effectively removing cells. Fibroblast viability and proliferation were scaffold-dependent, with reduced performance on scaffolds obtained using sodium dodecyl sulphate, likely because it is difficult to remove this cytotoxic surfactant. MSC-secretome release from the scaffolds varied with decellularization method, showing diffusion-controlled protein release and rapid lipid release. Proteomic and functional analyses demonstrated that MSC preconditioning modulates secretome composition and wound-healing efficacy in a cell type-specific manner, enabling tailored regenerative responses.
Conclusions
This study demonstrates in vitro that decellularized dermis scaffolds can deliver MSC-secretome, with different preconditioning methods of MSCs altering secretome composition and wound-healing efficacy, supporting future in vivo testing.

P.4

Adaptive mitochondrial quality control supports oxidative stress resistance in Muse cells

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Objective
Reactive oxygen species (ROS) exert context-dependent effects on adult stem cells: transient and controlled increases support activation and repair, whereas sustained oxidative stress compromises mitochondrial function and progressively erodes regenerative capacity. Effective tissue regeneration therefore relies on the ability of stem cells to rapidly sense oxidative challenges and engage adaptive mitochondrial quality control (MQC) responses. Multilineage-differentiating stress-enduring (Muse) cells, a rare SSEA-3–positive subpopulation of mesenchymal stromal cells (MSCs), display exceptional survival in ROS-rich environments, yet the molecular mechanisms underlying this phenotype remain incompletely defined.
Materials and Methods
Here, bulk MSCs, Muse cells, and Non-Muse cells were challenged with an acute oxidative insult (300 μM H_2O_2), and their responses were examined in terms of ROS handling, mitochondrial DNA (mtDNA) damage, antioxidant and Base Excision Repair (BER) activation, and MQC dynamics, with a specific focus on the PGC-1α/NRF2 signaling pathway and its pharmacological modulation.
Results
Muse cells exhibited a markedly enhanced capacity to resolve ROS accumulation, preserve mtDNA integrity, and prevent 8-oxo-dG buildup compared with MSCs and Non-Muse counterparts. Within one hour of stress exposure, Muse cells robustly induced key antioxidant enzymes (CAT, SOD1, GPX1) alongside BER components. Concomitantly, they maintained mitochondrial membrane potential and structural integrity, preserving fusion–fission homeostasis, while MSCs and Non-Muse cells underwent pronounced mitochondrial fragmentation and persistent depolarization.
Oxidatively damaged mitochondria in Muse cells were efficiently eliminated through PINK1-dependent mitophagy and rapidly replenished by mitochondrial biogenesis, as indicated by an increased COX-I/SDH-A ratio driven by PGC-1α activity. Pharmacological inhibition of this axis significantly blunted mitochondrial renewal, with a particularly strong impact in Muse cells, highlighting their reliance on tightly coordinated mitochondrial turnover.
Conclusions
Muse cells adapt to acute oxidative stress through an integrated response that combines early antioxidant and DNA repair activation with a temporally controlled mitophagy–biogenesis program driven by the PGC-1α–NRF2 axis. This efficient "clear-and-renew" strategy preserves mitochondrial functionality and supports the unique stress endurance of Muse cells.

P.5

Design of silk fibroin-based hydrogels functionalized with extracellular matrix and mesenchymal stem cell secretome for skin bioprinting

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Objective
This study aims to formulate bioactive silk fibroin (SF)-based bioinks for 3D bioprinting applications by incorporating mesenchymal stem cell (MSC)-secretome and decellularized extracellular matrix (dECM) to direct cellular proliferation and differentiation.
Materials and Methods
SF hydrogels were prepared by increasing K^+ concentration and applying sonication to induce b-sheet formation. 35 formulations were produced using a 2-level, 3-factor central composite design. Gelation kinetics were monitored at 600 nm and analyzed using 4-parameter logistic modeling. Neural network and Random Forest models predicted gelation parameters to define optimized formulation space. Dermal dECM was obtained by pepsin digestion, followed by enzyme inactivation through pH neutralization and incubation at 60 °C, and then lyophilized. MSC-secretome was obtained from IL-1b-stimulated MSCs cultured in serum-free medium. Green fluorescent protein (GFP)-expressing fibroblasts were seeded onto SF hydrogels with or without lyophilized dECM (1% w/v) and MSC-secretome (1 mg/mL), and monitored up to 14 days using confocal microscopy. Hydrogels with varying gelation levels were printed using a BioX bioprinter with 0.4-0.5 mm nozzles, producing serpentine or grid structures.
Results
Neural network modeling identified an operational region achieving 5-10 minute gelation suitable for bioink formation. Scanning electron microscopy revealed that sonication increased porosity, with higher amplitudes producing more sponge-like structures. All SF gels were non-cytotoxic to human fibroblasts. Regarding dECM, thermal inactivation + pH neutralization eliminated pepsin cytotoxicity, with concentrations up to 3.45 mg/mL non-cytotoxic for fibroblasts, MSCs, and human umbilical vein endothelial cells. IL-1b-stimulated MSC-secretome showed enhanced wound closure activity. Confocal microscopy revealed initial surface colonization at day 4, with complete penetration throughout gel thickness by day 14. Enhanced fluorescence was observed at all depths in hydrogels supplemented with both MSC-secretome and dECM. Preliminary bioprinting tests showed that intermediately gelled hydrogels performed better than fully gelled or ungelled formulations.
Conclusions
SF hydrogels enriched with dECM and MSC-secretome create a biocompatible, tissue-mimetic environment promoting fibroblast infiltration, demonstrating promise as bioinks for skin regeneration.

P.6

Cell-specific targeting via a gmp-ready microfluidic carrier-in-carrier system of silk fibroin nanoparticles encapsulated in extracellular vesicles

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Objective
This study aims to develop a carrier-in-carrier drug delivery system combining silk fibroin nanoparticles (SFNs) with extracellular vesicles (EVs) to integrate high drug loading and controlled release with enhanced targeting and cellular uptake, while enabling scalable and GMP-compliant production.
Materials and Methods
SFNs were prepared by desolvation of FITC-labeled silk fibroin extracted from *Bombyx mori* cocoons in acetone containing curcumin as a model drug, followed by purification and freeze-drying. EVs were isolated from mesenchymal stem or mesothelioma cells cultured in serum-free medium using tangential flow filtration. Carrier-in-carrier systems were generated by sonication or by a GMP-compliant microfluidic approach at defined SFN/EV ratios and flow conditions. Systems were characterized for structure (confocal microscopy, FRET), size, stability at 4°C, drug protection at 25°C, release kinetics, and cell-specific uptake in healthy and tumor cells.
Results
Confocal microscopy and FRET analyses confirmed successful EV coating of SFNs, revealing a spherical carrier-in-carrier structure with SFNs localized at the core and EVs forming the outer shell. The system showed high colloidal stability for up to 25 days at 4°C, unlike SFNs or EVs alone. The carrier-in-carrier significantly improved curcumin stability and slowed release compared with SFNs, with diffusion-dominated kinetics and a minor contribution from case-II relaxation. EV coating conferred cell-specific uptake in vitro, depending on EV origin, and the carrier-in-carrier architecture was reproducibly scaled up using a GMP-compliant microfluidic approach.
Conclusions
The carrier-in-carrier selective targeting represents a promising strategy for precision medicine, potentially enhancing therapeutic efficacy while minimizing off-target effects and reducing systemic toxicity associated with conventional drug-delivery methods.

P.7

Mesenchymal stromal cells - derived extracellular vesicles inhibit pyroptosis and protect tissue integrity in models of bronchopulmonary dysplasia

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Objective
Bronchopulmonary dysplasia (BPD) remains the most common complication of extreme prematurity, driven by prolonged exposure to hyperoxia that triggers oxidative stress and inflammation in both lung and brain. Pyroptosis, an inflammatory form of programmed cell death mediated by inflammasome activation, Caspase-1, Gasdermin D (GSDMD), and pro-inflammatory cytokine release, has emerged as a critical contributor to tissue injury. Mesenchymal stromal cell–derived extracellular vesicles (MSC-EVs) exert potent antioxidant and anti-inflammatory effects in BPD models, yet their ability to modulate pyroptosis is poorly defined. Here, we investigated whether Good Manufacturing Practice (GMP) grade MSC-EVs can suppress pyroptosis in experimental *in vivo* and *in vitro* models of BPD.
Materials and Methods
Hyperoxia-exposed neonatal rats were injected intratracheally with MSC-EVs or PBS. Lung and brain tissues were analyzed for oxidative stress, Nuclear factor erythroid 2-related factor 2 (NRF2) signaling, and activation of the pyroptosis pathway. Inflammatory responses were profiled using a cytokine array. In parallel, human lung organoid-like composed of human lung fibroblasts and alveolar epithelial cells, were exposed to hydrogen peroxide or rotenone to induce oxidative stress and were treated with MSC-EVs or vitamin C. Mitochondrial Reactive Oxygen Species (ROS), pyroptosis-related proteins, cell viability, and IL-18 in supernatants were quantified.
Results
MSC-EV administration promoted NRF2 nuclear localization and strengthened antioxidant responses in both the lung and brain, leading to pronounced inhibition of inflammasome activity. This effect was associated with reduced Caspase-1 activation, diminished GSDMD pore formation, and lower IL-18 levels, along with a broad suppression of inflammatory mediators as revealed by cytokine array analysis. In human lung 3D model, MSC-EVs markedly alleviated mitochondrial oxidative stress, suppressed pyroptosis, decreased cytokine secretion, and enhanced cell viability.
Conclusions
Our findings identify pyroptosis as a pivotal driver of hyperoxia-induced lung and brain injury in BPD. By boosting NRF2-dependent antioxidant responses and blocking pyroptosis, MSC-EVs preserve tissue integrity and cellular survival in *in vivo* and human 3D models. These results highlight MSC-EVs as a compelling, mechanism-based therapeutic strategy to limit inflammation and protect organ development in premature infants with BPD.

P.8

KLHL14 and its X1 isoform act as central regulators of epithelial plasticity by controlling epithelial-mesenchymal transition in amniotic epithelial stem cells and hepatocarcinoma cells

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Objective
Epithelial–mesenchymal transition (EMT) is a fundamental mechanism governing cellular plasticity during development, tissue regeneration and cancer progression. KLHL14, a member of the Kelch-like E3 ubiquitin ligase family, has been associated with EMT inhibition in malignant mesothelioma; however, while its full-length form has been partially characterized, the biological function of its splice variant X1 has remained entirely unexplored at the experimental level. This study aimed to define how KLHL14 and its X1 isoform cooperatively regulate epithelial plasticity by modulating EMT in physiological and pathological epithelial contexts.
Materials and Methods
The role of KLHL14 and X1 was investigated using two complementary models: amniotic epithelial stem cells, representing a physiological EMT context, and HepG2 hepatocarcinoma cells, characterized by high endogenous KLHL14 expression. Gain- and loss-of-function approaches were employed through isoform-specific overexpression and silencing. Protein localization, interaction and functional outcomes were assessed by immunofluorescence, co-immunoprecipitation and phenotypic analyses, and validated in situ in the native amniotic membrane.
Results
KLHL14 and X1 emerged as key and non-redundant regulators of epithelial plasticity through coordinated control of E-cadherin dynamics. Full-length KLHL14 predominantly localized at adherens junctions, promoting E-cadherin turnover through degradation pathways, whereas X1 displayed an intracellular distribution consistent with enhanced de novo E-cadherin synthesis. Disruption of either isoform compromised epithelial integrity, increased cell proliferation and facilitated EMT. Notably, these effects were conserved across both amniotic epithelial stem cells and hepatocarcinoma cells, indicating a shared regulatory mechanism underlying physiological and pathological epithelial plasticity.
Conclusions
This study identifies KLHL14 and its X1 isoform as central drivers of epithelial plasticity, acting through fine-tuned and opposing regulation of EMT. By integrating mechanisms that balance epithelial stability and transition, this regulatory axis represents a pivotal determinant of cell fate in stem cell biology and cancer, with potential implications for regenerative strategies and therapeutic modulation of epithelial plasticity.

P.9

Hypoxia-driven functional modulation of canine umbilical cord–derived mesenchymal stromal cells: implications for pulmonary vascular regeneration

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Pulmonary arterial hypertension (PAH) is a progressive vascular disease characterized by endothelial dysfunction and maladaptive vascular remodeling, leading to increased right ventricular afterload. Hypoxia plays a key role in disease progression by promoting oxidative stress and endothelial injury. Mesenchymal stromal cell (MSC)-based regenerative strategies represent a promising approach to restore vascular homeostasis through paracrine and angiogenic mechanisms.
While bone marrow– and adipose tissue–derived MSCs are commonly used, their clinical applicability is limited by invasive harvesting, age-related decline in cell yield and function, and reduced proliferation. Umbilical cord–derived MSCs (UC-MSCs) represent a valuable alternative, obtained from discarded tissues without ethical concerns, with higher proliferation, differentiation capacity, expansion stability, and lower immunogenicity.
This study aimed to isolate and characterize canine UC-MSCs and to evaluate their biological responses to hypoxia in a vascular regenerative context. Cells were assessed for growth kinetics, clonogenicity, cell–cell adhesion, migration, tri-lineage differentiation capacity, and surface marker expression. Cultures were exposed to normoxia or hypoxia (5% O_2 for 6 and 24 h). Cell viability, intracellular reactive oxygen species (ROS) level, and mitochondrial membrane potential (MMP) were evaluated using MTT, H_2DCFDA, and JC-1 assays. Angiogenic potential was assessed by *in vitro* tube formation assay followed by quantitative morphometric analysis using phalloidin staining and High Content Screening (HCS). Canine UC-MSCs showed a consistent doubling time of approximately 1.5 days and expressed mesenchymal markers (CD90, CD44), while lacking hematopoietic (CD34, CD45) and MHC class II (DLA-DQA1, DLA-DRA1) markers. Cells showed clonogenicity, stable 3D spheroid formation, strong migration, and successfully differentiated into adipogenic, osteogenic, and chondrogenic lineages. Hypoxia slightly increased viability at 24 h, reduced ROS level in most samples, and induced transient mitochondrial alterations at 6 h that normalized by 24 h. Hypoxic UC-MSCs showed enhanced formation of tubular-like structures, suggesting improved angiogenic potential and a possible perivascular-like role.
Overall, canine UC-MSCs exhibit robust functional properties and adapt to hypoxia relevant to pulmonary vascular disease, supporting their potential use in regenerative strategies for PAH.

P.10

Moda project: a randomized controlled trial to evaluate the decellularized human dermis in combination with orthobiologic stimuli for the arthroscopic augmentation of massive rotator cuff tears

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Objective
The primary aim of the research was to assess the efficacy of decellularised human dermis (ADM), alone or combined with orthobiological adjuvants such as concentrated humeral bone marrow (cHBM) and subacromial bursa (SAB), in reducing the retear rate (RTR) after rotator cuff (RC) arthroscopic repair. The secondary aim was the *in vitro* biological characterisation of cells harvested from SAB and cHBM residual materials.
Materials and Methods
The M.O.D.A. project was a controlled and randomised study that involved the arthroscopic repair of large to massive lesions. After the etic committee authorization (CE AVEC 154/2023/Sper/IOR), seventy-two patients were randomised into Group A (RC repair with ADM) or Group B (RC repair with ADM enriched with autologous orthobiological stimuli). RTR, clinical and functional scores were evaluated before surgery and at 1, 3, 6 and 12 months.
CHBM and SAB were cultured to characterise mesenchymal stromal cells (MSC) by analysing CD markers expression, colony-forming units (CFUs) and trilineage differentiation. Moreover, tenogenic commitment was assessed by decorin, collagen type I and tenascin-C gene expressions.
Results
All patients have been enrolled, 42 out of 72 have completed the 12-month follow-up. Preliminary MRI evaluation showed a RTR of 5% (1/20) in Group A and 14% (3/22) in Group B with functional and clinical improvements without side effects; all patients resumed normal daily activities.
Cells isolated from SAB and cHBM demonstrated mesenchymal stromal cell characteristics. Flow cytometry analysis showed expression of MSC-associated surface markers CD73, CD90, CD44, and CD105 in both cell populations, with higher expression levels in SAB: CD73 (99.3% *vs* 96.3%), CD90 (98.0% *vs* 76.2%), CD44 (99.6% *vs* 92.4%), and CD105 (98.8% *vs* 94.3%). The clonogenic potential of SAB was found to be significantly greater than cHBM (CFU: 37,58 *vs* 16,80). Furthermore, both SAB and cHBM presented multilineage differentiation capacity, including osteogenic, chondrogenic, adipogenic, and tenogenic lineages.
Conclusions
Preliminary data at 12-month follow-up showed good clinical and functional outcomes with low RTR, no postoperative complications and ADM integrated into the native tendon. SAB and cHBM were accessible sources of autologous mesenchymal stromal cells; SAB provided a population that supports its role as a biologically active mesenchymal stromal cell niche for the rotator cuff repair.

P.11

Patient iPSC-derived cerebral organoid as a multicellular model to mimic the complexity of the central nervous system

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Objective
2D cultures have major limitations in modeling complex tissue-like environments, in terms of heterogeneity, architecture and intercellular interactions. The study aims to develop a 3D cellular model derived from patient-induced pluripotent stem cells (iPSCs) that integrates neuronal and immune components to recapitulate key features of the central nervous system, providing a physiologically relevant *in vitro* platform for studying neurodegenerative diseases (NDDs).
Materials and Methods
Amyotrophic lateral sclerosis (ALS) has been chosen as an elective model for NDDs. Skin biopsies were obtained from ALS patients and healthy controls (CTRL) for fibroblast isolation and reprogramming into iPSCs. Validated iPSCs were used to generate brain organoids (BOs) using the liquid culture method. To improve model complexity, oligodendrocytes (obtained by stimulating BOs with specific growth factors) and microglial cells (generated from matched patient-derived iPSCs) were integrated. BOs were cultured for 50, 70, 80 and 100 days, and their growth and morphology were evaluated. Immunofluorescence and gene expression analyses were performed to evaluate neuronal and glial markers.
Results
Morphometric analysis showed regular growth of both CTRL and ALS BOs over time, suggesting morphological stability. Immunofluorescence and gene expression analyses confirmed correct neuronal differentiation and spatial organization in both groups. ALS BOs exhibited increased expression of mature neuron and progenitor markers. Motor neurons and oligodendrocytes were detected. Cytofluorimetric analysis confirmed microglia differentiation from iPSCs and its effective integration into both CTRL and ALS BOs. In ALS BOs, microglia predominantly displayed an amoeboid morphology, suggesting a more activated state compared with CTRL BOs.
Conclusions
Fibroblasts from ALS patients and CTRL were successfully reprogrammed into iPSCs. Optimized strategies for BOs generation enabled differentiation into neurons, motor neurons, and oligodendrocytes. An effective protocol for microglia integration was established, yielding a 3D platform suitable for investigating neurodegenerative and neuroinflammatory mechanisms in ALS.

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P.12

A three-dimensional bioprinted colorectal cancer model based on tumor-derived extracellular matrix, apelin-13 and type I collagen to study tumor–stroma interactions

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Objective
Colorectal cancer is one of the most prevalent malignancies and is associated with high mortality, mainly due to treatment resistance and disease recurrence, processes strongly driven by the tumor microenvironment. The aim of this study was to develop a biomimetic three-dimensional model able to overcome the limitations of conventional two-dimensional cultures and to reproduce functional interactions between tumor and stromal cells within a tumor-derived extracellular matrix context.
Materials and Methods
A three-dimensional bioprinted model was developed using a laminin-based bioink containing tumor-derived decellularized extracellular matrix, Apelin-13, and type I collagen. Human colon carcinoma cells (LoVo cell line) and cancer-associated fibroblasts were co-bioprinted and cultured for up to thirty days. Cell viability and proliferation were assessed by metabolic assays, while cell migration, tissue organization, and gene expression were analyzed by histological and fluorescence imaging and quantitative PCR.
Results
The three-dimensional constructs showed a significant increase in cell viability and proliferation, with up to a thirteen-fold increase in Apelin-13 and Collagen-enriched scaffolds compared to initial values ($p<0.0001$). Apelin-13 significantly promoted directional migration and proliferation of LoVo cells, whereas type I collagen provided biomechanical support and mechanotransduction cues. Transcriptomic analysis revealed a synergistic effect of Apelin and Collagen, with significant upregulation of stemness-associated genes SOX2 and SOX9 (4.28- and 5.13-fold, respectively, at 72 h). Morphological analyses confirmed the formation of well-organized cellular clusters and preservation of extracellular matrix architecture.
Conclusions
This three-dimensional model efficiently recapitulates key tumor–microenvironment interactions, including extracellular matrix–mediated mechanotransduction and paracrine signaling between stromal and cancer cells. The integration of patient-derived extracellular matrix components and bioactive peptides provides a relevant platform to bridge the gap between conventional two-dimensional cultures and patient-relevant tumor biology, enabling mechanistic studies on tumor–stroma interactions and Apelin-13 and YAP/TAZ-dependent signaling pathways, as well as the development of personalized therapeutic strategies in colorectal tumorigenesis.

P.13

Targeting nuclear lamina defects to impair epithelial-to-mesenchymal transition and stemness in glioblastoma

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Objective
Glioblastoma, the most common high-grade glioma, is characterized by poor prognosis due to therapy resistance and tumor recurrence, largely attributed to a group of cancer cells named glioma stem cells (GSCs). In glioblastoma cells, the structure of the nuclear lamina is completely altered, and recent evidence suggests that pharmacologically-induced accumulation of prelamin A, the precursor of lamin A, reduce GSCs aggressiveness and stemness. Interestingly, cells accumulating prelamin A show a decrease of expression of N-cadherin, a key factor involved in epithelial-to-mesenchymal transition (EMT). Moreover, recent findings indicate that a peculiar form of connexin, known as GJA1-20k (Cx20), which has also been involved in cancer progression, may act as a transcriptional regulator, binding to the N-cadherin promoter. Based on these assumptions, this project aims to investigate the possible role of prelamin A on EMT in GSCs, focusing on its potential impact on the intranuclear translocation of Cx20, similarly to other transcriptional factors, thereby indirectly affecting N-cadherin transcription.
Materials and Methods
Cx20 localization is analyzed in glioblastoma cell lines compared to human astrocytes, used as control samples. Prelamin A accumulation is induced using a farnesyltransferase inhibitor in glioblastoma cell lines and in patient-derived GSCs, coltured both as monolayer and as 3D spheroids. Immunofluorescence analyses are performed to evaluate the effects of prelamin A accumulation on Cx20 intracellular distribution.
Results
In control human astrocytes, Cx20 is predominantly localized in the perinuclear region, whereas in glioblastoma cells it shows a mainly nucleoplasmic localization, consistent with a transcriptional role. Drug-induced accumulation of prelamin A causes a redistribution of Cx20 toward a perinuclear localization in both glioblastoma cells and patient-derived GSCs, resembling control human astrocytes.
Conclusions
These findings suggest a possible link between a defect in the nuclear lamina, prelamin A accumulation, and a transcriptional pathway crucial for EMT and tumor progression, Cx20/N-cadherin. This could open new avenues for therapeutic strategies targeting GSCs, the specific cell population responsible for therapy resistance and relapse.

P.14

Pathological features of mesenchymal stromal cells isolated from human diseased vascular wall: implications for translational perspectives

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Objective
The vascular wall is a specialized niche of Mesenchymal Stromal Cells (MSCs) involved in vascular homeostasis. However, clinical settings characterized by unbalanced vascular remodeling, inflammation and neointimal formation may impair the reservoir and the regenerative potential of vascular MSCs. The present study was aimed to isolate and characterize MSCs from pathological vascular tissues.
Materials and Methods
Vascular specimens were obtained from aortic tissues of 12 patients undergoing abdominal aortic aneurysm repair (AAA-MSCs) and veins of 8 chronic kidney disease (CKD) patients subjected to arteriovenous fistula procedure for hemodialysis (AVF-MSCs). MSCs were isolated by enzymatic digestion, expanded in vitro and characterized by immunophenotype and multilineage differentiation. Immunomodulatory, proliferation and migration assays were performed to investigate whether the pathological microenvironment impairs the vascular MSCs. We further explored the translational potential of the VW-MSC model by testing the efficacy of pioglitazone, a peroxisome proliferator associated receptor (PPAR-g) agonist, in the modulation of AVF-MSC pathological features.
Results
MSCs isolated from AAA and AVF tissues expressed the typical mesenchymal marker panel (CD44, CD90, CD73, CD105). AAA-MSCs exhibited a pro-inflammatory property, by stimulating the activation and proliferation of peripheral blood mononuclear cells (PBMCs). Both AAA- and AVF- MSCs were endowed with the multilineage differentiation capacity, especially toward the osteogenic commitment, consistent with the calcification process found in both the pathological settings. Furthermore, AVF-MSCs displayed high proliferation and migration rates, reflecting the typical hallmarks of the neointimal lesion. These mechanisms were significantly mitigated by pioglitazone thorough a PPAR-g -mediated mechanism. Notably, pioglitazone also modulated the osteogenic differentiation process.
Conclusions
Our findings show that the pathological microenvironment shapes the vascular MSC fate. The altered vascular setting compromises the regenerative potential of MSCs, driving them toward a pro-inflammatory, calcific and migratory phenotype that contribute to disease progression. Additionally, these patient-specific MSCs are suitable for disease modeling and drug testing aimed at restoring the regenerative properties of vascular MSCs as possible therapeutic strategy.

P.15

In vivo evaluation of an ultrasound-stimulated smart piezoelectric nanocomposite hydrogel enriched with adipose-derived mesenchymal stromal cells for osteoarthritis treatment

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Objective
Osteoarthritis (OA) is a common degenerative joint disease characterized by cartilage degeneration, subchondral bone remodelling, and synovial hypertrophy, with limited long-term therapeutic options. The primary aim of this study was the efficacy evaluation of a personalized regenerative approach using a smart hydrogel enriched with piezoelectric nanomaterials (barium titanate nanoparticles and graphene oxide nanoflakes) and autologous adipose-derived stem cells (ASCs), designed to respond to low-intensity pulsed ultrasound (LIPUS) and promote chondrogenesis in two *in vivo* models of mild OA. The secondary aim was to investigate sex-related differences in outcomes.
Material and Methods
Knee OA was induced in rabbits and sheep via anterior cruciate ligament transection and lateral meniscectomy, respectively. Male and female rabbits received bilateral intra-articular treatments (Hydrogel+ASCs+LIPUS, Hydrogel+ASCs, LIPUS, or no treatment), while in sheep the left knee was treated with Hydrogel+ASCs+LIPUS and compared with the untreated contralateral joint. ASCs were harvested from the infrascapular region in rabbits and the infrapatellar fat pad in sheep. LIPUS was applied in five sessions over ten days. Outcomes included macroscopic and histological OA scores, histomorphometry, and local inflammatory responses at 1 and 3 months in rabbits and at 3 months in sheep.
Results
Macroscopic analysis showed improved cartilage integrity in both male and female rabbits treated with Hydrogel+ASCs+LIPUS at 3 months. At 1 month, OARSI score was higher in females than males receiving Hydrogel+ASCs+LIPUS. Histomorphometric parameters did not differ significantly between groups. Knees treated with Hydrogel+ASCs+LIPUS or Hydrogel+ASCs exhibited a stronger inflammatory response in females compared to males. In sheep, Hydrogel+ASCs+LIPUS–treated joints demonstrated improved OARSI scores and reduced tenosynovitis as assessed by ultrasonography.
Conclusions
The multi-approach therapy combining the nanocomposite hydrogel loaded with ASCs and stimulated with LIPUS effectively slowed down the progression of OA in both medium- and large-sized animal models, showing particularly improved outcomes in females, and paves the way for personalized treatments for knee OA.

P.16

Designing three-dimensional scaffolds to investigate limb girdle muscular dystrophy transportin 3 related

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Objective
Limb girdle muscular dystrophies (LGMDs) are a heterogeneous group of rare inherited myopathies characterized by progressive muscle weakness and degeneration of muscle fibers. This study focuses on Limb Girdle Muscular Dystrophy D2 (LGMDD2), an autosomal dominant form caused by mutations in the transportin 3 (TNPO3) gene and characterized by muscle atrophy. We employed tissue engineering approaches to develop three-dimensional (3D) tissue-like models to study the unknown pathogenic mechanism of this disorder.
Materials and Methods
We combined immortalized human myoblasts, derived from LGMDD2 patients, with biocompatible materials and biophysical cues within two distinct 3D scaffold systems designed to recapitulate key features of native skeletal muscle. The first model consisted of a bioprinted 3D collagen hydrogel, suitable for investigating myogenesis and exploring the role of TNPO3 in disease pathogenesis. The second model was a 3D micropillar-based platform that enabled real-time monitoring of cell contractility.
Results
Gene and protein analyses revealed a dysregulation of myogenic differentiation in LGMDD2 compared with control samples, characterized by increased expression of MuRF-1 and p62, respectively markers of muscle atrophy and autophagy. These molecular alterations were supported by morphological analyses showing impaired differentiation, reduced sarcomeric striations, and α-actinin aggregates in LGMDD2 myoblasts. Functional assessment using electrical stimulation in the micropillar model demonstrated that LGMDD2 cells generated higher contractile forces than controls. However, contractile force production was irregular in both frequency and amplitude, suggesting functional defects possibly related to altered calcium release.
Conclusion
Overall, this work presents innovative 3D in vitro platforms for modeling muscular dystrophies, offering versatile, ethical, and cost-effective alternatives to conventional 2D cell cultures and animal models for preclinical studies and drug testing.

P.17

Development and validation of clinically-relevant large animal model of meniscal degeneration

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Objective
Meniscal lesions are among the most common knee injuries that frequently progress to joint degeneration, especially within the avascular white zone, where intrinsic healing is limited. Mesenchymal stromal cells (MSCs) are key players in current meniscal regenerative strategies and in meniscus tissue engineering (MTE) approaches, but their clinical translation is still limited by the lack of robust, pathophysiologically relevant large-animal models. Therefore, this study aimed to develop and validate an ovine model that replicates human-like meniscal degeneration and provides a meaningful platform to evaluate MSC-based and cell-free MTE strategies.
Materials and Methods
Three surgical procedures were performed and compared in an ovine model (n=32 stifle joints): 1) direct arthroscopic mechanical injury in the medial meniscus; 2) peripheral devascularization and denervation; and 3) a full-thickness cartilage lesion in the medial femoral condyle. Contralateral knees served as controls. After three months, macroscopic, morphological, and histological analyses were performed to quantify the degeneration processes, with particular attention to meniscal alterations and early cartilage changes that can be relevant for MSC-mediated regeneration.
Results
All the three surgeries generated distinct patterns of meniscal and cartilage degeneration. The arthroscopic group showed a gradual and progressive degenerative environment that most closely mirrored human degenerative meniscal tears and early osteoarthritic changes. This model reproduced the heterogeneous environment into which MSCs or MSC-responsive scaffolds are expected to act, including matrix disruption, early inflammatory features, and region-specific biochemical and mechanical alterations.
Conclusions
The validated ovine model offers a robust, reproducible preclinical platform that addresses a key translational bottleneck in MTE research. Its degenerative trajectory is similar to what is observed in humans, making it suitable for testing direct MSC delivery, MSC–scaffold constructs, and next-generation cell-free scaffolds designed to recruit endogenous MSCs. By providing a controlled physiopathological environment, this model could accelerate the development of MSC-based regenerative therapies and clinically relevant solutions for meniscal repair.

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Injectable and bioprintable RGD-functionalized hydrogels support chondrogenic differentiation of adipose mesenchymal stromal cells

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Objective
Articular cartilage lesions represent a major clinical challenge due to limited regenerative capacity, particularly in osteoarthritis (OA), and tissue engineering approaches combining a range of biomaterials, cells and growth factors may offer promising alternatives. Hydrogels, by mimicking the extracellular matrix (ECM), may influence cell behavior. Therefore, we aimed to evaluate whether injectable and bioprintable hydrogels contribute differently to the in vitro chondrogenic differentiation of human adipose-derived stromal cells (hASCs).
Materials and Methods
Two commercial hydrogels functionalized with arginine-glycine-aspartic acid (RGD) motifs, VG-RGD (injectable) and VINK-RGD (bioprintable) were evaluated using human adipose-derived stromal cells. Rheological and mechanical properties, printability, and cytocompatibility were assessed. Printing parameters were chosen to print 3D cell-embedded construct (Discovery 3D, RegenHu) with interconnected pores to guarantee nutrients permeability using extrusion technology. Chondrogenic differentiation was evaluated at multiple time points (day 2, 10, 28) via real-time PCR, immunohistochemistry, transmission electron microscopy (TEM) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR).
Results
ATR-FTIR showed that RGD-functionalized hydrogels exhibit the characteristic bands of sodium alginate. Rheological analysis revealed shear-thinning behavior in both VG-RGD and VINK-RGD, with stiffness range from 1.30 to 3.66 kPa, respectively. VINK-RGD exhibited superior shear-thinning behavior and structural fidelity post-printing. Both hydrogels well supported hASCs viability and did not show cytotoxic effects. VG-RGD and VINK-RGD supported the expression of key chondrogenic markers COL2A1, SOX9 and ACAN, which significantly increased over time, while COL1A1 expression decreased in VINK-RGD by day 28. ATR-FTIR confirmed the formation of collagen type II in both hydrogels, while TEM revealed more organized fibrillar collagen structures in VG-RGD.
Conclusions
VG-RGD and VINK-RGD hydrogels both effectively support chondrogenic differentiation and extracellular matrix remodeling. This study provides the first direct comparison of injectable and bioprintable RGD-modified hydrogels under identical cell and culture conditions, offering critical insights for the rationale selection of materials in cartilage tissue engineering.

P.19

Mesenchymal stem cell fate across monoclonal gammopathies: from single-cell profiling to 3D niche modeling

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Objective
In multiple myeloma (MM) and its precursor conditions (monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM)) malignant plasma cells remodel the bone microenvironment (BME) cells, mesenchymal (MSCs) and osteoblastic cells (OBs), but mechanisms remain incompletely defined. We profiled non-hematopoietic BME cells at single-cell level across disease stages and investigated WISP2, a modulator of Wnt and TGF-β signaling.
Materials and Methods
Rare non-hematopoietic BME cells from 16 bone biopsies (MGUS, SMM, newly diagnosed MM) were analyzed by single-cell RNA sequencing (10x Genomics Chromium). Differential expression was assessed with Scanpy. Pathway/biological process enrichment was inferred by ORA and GSVA. For *in vitro* validation, hTERT-MSCs and primary MSCs were differentiated to OBs ±conditioned media (CM) from human myeloma cell lines (HMCLs); osteogenic markers and *WISP2* expression were quantified by qPCR. Additionally, 3D hydrogel and bioprinted models of MM niche were developed by embedding MM cells (RegenHu 3D Discovery) and culturing constructs for up to 10 days.
Results
Across 42,823 BME cells, we identified 14 clusters that recapitulate stromal to osteoblast differentiation trajectories. Two pre-osteoblastic states emerged with opposite programs: (i) dysfunctional, immunosuppressive *pre-OBs* and (ii) *pre-OBs WISP2+* with a pro-osteogenic signature. Across disease stages, we observed a significant, progressive depletion of *pre-OBs WISP2+ cluster*, inversely correlated with tumor burden, and a reduction of *WISP2* expression in MM. In primary MSCs, *WISP2* was significantly upregulated in SMM-derived but not in MM-derived OBs. Exposure to HMCL-CMs suppressed *WISP2* in hTERT-derived and SMM-derived OBs, whereas MM-derived OBs displayed limited transcriptional responsiveness. Preliminary *WISP2* overexpression experiments partially attenuates CM-driven inhibition, increasing functional OB marker expression. 3D hydrogel models preserved cell viability and morphology, showing low cytotoxicity and sustained proliferation over 10 days.
Conclusions
Our single cell analysis on bone biopsies deciphers the OB populations complexity highlighting their altered dynamics through the progression from precursors diseases to MM. By integrating with our 3D niche models, we can perform a physiologically relevant platform for mechanistic studies and preclinical testing, potentially informing therapeutic strategies and early intervention.

P.20

Human umbilical vein endothelial cells-derived conditioned medium for the endothelial differentiation of induced pluripotent stem cells: a cost-effective human-based protocol

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Objective
The lack of adequate vascularization remains a critical bottleneck in the development of multicellular 3D structures and advanced in vitro models. Current strategies to induce endothelial differentiation rely on expensive protocols based on exogenous growth factors. To address this challenge and provide a sustainable human-based alternative, this study established an experimental protocol leveraging conditioned medium from a widely used cell line - Human Umbilical Vein Endothelial Cells (HUVECs) - to guide human Induced Pluripotent Stem Cells (hiPSCs) toward the endothelial lineage.
Materials and Methods
The protocol involves exposing hiPSCs to HUVEC-derived conditioned medium over a five-day period. Samples were analyzed at three specific timepoints (after 24h, 72h and 120h) to monitor the transition in gene and protein expression profiles. Differentiation milestones were assessed via qPCR for pluripotency (Nanog, Oct4), lineage commitment (Gata2, Sox17, Pax6), hemangioblast induction (Brachyury, VEGFR2), and advanced endothelial differentiation (CD34, Endoglin, CD31). Parallel immunofluorescence assays evaluated the protein expression of Nanog, Oct4, and Brachyury.
Results
Significant transcriptomic shifts were observed from 72h of treatment, characterized by the downregulation of pluripotency markers and the onset of mesodermal commitment, evidenced by Gata2 upregulation. At day 5, Brachyury overexpression indicated progression toward mature mesoderm. While CD31 and CD34 showed significant temporal upregulation by the final timepoint, their expression levels, when evaluated alongside Endoglin, suggested that full endothelial maturation was still ongoing. Immunofluorescence confirmed the loss of Oct4 and Nanog starting from day 1. Brachyury expression was detected as early as day 1, followed by a decrease in more differentiated cells by day 5 as they progressed toward endothelial specification. Furthermore, cells exhibited clear morphological transitions toward the “cobblestone-like” appearance typical of endothelial cells.
Conclusions
This protocol successfully initiates the differentiation of hiPSCs toward the endothelial lineage, offering a promising and accessible methodology for generating endothelial cells in vitro. By utilizing two standard commercial cell lines, we have developed a cost-effective strategy that facilitates the production of potentially autologous endothelial elements for regenerative medicine and tissue engineering.

P.21

Advanced Therapy Medicinal Products: towards cost-effective manufacturing and sustainability

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Objective
Advanced Therapy Medicinal Products (ATMPs) represent some of the most promising approaches for treating a wide range of diseases by modifying genetic material or utilizing cells to restore lost function. However, the high costs and complex regulations associated with these therapies pose significant challenges due to their scientific sophistication, rapid technological advancements, and varying regulatory frameworks across different regions.
Addressing these issues requires harmonized international regulations, closer collaboration between regulators and developers, and the adoption of innovative economic models to ensure timely and sustainable access for patients. Establishing and maintaining Good Manufacturing Practice (GMP) compliant facilities for ATMPs is particularly costly, both economically and technically. These high costs can limit the number of centres capable of developing and manufacturing such therapies.
Materials and Methods
In this study, we compared "open" and "closed" manufacturing systems to assess their efficiency in terms of cost effectiveness and sustainability.
The comparison was performed by analyzing key parameters, including direct and indirect production costs, consumption of materials and reagents, contamination risk, requirements for classified environments, staff workload, and waste generation.
Results
Open systems, while often more flexible and less expensive at start-up, are associated with higher operational risks, greater resource consumption, and increased waste production. Moreover, environmental monitoring in open systems requires substantial resources, including personnel (cleaning staff, monitoring operators, quality control staff), sanitizing agents, and microbiological testing. In contrast, closed systems require a higher initial investment but offer substantial long-term reductions in operational costs, improved quality control, and lower environmental impact.
Conclusions
Innovative technologies and new manufacturing models should be explored with the aim of improving clinical outcomes, taking in account costs and sustainability for the health system.

P.22

Ex vivo study of neural stem cells from the subventricular zone in neonatal encephalopathy

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Objective
Neonatal hypoxic-ischemic encephalopathy (HIE) is a multifactorial pathology characterized by brain damage resulting from a reduced supply of oxygenated blood to the brain during the perinatal period. HIE is one of the principal causes of death and neurological disability in children. In addition to standard supportive therapies, therapeutic hypothermia (TH) is currently the main clinical measure to improve the prognosis of children with HIE, but it is only partially effective. The current challenge is to improve outcomes by investigating neuroprotective agents that support hypothermia. Nerve Growth Factor (NGF), the first identified neurotrophin, has demonstrated neuroprotective potential in preclinical and clinical studies on neonatal HIE. This study aims to evaluate NGF as a complementary neuroprotective strategy to therapeutic hypothermia in neonatal HIE.
Materials and Methods
The present study developed an ex vivo model of neonatal HIE by evaluating the effect of hypoxic-ischemic conditions (hypoxia/ischemia; H/I) on neural stem cells (NSCs) isolated from P7 rats subjected to unilateral carotid ligation and incubation in a hypoxic chamber. After the insult, the animals were treated with TH and mouse NGF, either alone or in combination. Subsequently, 15 days after the insult, NSCs were isolated and the expression of differentiation and maturation markers of the three neural lineages (astrocytes, neurons, and oligodendrocytes) was evaluated.
Results
The ex vivo model developed, found that H/I insult causes an increase in the proliferation of neural precursors, resulting in a boost to neuronal and astrocytic differentiation 21 days after the induction of spontaneous differentiation, coupled with reduced neuron maturation. In contrast, the oligodendrocyte lineage is severely compromised, with a reduction in the percentage of mature oligodendrocytes. Furthermore, it has been shown that treatments, especially in combination, are effective in restoring differentiation deficits induced by H/I damage. Instead, single treatments are more effective in increasing the degree of maturation of differentiated neurons.
Conclusion
The results obtained suggest that NGF, used in combination with TH, may be a promising molecule for improving the prognosis of neonatal HIE by acting on the three neural lineages affected by the damage.

P.23

Comparative bioinformatic analysis of ASC and BMSC secretomes reveals shared functional programs and condition-specific enrichment patterns

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Objective
A significant proportion of the therapeutic benefits of mesenchymal stromal cells (MSCs) are derived from their secretome. However, a comprehensive description of the shared and condition-specific functional characteristics of MSC secretomes remains to be achieved. A bioinformatic analysis of a large-scale proteomic dataset has been conducted to identify a general and distinct secretome signature between adipose-derived MSCs (ASCs) and bone marrow-derived MSCs (BMSCs).
Materials and Methods
The proteomic data analysed was derived from 3,485 quantified proteins from five ASCs and five BMSCs donors. Exploratory distribution analysis was used to evaluate data integrity and possible donor effects. The globally abundant core was then subjected to over-representation analysis (ORA) to identify common biological processes. A linear model integrated within the limma framework was used to assess the variations in protein abundance between ASCs and BMSCs and the limma moderated t-statistic was employed for the ranking. Gene Set Enrichment Analysis (GSEA) was used to identify pathways that were differentially enriched between conditions across GO, KEGG, and Reactome databases.
Results
Downstream comparison studies were supported by exploratory analysis which revealed no significant donor-driven effects. ORA of the core secretome shared by MSCs showed enrichment of several biological pathways linked to extracellular organisation, signalling, metabolism, and regulatory functions. However, given the wide range of functions, it was challenging to identify a single global signature. Condition-specific proteomic patterns were confirmed by differential analyses that clearly distinguished ASCs and BMSCs using PCA and heatmap visualization. While BMSC secretomes demonstrated enrichment for extracellular matrix organisation, cell adhesion, and activation-related pathways, ASC secretomes were enriched for pathways linked to transcriptional and RNA regulatory activities, according to GSEA.
Conclusions
This comprehensive bioinformatic analysis reveals that while MSC secretomes exhibit a complex and multifunctional global proteomic landscape, the most biologically informative differences emerge at the condition-specific level, suggesting differential mechanisms of action. While experimental validation is required to fully support these findings, they provide a valuable framework for the rational and personalised use of MSC-derived secretomes in regenerative medicine.

P.24

Secretome and extracellular vesicle signatures in bone marrow-derived mesenchymal stromal cells after expansion in standard and next-generation media

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Objective
Mesenchymal stem cells (MSCs) are a promising therapeutic strategy for osteoarthritis (OA), largely due to their regenerative potential, which is attributed in part to their secretome. The secretome includes soluble factors and extracellular vesicles (EVs). Given that MSCs are sensitive to various culture conditions, this study aims to investigate the effects of different media supplemented with either fetal bovine serum (FBS) (F), platelet lysate (P), or serum/xeno-free (S/X) on the composition and therapeutic potential of the secretome from bone marrow-derived MSCs (BMSCs).
Materials and Methods
BMSCs were cultured in F, P, or S/X media, with secretomes collected after starvation. The secretomes were analyzed for soluble factors, EVs, and miRNAs. Inflammatory responses were assessed in an *in vitro* OA model using inflamed chondrocytes and gene expression was evaluated by qRT-PCR.
Results
The secretomes from all conditions exhibited a similar molecular fingerprint. Proteomic analysis identified 98 common proteins encompassing growth factors and inflammatory mediators. EVs showed similar size and phenotype, with a slight difference in CD44 expression in EVs derived from P-expanded MSCs. Despite the high overall similarity, miRNA profiling identified 13 key players, with subtle differences in the miRNA composition of EVs from FBS-expanded BMSCs. All secretomes exhibited anti-inflammatory effects, with the FBS-expanded secretome showing the most pronounced therapeutic potential.
Conclusion
The secretomes derived from different culture conditions share key molecular components. EVs may contribute to variations in therapeutic outcomes through their cargo. Optimizing MSC expansion conditions is crucial for enhancing the therapeutic potential of MSC-derived secretomes in OA treatment. Further research is needed to clarify the specific role of factors, miRNAs, and EVs in modulating OA pathology.

P.25

Selection of mesenchymal stem/stromal cell-derived secretome for osteoarticular diseases through tissue source and priming strategies

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Objective
In osteochondral pathologies, MSC-derived secretome, rich in immunomodulatory and pro-regenerative proteins and microRNAs, represents a promising cell-free therapeutic approach. However, tissue source and priming markedly shape secretome composition. This study compares secretomes from placenta-derived MSCs (PSCs) and adipose-derived MSCs (ASCs) and evaluates priming strategies to identify the most tailored secretome for osteoarticular applications.
Materials and Methods
Secretomes from ASCs and PSCs were obtained from human biological triplicates and assessed by pathway enrichment to identify signatures related to immunoregulation, extracellular matrix organization, osteogenesis, and chondrogenesis. Based on screening results, PSCs were primed with hypoxia, interferon-γ (IFNγ), or interleukin-1β (IL1β). Primed versus unprimed PSC secretomes were compared for proteomic and exosomal miRNA content to identify the optimal priming strategy.
Results
Proteomics revealed clear differences between PSC and ASC secretomes. The top upregulated proteins in PSC secretome were enriched in pathways linked to immunomodulation and osteochondral regeneration, while ASC secretome showed limited enrichment in these processes. In PSCs, cytokine priming induced stronger changes than hypoxia. IL1β priming enhanced protein signatures associated with osteochondral processes and immune regulation, whereas IFNγ priming yielded the broadest exosomal miRNA cargo and the strongest enrichment of predicted targets in osteochondral and immunoregulatory pathways.
Conclusions
PSCs provide a more disease-relevant secretome profile than ASCs for osteoarticular applications. Priming enables differential tuning: IL1β primarily boosts protein-driven osteochondral and immunomodulatory signatures, while IFNγ preferentially enriches exosomal miRNA cargo linked to regenerative and immune-regulatory pathways. These findings support PSC-derived, primed secretomes as customizable candidates for cell-free strategies in osteoarticular disease.

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P.26

New perspectives for the exploitation of female reproductive potential in mammals: generation of granulosa-like cells from human adipose mesenchymal stem cells

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Objective
Age, diet, and environmental factors, as well as metabolic and endocrine disorders, significantly impact female reproduction. For this reason, many efforts have been made to study *in vitro* oocyte production by generating primordial germ cells (PGCs). However, the correct meiotic maturation and growth of a PGC depend primarily on the stage-specific support provided by granulosa cells (GCs). This study aims to generate granulosa-like cells (GLCs) from human adipose mesenchymal stem cells-derived iPSCs (hASC-iPSCs), which share the same embryonic origin as native GCs. These starting cells can more accurately mimic the developmentally regulated differentiation path of the GC lineage than others.
Materials and Methods
Human ASC-iPSCs were previously established in our laboratory. Two protocols for GLC production published in mice were adapted for use with human cells. In the first protocol, stepwise differentiation recapitulates embryonic development: hASC-iPSCs were treated with BMP4 and FGF9 to induce epiblast; CHIR99021, BMP4, and EGF to promote intermediate mesoderm (IMM); exposure to retinoic acid, Sonic Hedgehog, PD0325901, EGF, and BMP4 induced coelomic epithelium (CE); finally, BMP4 and FGF9 generated GLCs. In the second protocol, hASC-iPSCs were cultured with vitamin C and AM580 to directly induce GLCs. Differentiation was assessed at defined time points by morphological analysis and evaluation of lineage-specific markers using real-time PCR and immunofluorescence.
Results
The first protocol successfully recapitulates embryonic developmental stages. Expression of Brachyury confirmed epiblast formation; OSR1, IMM differentiation; and GATA4, CE specification, while FOXL2, FSHR, GATA4, LGR5, and WNT4 indicated GLC differentiation. Using the second protocol, treatment with vitamin C and AM580 induced direct differentiation into GLCs, as demonstrated by the expression of the same GC markers described previously. All results were statistically significant compared to controls. In both protocols, evident morphological changes accompanied molecular differentiation.
Conclusions
The two protocols efficiently generate cells displaying a granulosa-like phenotype. These findings support the use of hASC-iPSCs as a possible source for *in vitro* production of GLCs and provide a foundation for ongoing functional studies comparing the two GLC populations in their ability to support oocyte growth and maturation following ovarian follicle reconstitution *in vitro*.

P.27

Enhanced osteoinductive and osteoconductive potential of human adipose-derived mesenchymal stromal cells on trabecular titanium compared to chromium cobalt scaffolds

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Objective
The study aimed to evaluate and compare the osteoinductive and osteoconductive potential of two metallic scaffolds, trabecular titanium and chromium cobalt alloy. Human adipose-derived mesenchymal stromal cells were used to assess adhesion, viability, osteogenic differentiation and matrix mineralization.
Materials and Methods
Scaffolds were characterized by scanning electron microscopy before and after cell seeding. Cultures were maintained for up to 28 days in either control medium or osteogenic medium. Cell morphology, proliferation and viability were assessed by microscopy and metabolic assays. Osteogenic differentiation was quantified by alkaline phosphatase activity, calcium deposition, nutrient depletion assays and gene expression of alkaline phosphatase and runt related transcription factor 2 measured by quantitative real-time polymerase chain reaction.
Results
Cells adhered and proliferated on both scaffolds with sustained viability. Trabecular titanium induced early increases in alkaline phosphatase activity and calcium deposition under control conditions and further enhanced mineralization in osteogenic medium, demonstrating both osteoinductive and osteoconductive behavior. Gene expression analysis confirmed higher alkaline phosphatase transcription in titanium cultures. Chromium cobalt supported adhesion and viability but showed minimal osteogenic differentiation, with low alkaline phosphatase activity, absence of calcium deposition and reduced expression of osteogenic transcription factors compared with titanium.
Conclusions
Trabecular titanium effectively promoted mesenchymal stromal cell commitment toward the osteogenic lineage even without exogenous stimuli, supporting both bone induction and bone conduction. Chromium cobalt lacked these properties and may exert an inhibitory influence on osteogenesis. These data support preferential clinical use of titanium scaffolds in orthopaedic applications requiring reliable bone integration and regeneration.

P.28

Effects of low-intensity pulsed ultrasound frequencies on chondrogenic differentiation of adipose-derived stromal cells embedded in hydrogels

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Objective
In cartilage tissue engineering (TE) new solutions are needed to effectively drive chondrogenic differentiation of adipose mesenchymal stromal cells (ASCs). Hydrogels are promising biomaterials capable of embedding ASCs by providing an instructive biomimetic environment which can be stimulated by low-intensity pulsed ultrasound (LIPUS) to regulate cell differentiation. Since the role of ultrasound wave frequency has been poorly explored in this context, the aim of this study was to evaluate three different dose-controlled LIPUS frequencies on the chondrogenic differentiation of ASCs in an RGD-modified hydrogel.
Materials and Methods
Human ASCs at $2*10^6$ cells/mL were embedded in a VitroGel RGD® hydrogel and stimulated with LIPUS (1, 3 and 5 MHz frequencies) every 2 days until day 10 of culture and chondrogenic differentiated for 28 days. Viability tests were conducted on day 2 and 10, while the chondrogenic extracellular matrix (ECM) formation was evaluated using specific biological (gene expression and immunohistochemistry), ultrastructural (transmission electron microscopy) and mechanical assessments on day 2, 10, and 28. Also, integrin β1 receptor blocking experiments were performed using GRGDSP to evaluate LIPUS-mediated integrin mechanotransduction effects using Western Blot analyses on day 10.
Results
In all hydrogels, LIPUS did not affect the viability of the embedded cells. From day 10 to 28, the 5 MHz frequency was the most effective treatment for increasing the typical genes and proteins of the hyaline cartilage ECM (collagen type 2, aggrecan) and for reducing the ones associated with fibrosis (collagen type 1) and hypertrophy (collagen type 10). Interestingly, electron microscopy evidenced the formation of a well-organized ECM with fibrils having the structural features of mature collagen fibers. Rheological and mechanical evaluations showed the highest compressive resistance and toughness in hydrogels treated with 5 MHz. Also, blocking experiments confirmed the direct involvement of the integrin β1 receptor and FAK/ERK pathway in the response to LIPUS stimulation.
Conclusions
Overall, among the LIPUS frequencies tested, the 5 MHz proved to be the best at increasing the chondrogenesis of human ASCs in RGD-modified hydrogels, representing a promising and non-invasive mechanical stimulation strategy for future clinical applications.

P.29

Growing bones in a dish: a new human *in vitro* model of endochondral ossification for the study of bone metastasis

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No available *in vitro* model can fully mimic bone formation, impairing our ability to study skeletal diseases and cancers with bone involvement. Metastasis to the bone are often lethal and no treatment is available. Most bones form via endochondral ossification, whereby a template of cartilage tissue is mineralised, vascularised and remodeled into bone. We previously recapitulated endochondral ossification in the tissue engineering setting by implanting mesenchymal stromal cells (MSCs)-derived cartilage in mice, which leads to bone formation. Inspired by this system, we set out to engineer a new fully human model to recapitulate endochondral ossification *in vitro*, and apply it for studies on bone metastasis.
Human MSC aggregates/pellets were cultured with TGF-β3 to induce cartilage formation and subjected to RNA-sequencing. *In vitro* cartilage mineralisation was induced by adding β-glycerophosphate. $CD14^+$-human monocytes were seeded on the pellets prior to or during mineralisation, and cultured with RANKL/M-CSF. To induce vessel formation, pellets seeded with monocytes were embedded in fibrin hydrogels containing human adipose stromal cells and umbilical vein endothelial cells. For dynamic experiments, the co-cultures were formed in the tissue chambers of a 3D-printed device with a central channel, and MDA-MB-231 metastatic breast cancer cells were perfused in the system. Cancer cells were detected by measuring bioluminescence.
RNA-sequencing of chondrogenic pellets demonstrated induction of angiogenesis- and remodeling-related genes, which may support these processes *in vitro*. $TRAP^+$-osteoclasts formed on and infiltrated both unmineralised and mineralised cartilage, as evidenced by gene expression and histological analyses. When vessel-forming cells were added to the co-cultures, confocal microscopy revealed the formation of microvascular networks, with contact between $laminin^+$-vessels with lumina and mineralised pellets. Finally, experiments performed in a fluidic device evidenced the ability of the pellet/osteoclasts/vessels co-cultures to attract MDA-MB-231 cancer cells and support their survival and proliferation *in vitro*.
We developed a new *in vitro* model which simultaneously captures cartilage formation, mineralisation, vessel formation and osteoclastogenesis. This integrated approach pushes the boundaries of the *in vitro* modeling of bone formation, laying groundwork for a range of skeletal disease modeling studies, including those on bone metastasis.

P.30

Influence of mesenchymal stem cell-derived extracellular vesicles on the injured skeletal muscle microenvironment

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Objective
Traumatic events can severely damage skeletal muscles, leading to serious consequences such as insufficient muscle regeneration, muscle loss, fibrosis and impaired muscle function. Although various therapeutic approaches, including cell transplantation, have been explored to improve muscle regeneration, so far none have produced satisfactory results. Cell therapy using mesenchymal stem cells (MSCs) shows great potential for muscle regeneration, particularly through paracrine and immunomodulatory factors released by extracellular vesicles (EVs). Although the cellular and molecular mechanisms underlying skeletal muscle regeneration are well understood, the role of EVs in intercellular communication to coordinate the repair and regeneration of damaged myofibers remains a matter of study. Furthermore, the impact of EVs released into the microenvironment by MSCs on myogenic repair and regeneration has not been thoroughly studied. Therefore, the aim of this study is to explore the role of EVs isolated from MSCs in muscle repair and regeneration.
Materials and Methods
EVs were isolated from murine myoblast culture medium using a polyethylene glycol precipitation protocol. EVs derived from myotubes, control myoblasts and MSCs were characterized for their size by dynamic light scattering, electron microscopy and membrane markers. The expression of cytokines was tested by western blotting, real time PCR and ProQuantum immunoassays. The ability to produce myotubes was assessed by inverted light microscopy, and the expression of muscle differentiation markers, such as MyoD and myogenin, was assessed by Western blotting.
Results
The results demonstrated that damaged myotubes could release inflammatory factors encapsulated in EVs and that EVs isolated from MSCs played a role in reducing the inflammatory microenvironment, promoting myogenic repair and promoting muscle regeneration.
Conclusions
In conclusion, our findings highlight the significant role of MSC-derived EVs in regulating myogenic differentiation and regeneration following muscle damage.

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P.31

Role of Glypican3 in tumor dissemination and proliferation: study of extracellular vesicles isolated from the mesenchymal rhabdomyosarcoma cells expressing glypican3

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Objective
The aim of this work is twofold: (1) to unveil the role of glypican 3 (GPC3) in pediatric rhabdomyosarcoma (RMS) dissemination and proliferation by silencing GPC3 in RMS cells using classical 2D cell culture and 3D hyaluronic acid-based hydrogel models; (2) to investigate the role of extracellular vesicles (EVs) in the transport of GPC3.
RMS is a pediatric soft tissue sarcoma of mesenchymal origin, that mainly affect muscle and it is classified into two subtypes, the embryonal (ERMS), less aggressive, and the alveolar RMS (ARMS), more metastatic. Approximately 20–25% of RMS cases have metastasis at diagnosis, particularly in the lung and bone marrow where the microenvironment (i.e., supporting cells and extracellular matrix -ECM- components) contributes significantly to the growth potential of cancer cells. RMS produce their own ECM with minimal involvement of cancer associated fibroblasts. The role of ECM in the growth and migration of RMS, as in other cancers, is becoming increasingly important. The ECM components of RMS include GPC3, a proteoglycan that is naturally turned off in healthy muscle but in RMS is abnormally present. Furthermore, GPC3 has shown to drive cell growth and inhibit cell differentiation in other tumors via cell-signalling pathways.
Materials and Methods
GPC3 was silenced in the RMS cell lines, RH30(ARMS) and RD(ERMS) which were cultured in 2D and 3D models (home-made hyaluronic hydrogel with RMS ECM proteins). The adhesion, proliferation, matrix degradation, and consequent cell motility assays were performed. Functional assays were achieved with the antineoplastic drug doxorubicin and the WNT3a inhibitor, ipafricept. EVs were isolated from RMS wild type cells and added in GPC3 silenced RMS cells in order to perform motility test.
Results
Both in 2D and in 3D model, cell motility and proliferation were significantly impaired after GPC3 silencing. When the in vivo cell-ECM interactions were mimicked in the hyaluronic acid-based hydrogel, doxorubicin and ipafricept were particularly effective against the growth of GPC3-silenced RMS cells. When EVs from RMS wild type cells were added in GPC3 silenced cells, the wound healing was significantly improved.
Conclusions
This study lay the foundation for a different therapeutic approach against pediatric RMS that aim to dysregulate the protein microenvironment not only beat the cancer cells.

P.32

Stem cell derived extracellular vesicles ameliorate the neuron mitochondrial damage induced by ROS- LPS- exposure: *in vitro* model of neuron, microglia, and astrocyte triple co-culture

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Oxidative stress can cause brain damage through neuronal apoptosis, neuroinflammation, and synaptic dysfunction, contributing to the effects of neurodegenerative and vascular diseases. Systemic acute or chronic inflammation is closely related to the increase in reactive oxygen species (ROS) and the presence of lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, plays a crucial role in neuroinflammatory and neurodegenerative mechanisms. Significantly increased oxidative stress is observed in the brains of Alzheimer disease (AD) patients, where mitochondrial damage is both a cause and a consequence of the accumulation of Aβ and hyperphosphorylated tau. Reactive oxygen species (ROS) play a central role also in brain damage during stroke, especially in the ischemia-reperfusion phase, leading to blood-brain barrier impairment. Moreover, ischemic events can occur in patients with AD. In fact, there is a link between vascular pathology and AD, especially in the elderly.
To investigate perturbations in brain cells occurring in mixed dementia (AD plus vascular dementia components), we used a model a triple culture system containing neurons, astrocytes, and microglia, which better recapitulate the human disease pathology. Thus, we induced neuronal injury combining LPS and H_2O_2 exposures.
Cell viability test showed that neuronal death occurred mainly through apoptosis and DNA damage. In neurons and astrocytes exposed to LPS/H_2O_2, the expression of NADPH oxidase isoform 2, a source of ROS, increased as well as FOXO3 and SOD2, as mitochondrial ROS scavengers. Indeed, mitochondria changed in their morphology and integrity, meanwhile neurite extension and thickness decreased.
The treatment with extracellular vesicles (EVs) derived from amniotic fluid stem cells was tested due to their rich content of antioxidant molecules. Interestingly, EVs reversed the negative effects of LPS/H_2O_2 suggesting a protective role on neuronal injury *in vitro* associated with the EV- cargo, such as SOD proteins and miR-21-5p and miR-181b.

P.33

Large-scale manufacturing of extracellular vesicles from adipose tissue derived-mesenchymal stromal cells using a bioreactor

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Objective
Mesenchymal Stromal Cell-derived Extracellular Vesicles (MSC-EV) retain the therapeutic benefits of MSC without the drawbacks associated with cell-based therapies. However, their clinical use is limited by the lack of large-scale isolation protocols. We developed a GMP-compliant standardized protocol for the large-scale preparation of MSC-EV in a bioreactor and investigated their identity in terms of number, morphology, protein content and surface antigens expression in comparison with those obtained in flasks.
Materials and Methods
Adipose tissues from 4 healthy donors were used to isolate and expand MSC both in flasks (small scale) and in the bioreactor (large scale). Culture supernatants were used to isolate EV by ultracentrifugation. EV identity was verified in terms of concentration/size by Nanoparticles Tracking Analysis (NTA), protein content by microBCA, morphology by Transmission Electron Microscopy (TEM), and expression of both EV and MSC typical surface markers by flow cytometry.
Results
Starting from $9,97\pm4,82x10^6$ MSC at 50-60% confluence, cultured in flasks, we were able to obtain $4,62\pm1,4x10^9$ total EV from 108 mL supernatants; starting from $92,94\pm39x10^6$ MSC at the same confluence cultured in a bioreactor, we were able to obtain $6,19\pm1,8x10^{10}$ total EV. Purified populations were homogeneous with sizes ranging from 186 to 211nm for EV from flasks and 189 to 248nm for EV from bioreactor. Protein content was 40,19±13,30 µg/ml for EV from flasks and 53,9±12,6 µg/ml for EV from bioreactor. EV obtained both in bioreactor and flasks showed an intact morphology, as analyzed by TEM, and high levels of tetraspanin markers CD9, CD63, CD81 and MSC markers CD105, CD49e, CD146, CD44, CD29. Specific leucocyte-and platelet-markers, as well as endothelial markers were expressed at very low levels on all the EV samples, proving the absence of platelet lysate-derived EV. Although the results were normalized to 108 mL of supernatant, the bioreactor system enabled the collection of 230 mL of supernatant within one week, highlighting its enhanced scalability, reproducibility and suitability for large-scale and clinically relevant EV production.
Conclusions
Our data demonstrate that large-scale, GMP-compliant MSC-EV preparation protocol in a bioreactor is successful and permit to obtain a high number of MSC-EV and to maintain product integrity/identity. Studies on the MSC-EV efficacy and safety, in terms of absence of microbial contaminants, are ongoing.

P.34

Advanced therapies: the sustainability challenge

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Objective
This study qualitatively evaluates the different design options and operational practices of an Advanced Therapy Medical Product (ATMP) laboratory in order to minimize environmental impact. An oriented Life Cycle Assessment (LCA) approach was adopted to compare the various guidelines with the specific context of the Laboratorio Cellule Staminali, Cell Factory e Biobanca (CF), in order to identify potential improvements tailored to our setting.
Materials and Methods
Data were collected from the literature through searches of major scientific databases, including Elsevier, MDPI, and PubMed, and were subsequently integrated with information obtained from various corporate sustainability reports. The LCA-oriented comparison considered several factors, including water and waste management, CO_2eq emissions, and energy consumption, evaluated across the different proposed options. The aspects analyzed in this study include laboratory design (open vs. closed), the usage strategy of consumable materials (washable vs. single-use) and their material origin (fossil-based vs. bio-based).
Results
The best design solution emerging as the most sustainable is the closed one, both in terms of energy consumption and installation impacts, in contrast to the traditional layout of our CF. Nevertheless, both solutions considered require a high level of energy input for HVAC operation and maintenance. In order to offset this excessive energy consumption, single-use consumables are preferred over washable ones, as they reduce autoclave use for sterilization and, consequently, the laboratory's overall energy demand. An additional strategy to enhance facility sustainability involves the use of consumables made of polypropylene derived from used cooking oil (UCO) rather than petroleum, with benefits extending beyond laboratory waste management to the materials' production supply chain.
Conclusions
One of the most challenging aspects in the field of ATMPs is sustainability, particularly in the effort to balance it with the various production requirements. This study aims to analyze the internal procedures of the CF in relation to existing guidelines, with the goal of implementing sustainable alternatives from both an ecological and an economic perspective, without compromising laboratory productivity.

P.35

The influence of standard hepatocyte medium and/or interleukin 13 on the phenotype of non-parenchymal hepatic cell lines in monoculture and co-culture *in vitro*

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Objective
The aim of this study was to evaluate whether non-parenchymal liver cells – specifically hepatic stellate cells (HSCs) and Kupffer cells (KCs) – undergo phenotypic modulation when cultured in standard hepatocyte medium (Williams' E) and/or with the addition of interleukin-13 (IL-13) in *in vitro* inflammatory model. These conditions were assessed in both monocultures and co-cultures. The research results are intended to facilitate the development of a dynamic *in vitro* model for studying liver fibrosis.
Materials and Methods
HSCs and KCs were initially cultured in specialized media in 24-well plates. In monocultures, seeding densities were 10,000 cells/cm² for KCs and 100,000 cells/cm² for HSCs. For co-cultures, cells were seeded at a 1:1 ratio with a total density of 100,000 cells/cm² using a 1:1 mixture of their respective specialized media. Upon reaching 80% confluency, the media were replaced with standard hepatocyte culture medium (Williams' E supplemented with Fetal Bovine Serum and Antibiotic-Antimycotic) containing IL-13 at concentrations of 0, 5, 10, or 50 ng/ml. After 72 hours, cells were harvested and analyzed via flow cytometry. Similar cell lines and culture conditions were also applied in a dynamic bioreactor monocultures and co-cultures, with peristaltic flow set to 180µL/min. Results were compared against baseline data from freshly thawed cells.
Results
The analysis demonstrated that the expression of key phenotypic markers – α-SMA for stellate cells and CD68 for Kupffer cells–remained stable despite the transition to Williams' E medium. The effect of medium with IL-13 on cell phenotype in both monocultures and co-cultures was also assessed.
Conclusions
These results seem to indicate that neither standard hepatocyte medium nor the addition of IL-13 (simulating an inflammatory environment) significantly affects the phenotypic stability of HSCs and KCs. As a result, this experimental setup provides a robust framework for building a multi-population in vitro model for studying liver fibrosis.

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P.36

Modelling CLCN7-associated osteopetrosis: iPSC/iMSC-based approach and PBMC-derived osteoclast differentiation in patient and control

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Objective
Osteopetrosis is a rare group of genetic skeletal disorders characterized by the accumulation of abnormally dense and brittle bone. The CLCN7 gene encodes ClC-7 protein, a chloride/proton antiporter, that localizes to the ruffled border of osteoclasts, fundamental in lysosomal acidification and osteoclast-mediated bone resorption.
The study aimed to develop patient-specific cellular models to investigate the molecular mechanisms of CLCN7 mutations responsible for osteopetrosis.
Materiald and Methods
We established patient-specific cellular models to investigate the impact of CLCN7 mutations and to elucidate the molecular basis of CLCN7-related osteopetrosis. Osteoclasts were generated from PBMCs, and in parallel PBMCs were reprogrammed into iPSCs, that subsequently differentiate into iMSCs.
Results
PBMCs carrying *CLCN7* mutations exhibited significantly higher expression of *RANKL, RANK,* and *CTSK* compared to controls. We obtained fully mature osteoclasts derived from PBMCs for CTRL line while the CLCN7-mutated line presents fused osteoclasts aggregates. We validated iPSCs for both populations, and iMSCs were derived from them. iMSC-derived from iPSCs exhibited the characteristic mesenchymal surface markers and retained the ability to differentiate into adipocytes, chondrocytes, and osteoblasts confirming their multipotent differentiation capacity. We analysed the master gene of each specific differentiation commitment and observed a significant reduction of PPARG in CLCN7 line while SOX9 was highly expressed, instead of its protein levels were reduced. RUNX2, SP7 and COL1A1 showed an increase in protein level, while TGBF1 levels, which is associated with an impairment of lysosomal integrity, leading to the dysregulation of the autophagic pathway, was lower.
Conclusions
In our study we generated stable cellular systems for *in vitro* modelling of osteopetrosis. CLCN7 mutations lead to a general impairment of final cellular maturation. The dysregulation cause by CLCN7-mutations is confirmed by an altered commitment of the mesenchymal stem, toward adipocytic, chondrocytes and osteogenic lineages. Our work suggests new ways to investigate *in vitro* rare diseases, without the involvement of *in vivo* models.

P.37

Effect of the microenvironment on lyo-secretome from mesenchymal stromal cells: extensive characterization and *in vitro* biological activity

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Objective
This study aims to act on the microenvironment to "educate" mesenchymal stem cells (MSCs) to produce a secretome with biological activity optimized to treat chronic and degenerative lung diseases. Serum starvation, inflammatory stimulus by IL-1b, and hypoxia (5% O_2) were selected as variables to modulate secretome properties.
Materials and Methods
Three lyo-secretome batches of each condition were produced from human adipose-derived MSCs. Cells were cultured at 37 °C and 5% CO_2 until sub-confluence was reached; then secretome release was induced by serum starvation (SSer), IL-1β (10 ng/ml) + serum starvation (IL-1β + SSer), or hypoxia 5% O_2 + serum starvation (Hyp 5% O_2 + SSer). For each condition, the cells were grown in serum-free medium for 48 h. Secretome was purified by tangential flow filtration using the KrosFlo® Research 2i system equipped with a 5 kDa Molecular Weight Cut-off filtration module, supplemented with cryoprotectant (mannitol 5 mg/ml), and freeze-dried, yielding lyo-secretome. During the ultrafiltration step, each batch was concentrated to 115 µg of proteins/mL.
Results
The protein content of freeze-dried EVs revealed standardized batches (22.5 ± 4.05 µg/mg of protein, n=3), while the lipid composition was higher for IL-1β + SSer (23.28 ± 8.9 µg/mg) and Hyp 5% O_2 + SSer (20.62 ± 1.9 µg/mg) than SSer (5.56 ± 4.29 µg/mg) ($p<0.05$, n=3). The particle size distribution of EVs showed that the d_{50}, d_{90}, and the mean diameter of IL-1β + SSer batches were significantly higher than Hyp 5% O_2 + SSer and SSer-EVs. The morphology and ultrastructure, as observed by Transmission Electron Microscopy, showed that EVs were preserved at the end of ultrafiltration and freeze-drying. All three conditions fully inhibited elastase activity, whereas IL-1β + SSer and SSer showed higher anti-hyaluronidase activity at higher concentrations. Proteomics revealed the most significant pathway-related protein enrichment in IL-1β + SSer-EVs and the least in Hyp 5% O_2 + SSer. At 1.5 mg/mL, Hyp 5% O_2 + SSer-EVs showed the highest alveolar epithelial cell viability (95%), followed by SSer (79%) and IL-1β + SSer (63%). SSer-EVs demonstrated superior wound healing properties and angiogenic activity in vitro.
Conclusions
Based on the results obtained, SSer-EVs appear to be the most promising condition for both ex vivo and in vivo evaluations of chronic and degenerative lung disease.

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A lung organoid model to study oxidative stress and inflammation in pulmonary diseases

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Objective
Inflammatory lung diseases driven by oxidative stress significantly contribute to global mortality. The pediatric and adult lung conditions such as Bronchopulmonary Dysplasia, Chronic Obstructive Pulmonary Disease and COVID-19 are pathological states in which reactive oxygen species (ROS) activate inflammatory pathways. Due to the limited effectiveness of current treatments, this project aims to study ROS generation in organoids with oxidative damage. The antioxidant and anti-inflammatory properties of extracellular vesicles (EVs) from bone marrow mesenchymal stem cells will be tested through the study of NF-kb and Nrf2-ARE pathways. Therefore, this model could advance understanding of oxidative stress and support new therapies for lung inflammation.
Methods
The generation of a mouse lung organoid model (mLO) derived from adult stem cells and the induction of an oxidative damage by hydrogen peroxide (H_2O_2) administration was performed. EVs were administered, and Vitamin C was used as positive control. Immunofluorescence, gene expression analysis and viability assays were performed, along with assessments of cell death and proliferation.
Results
The characterization of mLOs showed stemness markers like SFTPC and CC10 present at early passages of the organoid culture. Gene expression analysis confirmed the formation of a mixed population of airway- and alveolar-like organoids, as indicated by the expression of MUC5AC b-TUB IV, SFTPC and RAGE markers, at late timepoints. The oxidative damage was proven by viability assays, the expression of 8-OXO-dG marker and the high proliferation of cells (KI67+cells). The antioxidant NRF2 protein was markedly present after damage but significantly reduced after EVs treatment. Moreover, qPCR analysis indicated a modulation of *Tnf-a*, and *Tgf-b* gene expression in damaged organoids and a recovery after EVs administration, confirming their anti-inflammatory and anti-oxidative action.
Conclusions
This *ex vivo* mLO model could be useful both for disease development studies and as drug platform to test new therapeutic strategies for several pediatric and adult pulmonary diseases linked to oxidative stress.

P.39

Lipidomic basis of enhanced paclitaxel efficacy via MFAT in glioblastoma

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Objective
Micro-fragmented adipose tissue (MFAT) has emerged as a promising natural scaffold for drug delivery. This study explores the potential of MFAT as a platform for Paclitaxel (PTX) delivery, investigating how the MFAT-derived secretome and its lipidomic composition influence PTX antitumor efficacy against glioblastoma.
Materials and Methods
MFAT was processed from human lipoaspirates (Lipogems®) and loaded with PTX (1 mg/mL). After 72h incubation, PTX release and the secretome lipid profile were analyzed via targeted UPLC-MS/MS and untargeted UPLC-HRMS, respectively. Antitumor activity was evaluated on U87-MG glioblastoma cells (MTT assay). The modulatory roles of specific ceramides (CerEos and C16Cer) identified in the secretome were further investigated through co-treatment and pretreatment assays.
Results
MFAT effectively released biologically active PTX. Notably, MFAT/PTX-conditioned media exhibited significantly lower IC_{50} values compared to free PTX, suggesting an enhanced antitumor activity. Lipidomic analysis showed that while triglycerides were reduced in the secretome compared to native MFAT, specific ceramides were enriched. Functional assays revealed that while CerEos had minimal impact at physiological doses, C16Cer significantly potentiated PTX activity, reducing its IC_{50} and increasing U87 cell sensitivity to the drug.
Conclusions
MFAT is an effective carrier for paclitaxel, enhancing its antitumor activity compared with the free drug. PTX loading selectively modifies the MFAT secretome, enriching of specific ceramides that may influence drug delivery. While CerEos did not enhance PTX efficacy at relevant concentrations, C16Cer markedly increased PTX sensitivity. These findings support MFAT/PTX as a promising localized drug-delivery system and highlight the importance of lipid–drug interactions in advanced MFAT-based therapeutic strategies.

P.40

In vitro expansion of human adipose-derived mesenchymal stromal cells for regenerative applications reveals dynamic small-variant changes within a stable genetic background

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Objective
The clinical use of *in vitro*–expanded mesenchymal stromal cells (MSCs) requires careful evaluation of genomic stability to ensure safety prior to therapeutic application, in accordance with Good Manufacturing Practice requirements. While current assessments mainly focus on large cytogenetic alterations, small genetic variants are often overlooked. Here, we evaluated the suitability of Next Generation Sequencing (NGS) as a quality control and safety surveillance tool to monitor genomic stability at single-base resolution. Using NGS and digital PCR, we tracked somatic mutations and copy number variations in cancer-related genes of adipose-derived stromal cells (ASCs) from knee osteoarthritis patients at different stages of in vitro expansion (P0–P10), providing a more detailed assessment of genetic stability during culture.
Materials and Methods
Human ASCs from 23 osteoarthritis patients were analyzed at multiple time points from passage 1 to passage 10, with one case extended to passage 14. Targeted NGS of a panel of cancer-related genes was performed to detect somatic single-nucleotide variants and small insertions/deletions. Copy number variations were assessed across all panel regions using coverage-based NGS analysis, and digital PCR was performed specifically for the p53 gene.
Results
ASCs largely maintained overall genetic stability throughout in vitro expansion. Nevertheless, sporadic small genetic variants emerged over time, including alterations with potential pathogenic relevance or variants of uncertain significance, indicating dynamic genetic changes during culture. No copy number variations were detected at any passage.
Conclusions
These findings confirm culture duration as a critical factor influencing the accumulation of genetic alterations in mesenchymal stromal cells, highlighting the importance of minimizing *in vitro* expansion. Given the potential functional impact of small genetic variants and the high sensitivity and throughput of NGS, systematic monitoring of emerging mutations represents not only a valuable research approach but also a robust strategy for genetic surveillance. Incorporating NGS-based analyses into quality control frameworks could improve standardized assessment of genomic integrity in MSCs prior to clinical application.

P.41

Dissecting the role of amniotic epithelial and mesenchymal cells in amniotic membrane–mediated chronic wound healing

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Objective
Chronic wounds, including diabetic foot ulcers, frequently fail to progress through the normal stages of wound healing, resulting in impaired re-epithelialization. The clinical application of human amniotic membrane (AM) has proven effective in reactivating these stalled wounds. AM is composed mainly of human amniotic epithelial cells (hAECs) and human amniotic mesenchymal stromal cells (hAMSCs), but the specific contribution of each cell population to the therapeutic effects of AM remains unclear. The objective of this study was to evaluate the relative roles of hAECs and hAMSCs in key processes involved in wound repair.
Materials and Methods
The effects of hAECs and hAMSCs were compared using a panel of in vitro experimental models designed to recapitulate critical events of the wound healing process, including cell migration, angiogenic-related responses, and re-entry of quiescent cells into the cell cycle. To assess paracrine mechanisms, conditioned media were generated from both cell types using different culture strategies and tested across the experimental systems.
Results
Conditioned media derived from hAECs reproduced some of the biological effects associated with intact AM, particularly those related to cell migration and the expression of migration-associated proteins. However, these effects were markedly enhanced by hAMSC-conditioned media, which consistently surpassed the migratory response induced by AM itself. In addition, hAMSC-conditioned media promoted cell proliferation and effectively antagonized TGF-β–mediated cell cycle arrest, a characteristic feature of chronic non-healing wounds. These effects were comparable to those induced by AM and were absent in. Analysis of cellular senescence revealed a clear response following treatment with hAMSC-conditioned media or AM, whereas no significant effect was observed with hAEC-derived conditioned media. hAMSC-conditioned media test on human fibroblast produced a significant antifibrotic response, not exhibited by hAEC-conditioned media.
Conclusions
These findings indicate that the mesenchymal component of the amniotic membrane plays a predominant role in mediating the regenerative effects observed in chronic wound healing. Nevertheless, a contribution of amniotic epithelial cells cannot be excluded, particularly in biological processes not addressed in this study, such as immune modulation, endothelial regulation, or control of excessive fibrosis.

P.42

Secretome from micro-fragmented human adipose tissue modulates inflammatory activity of monocytes/macrophages via ICAM-1

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Objective
Micro-fragmented adipose tissue (MFAT) is a widely used biological product in regenerative medicine. While its clinical efficacy is well-documented, the underlying paracrine mechanisms remain to be fully elucidated. This study investigates the biological activity of the MFAT-derived secretome, focusing on its immunomodulatory potential and its impact on cellular proliferation.
Materials and Methods
Lipoaspirate samples were obtained from subcutaneous adipose tissue using the Lipogems® system and processed to generate MFAT. Fresh MFAT from six donors was cultured in serum-free DMEM for 5–6 days. The conditioned medium (secretome) was collected, centrifuged, and stored at −20°C prior to freeze-drying. U-937 monocytes were treated with donor-derived secretomes, and the expression of six adhesion molecules (VCAM, NCAM, ITGB3, ICAM-1, MCAM, and ICAM-2) was assessed by flow cytometry. Anti-inflammatory activity was assessed by measuring RANTES and MCP-1 levels following LPS stimulation by ELISA. Proliferation assays were performed on both normal and cancer cell lines to evaluate safety.
Results
Flow cytometry revealed low basal expression of NCAM, MCAM, VCAM, and ICAM-2 in U-937 cells, with no significant modulation by the secretomes. ITGB3 and VCAM showed a slight, non-significant reduction with one donor secretome. In contrast, ICAM-1 expression was strongly and consistently inhibited by all MFAT secretomes. ELISA analysis confirmed that LPS stimulation significantly increased RANTES and MCP-1 production. Secretomes displayed donor-dependent effects on RANTES secretion, with half of the samples showing no stimulation and three inducing a significant increase. Overall, mean RANTES levels were comparable to unstimulated controls and significantly lower than LPS-stimulated cells. Notably, MCP-1 production was markedly inhibited by most secretomes, with 83% of donor samples showing significant suppression. Additionally, the MFAT secretome did not affect the proliferation of either normal or cancer cells.
Conclusions
These findings demonstrate that MFAT releases bioactive factors capable of modulating inflammatory signaling pathways, supporting its therapeutic potential in inflammatory conditions. Beyond its anti-inflammatory properties, the MFAT secretome appears to be biologically safe and suggests that the clinical efficacy of MFAT in joint inflammation may be partly mediated through paracrine mechanisms.

P.43

Perinatal stem cell spheroids for cell-based therapy in type 1 diabetes: immunomodulatory and differentiation properties

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Objective
Type 1 diabetes mellitus (T1DM) is an autoimmune disease charachterized by immune-mediated β-cells disruption, leading to persistent insulin deficiency. Among the different sources of stem cells available in regenerative medicine, perinatal stem cells (PSCs) represent an attractive source of cells due to their low immunogenicity and ethical accessibility. In particular, Wharton's Jelly Mesenchymal Stem Cells (WJ-MSC) and Amniotic Epithelial Cells (AEC), have demonstrated immunomodulatory and pancreatic differentiation properties, respectively, making them suitable for cell-based therapy in TIDM. Moreover, PSCs are able to generate 3D structures in vitro, which better recapitulate the pancreatic microenvironment.
This study aims to evaluate the immunomodulatory and differentiative capacities of perinatal spheroids as potential candidates for T1DM therapy.
Materials and Methods
After isolation from the placental tissue, WJ-MSCs and AECs were aggregated in ultra-low attachment plates to generate three-dimensional spheroids. Spheroids were subsequently co-coltured with activated Pheripheral Blood Mononuclear Cells (PBMCs) to evaluate the immunomodulatory capacities of the model. After the co-culture, PBMCs were collected and analyzed by flow cytometry to evaluate proliferation, apoptosis, and activation status, as well as the distribution of T cytotoxic cells and T regulatory cells (Tregs). The differentiative capacity of AECs was evaluated using a commercial differentiation kit and the yield was evaluated through immunofluorescence staining.
Results
The perinatal spheroids exhibited significant immunomodulatory properties, reducing pro-inflammatory cell activation and promoting anti-inflammatory responses. AEC differentiated into insulin producing cells showed positivity for pancreatic markers. Spheroids formed with differentiated AECs, combined with undifferentiated WJ-MSCs successfully formed cohesive structures and demonstrated potential for insulin production.
Conclusions
Perinatal spheroids represent a promising dual approach for developing new T1DM treatment. Their ability to modulate the immune system and differentiate into insulin-producing cells may address both the symptoms and underlying causes of T1DM, offering a comprehensive cellular therapy beyond traditional insulin replacement.

P.44

Multilineage-Differentiating Stress-Enduring (MUSE) cells as an innovative *in vitro* cellular model to study mood and psychotic disorders

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Psychotic and mood disorders, such as schizophrenia (SCZ), bipolar disorder (BD) and autism, share anomalies in neurodevelopmental processes, often linked to dysfunctions in synaptic transmission. While induced pluripotent stem cells (iPSCs) have been valuable tools for studying these conditions, they present some limitations in replicating physiological environments.
Multilineage-differentiating stress-enduring (MUSE) cells represent a promising alternative in vitro model due to their endogenous origin, stress resistance, and ability to differentiate into cells from the three embryonic germ layers. In this study, we used fibroblast-derived MUSE cells, obtained via skin biopsy from healthy individuals and patients with SCZ and BD, to develop an in vitro neuronal model. Cells were differentiated through neuro-glial induction protocols, and the resulting neurons were analyzed for morphology, gene expression, protein expression, and functionality. Preliminary findings show that MUSE cells can differentiate into GABAergic, dopaminergic, and glutamatergic neurons, displaying morphological and molecular features comparable to brain neurons.
MUSE cells derived from patients showed reduced expression of stemness markers and increased levels of apoptosis and senescence compared to those from healthy subjects. Following neural differentiation, we observed alterations in neuronal morphology and gene expression in patient-derived cells.
These findings highlight the potential of MUSE cells as an innovative and physiologically relevant model for studying neuropsychiatric disorders. This approach may contribute to a better understanding of the molecular and cellular mechanisms underlying psychotic and mood disorders and support the development of future targeted therapies.

P.45

The potential of decellularized Wharton's jelly as a bioscaffold in stimulating cellular functional recovery

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Objective
The principal aims of the study are i. to create an experimental model using bioinspired scaffolds based on umbilical cord decellularized Wharton's jelly (DWJ) for application in human intervertebral disc and cartilage tissue engineering, and ii. to provide a standardized protocol for the production of high-performance DWJ-based millicylindrical scaffolds to be used to study the behavior of chondrocytes and chondrocyte-like cells such as IVD cells under controlled conditions.
Materials and Methods
We focused on A075W3, a composite hydrogel containing 0.75 % (w/v) alginate and 3% (w/v) DWJ, produced through various steps including DWJ homogenization, meshing and freeze-drying. The hydrogel was subjected to compression loading with a FlexCell system, achieving a compromise between absorbing a mechanical stimulus and maintaining structural integrity to allow subsequent evaluation of the effects on the cells combined with it. The optimized protocol involved maintaining A075W3 in culture medium for 7 days, followed by loading for 2 days (range 0–4 kPa, frequency 0.5 Hz, duty cycle 50%, time/day 30 min, twice daily with a 3-hour break). Cells expanded were then combined with A075W3 by surface seeding and monitored for viability, eATP production, expression of genes related to discogenic differentiation, maintenance of IVD homeostasis and mechanical load signaling.
Results
We demonstrated that the 3D condition, the presence of DWJ, and exposure to adequate mechanical loading promote the functional recovery of damaged IVD cells, which are viable and regain a chondrocyte-like phenotype lost during tissue degeneration. Immunohistochemical analysis showed that mechanical loading positively affects the expression of SOX9 (the master regulator of chondrogenesis), Brachyury, and Integrin 1β, only partially SIRT1, while induces a decrease in Forkhead Box O3 (FOXO3a) and its target, superoxide dismutase-2 (SOD2). The purinergic receptor P2X7 expression remained unchanged.
Conclusions
Through the development of a meticulous protocol, we obtained DWJ-based scaffolds capable of maintaining structural integrity, responsiveness to mechanical stimuli, and pro-anabolic properties over time. Understanding the quantitative relationship between mechanical loading and gene expression can contribute to a better understanding of IVD mechanobiology, with the aim of optimizing repair techniques through cellular, molecular, and tissue engineering approaches.

P.46

Characterization of human dermis-derived mesenchymal stem cells during long-term *in vitro* culture and stress conditions

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Objective
Stem cells (SCs) are widely used in regenerative medicine due to their ability to migrate to injured tissues and promote repair. Among SCs, mesenchymal stem cells (MSCs) are particularly promising due to easy isolation and low donor risk, but they undergo replicative senescence during in vitro expansion, leading to irreversible growth arrest and reduced survival and differentiation. Understanding the mechanisms of MSC senescence is therefore essential to improve therapeutic outcomes. In this context, this study aimed to investigate the molecular and physical changes of human dermis-derived MSCs (hDMSCs) during long-term culture and to compare them with cells treated with a senescence inducer.
Materials and Methods
hDMSCs were isolated from the dermis of 3 healthy donors after informed consent and were characterized before use. All investigations were performed on cells at passages (p) 4-28. In addition, p4 cells were treated for 3 hours with tert-butyl hydroperoxide (t-BHP) 50 μM, a well-known senescence inducer. Cell proliferation was assessed by bromodeoxiuridine (BrdU) assay combined with Ki-67 gene expression analysis. The expression of cell cycle and senescence markers was analyzed by real-time PCR and immunofluorescence. The senescence phenotype was further evaluated by senescence-associated β-galactosidase (SA-β-Gal) staining, as well as by cell stiffness analysis. The mechanical response of the cell membrane was investigated by atomic force microscopy (AFM) indentation experiments on young (p4-p8) and old (p24-p28) hDMSCs.
Results
Our data show a progressive increase in hDMSC senescence during long-term in vitro culture, supported by reduced proliferative capacity and altered expression of cell cycle markers (p53, p21, p16, and Cyclin D1). Senescence was further confirmed by increased β-gal+ cells, changes in γH2AX and HMGB1 expression, elevated vimentin levels, and specific alterations in the mechanical properties of cell membrane. Notably, differences in marker modulation between t-BHP-treated and long-cultured cells could suggest the involvement of distinct molecular pathways.
Conclusions
hDMSCs maintain a youthful phenotype and sustained proliferative capacity during prolonged in vitro culture. Senescence-associated changes emerge mainly at late passages, with a greater proliferative decline than in t-BHP-treated cells, highlighting fundamental differences between replicative and chemically induced senescence.

P.47

A humanized 3D bioprinted model for studying breast cancer bone metastasis

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Objective
Breast cancer shows a marked propensity to metastasize to bone, where tumor–stroma interactions contribute to osteolytic lesions and disease progression. This study aimed to develop a humanized, physiomimetic *in vitro* platform capable of reproducing key biological features of breast-to-bone metastasis, while enabling future vascularization and advanced microenvironmental modeling.
Materials and Methods
Breast tumor constructs were generated by 3D bioprinting Laminink 111 bioink enriched with metastatic breast cancer cells (MDA-MB-231), non-tumorigenic mammary epithelial cells (MCF-10A), and decellularized human mammary extracellular matrix to better recapitulate biochemical and mechanical tumor cues. Bone tissue was modeled using two complementary approaches: a 3D bioprinted Cellink Bone scaffold containing human mesenchymal stem cells, and a β-tricalcium phosphate/hydroxyapatite ceramic scaffold combined with fibrin, both supporting osteogenic differentiation and matrix mineralization. Tumor and bone constructs were integrated in a Transwell co-culture system to allow paracrine signaling and directional tumor cell migration.
Results
The integrated system successfully reproduced breast cancer osteotropism, as evidenced by the migration of GFP-labeled MDA-MB-231 cells toward bone constructs and the activation of tumor–bone crosstalk. Both bone models maintained osteogenic features in co-culture conditions. To further enhance model complexity, *in ovo* chorioallantoic membrane (CAM) assays were initiated to promote pre-vascularization of bone scaffolds, supporting vessel ingrowth and improved nutrient diffusion. In parallel, decellularization of neoplastic mammary tissue was performed to generate tumor-specific ECM-based bioinks.
Conclusions
This humanized and modular 3D bioprinted platform provides a reproducible tool for modeling breast cancer bone metastasis. The integration of vascularization strategies and tumor-derived extracellular matrices further enhances physiological relevance, supporting applications in metastasis research, drug screening, and translational oncology while reducing reliance on animal models.

P.48

When contamination is not an option: microbiological monitoring in ATMPs

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Objective
The primary objective of this study was to demonstrate the effectiveness of the microbiological contamination control strategy in the context of ATMPs, which are associated with a heightened microbiological risk due to their biological nature and complex manufacturing processes. This strategy was implemented during the manufacturing of a Phase I clinical trial for secondary progressive multiple sclerosis using neural stem cell–based products (2017–2020) at the Laboratorio Cellule Staminali, Cell Factory e Biobanca, Azienda Ospedaliera Santa Maria di Terni (CF), and proved effective in maintaining consistently low levels of microbiological contamination in critical Grade A–D cleanrooms in compliance with GMP requirements.
Materials and Methods
A retrospective evaluation of monitoring data collected at CF during the trial was performed. The microbiological monitoring program followed Eudralex Volume 4 Annex 1, classifying cleanrooms as Grades A-D. Monitoring included airborne sampling, settle plates, surface and personnel monitoring. Microorganisms were identified to species level by an external GMP laboratory using 16S rRNA gene sequencing.
Results
Between 2017 and 2020, a total of 21,829 samples were collected, including 5,569 from operators and cleanroom areas A-D. The overall positivity rate was 2.0%, ranging from 2.2% in 2017 to 1.1% in 2020, indicating consistently low microbial levels.
In relation to Grade A-D and personnel, their deviations above limits were limited, occurring in 16 of 387 positive samples (4.1%). Personnel monitoring showed 0.3% positivity; deviations were rare (8 events), mostly in 2019. *Aspergillus fumigatus* was the predominant isolate in Grade B areas, likely due to the presence of incubators creating conditions favorable to fungal growth (es. humidity). Other isolates were Gram-positive bacteria from skin or environment. No Gram-negative or high-risk microbes were found in Grade A or B, and all cell therapy batch products were tested negatively for sterility, mycoplasma and endotoxins.
Conclusions
The data support the effectiveness of an Annex 1–compliant contamination control strategy implemented at the CF in ensuring microbiological control across Grade A–D cleanrooms, as evidenced by the very low frequency of out-of-limit deviations in Grades A and B and the absence of microbiological contamination in clinical-grade stem cell products.

P.49

New players in bone anabolism by skeletogenic stem cells: the desmin/myo-inositol duo and its translational relevance

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Objective
The purpose of this study was to investigate the protein-protein interaction network or interactome involved in osteogenic activation of multipotent, mesenchymal stromal cells by the cytoskeletal filament desmin (DES), and the role of myo-inositol (MYO) in favoring this response.
Materials e Methods
The DES osteogenic interactome was based on STRING12 and STITCH5 databases whereas interactions between DES, cytoskeletal proteins, and osteogenic enzymes/trascription factors±MYO were topologically analyzed using the Gephi software. *In vitro* studies were conducted using the embryonic murine fibroblasts NIH 3T3 (courtesy of Lucio Cocco), and human primary MSCs. DES was identified by immunocytochemistry and western blotting, using rabbit polyclonal and mouse monoclonal Abs (1:50-1:800), Fast Red-Alkaline Phosphatase (AP), ABC/DAB, and chemiluminescence, respectively. Osteogenesis (2.5-100x10^3cc/monolayer) was studied between 48h-28 days, MYO administered in a 320-640 μM range, and mineralization evaluated with calcium spectrophotomery. An *in vivo* study was done using MYO (3 gr/day x 3 months) in a human case of tibial osteomielitis, and grey level densitometry on X-ray films. Differences between control and treated samples (cells and tibial lesion) were considered significant if $p<0.05$.
Results
Topological analysis of the DES interactome predicted that the DES signal may reach the enzyme AKT via VIM, VINC, β-actin, ROCK-Rhoa-SMAD, and Hspb1 (Hsp27). AKT acts as an "hub" and "bottleneck" for the DES signal *en route* to RUNX2 and AP, and links to GSK3β. GSK3β interacts with YAP/TAZ, and is accessed by MYO through AKT-mTOR. The DES signal may reach RUNX2/AP also through Gal1-SPARC. Consistently, both mouse and human osteoprogenitors were positive for DES (83-85% of cells), gave rise to osteoid nodules rich in DES and, when NIH 3T3 cells were challenged with MYO significantly increased both DES and mineral deposition. Finally, *in vivo* MYO administration significanlty increased bone density in the lesioned, distal human tibia.
Conclusions
DES represents a new and early signal to trigger differentiation of mammalian skeletogenic stem cells, and involves the AKT-GSK3β complex. Since activation of AKT by MYO inhibits GSK3β, and GSK3β deactivates DES through phosphorylation, we believe that the observed *in vitro* and *in vivo* bone anabolic actions of MYO occur by preserving DES in a functional dephosphorylated state. Grants PRIN PNRR P20224TAETP, P2022H74YP.

P.50

Inflammatory priming activates the healing ability of adipose-derived stem cells in osteoarthritis

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Objective
Osteoarthritis (OA) is a chronic joint disease characterized by cartilage degradation and inflammation, where macrophages play a pivotal role in pathogenesis. Intra-articular injection of adipose-derived stem cells (ASCs) is a promising therapy, but the therapeutic efficacy may require improvement. This study aims to evaluate whether priming strategies (inflammatory or hypoxic) can "unlock" the immunomodulatory and regenerative potential of ASCs to better counteract the OA environment.
Materials and Methods
We isolated human ASCs and co-cultured them with macrophages from healthy donors to test their effect on macrophage polarization. To identify the most effective activation stimulus for enhancing their therapeutic efficacy in the OA environment, ASCs were exposed to three different priming conditions: hypoxia (1% O_2), interferon γ (IFNγ), or interleukin-1β (IL-1β). The molecular changes were characterized by using a multi-omics approach: mass spectrometry for the secretome, TaqMan Arrays for exosomal miRNAs, and Next-Generation Sequencing (NGS) for gene expression.
Results
Under basal conditions, ASCs did not induce an anti-inflammatory phenotype in macrophages. However, IL-1β priming, unlike hypoxia or IFNγ, triggered a massive proteomic and transcriptomic remodeling. Specifically, IL-1β- upregulated proteins related to immune regulation (chemotaxis) and cartilage matrix organization. The exosome cargo is changed by this priming, enriching specific classes of miRNAs. These molecules are predicted to target and downregulate inflammatory signals, while supporting pathways involved in matrix remodeling.
Conclusions
ASCs require specific signals to become active because they are not inherently anti-inflammatory. IL-1β priming promotes both inflammation resolution and tissue repair. This study suggests that priming could be a key strategy to develop more effective, tailored treatments for OA.

P.51

Good manufacturing practice-compliant process optimization for the production of safe and effective cartilage cells to treat diffuse cartilage lesions

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Objective
The main purpose of this study was to develop a robust method for obtaining cartilage cells enriched in progenitors under Good Manufacturing Practice (GMP) conditions in order to treat diffuse cartilage lesions. To achieve this, a low-density culture system supplemented with human platelet lysate (hPL) was employed.
Materials and Methods
Cartilage cell production under GMP-compliant conditions was performed, and risks associated with the production process were evaluated and identified. The study evaluated two distinct types of hPL: an in-house produced lysate (hPL A), qualified for use in GMP-compliant cell production (hPL A) and a commercial available alternative (hPL B). A comparison between their protein composition and biological activity was performed through proteomic profiling. Knee cartilage cells were characterized for proliferation, surface marker expression, karyotype stability, and used for the spheroid production. The obtained spheroids were characterized for cellularity and matrix production.
Results
The study identified suitable clinical-grade reagents for the production process and defined corrective measures for the risky phases. Proteomic analysis showed substantial differences for the supplements, in particular hPL A was enriched in extracellular matrix (ECM)-related proteins, while hPL B contained a higher abundance of cytoskeletal and immune-associated proteins. Cells cultured at low density in hPL B showed a significant increase in CD146 expression compared to those in hPL A. High proliferation, CD166 expression, a low immunogenicity profile and a stable karyotype were observed in cells cultured in both types of supplements. Spheroids cultured in hPL A showed lower cell density, higher glycosaminoglycan production and lower type I collagen synthesis in comparison with spheroids cultured in hPL B, while type II collagen was produced equally in both culture conditions.
Conclusion
The study successfully identified reagents and procedures for GMP-compliant production of cartilage cells. Although distinct hPL protein profiles influenced spheroid matrix properties, both supplements ensured an enrichment in progenitor cells. This allows the production of a large number of cartilage cells meeting safety limits and suitable for the treatment of diffuse cartilage lesions.

P.52

Side-to-side characterisation of soluble factors, cellular content and *in vitro* potential on chondrocytes for MSCs based adipose-derived stromal vascular fraction and bone marrow aspirate concentrate

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Objective
Orthobiologics are increasingly used for musculoskeletal disorders, including osteoarthritis (OA). Bone marrow aspirate concentrate (BMAC) and adipose-derived stromal vascular fraction (SVF) have been reported to reduce OA symptoms and contribute to joint homeostasis due to their mesenchymal stromal cells (MSCs) content. However, variability in production protocols and limited characterisation hinder clear indications for choosing one product over the other. This study aimed to characterise BMAC and SVF side by side, obtained using the same family of devices, by assessing cell immunophenotype, soluble factor release, and anti-inflammatory activity in a pathological *in vitro* chondrocyte model.
Materials and Methods
BMAC (iliac crest) and SVF (abdominal liposuction) were obtained from 28 (55 ± 8 years) and 39 patients (56 ± 9 years), respectively, using Hy-Tissue BMAC and Hy-Tissue SVF systems. Cellular composition was analysed, and MSCs were quantified by flow cytometry ($CD45^-CD31^+CD34^+CD90^+CD105^{-/low}CD146^-$ for adipose-derived MSCs; $CD45^-CD271^+$ for bone marrow-derived MSCs). Two hundred soluble factors (cytokines, chemokines, receptors, growth factors, and inflammatory molecules) were assessed by enzyme-linked immunosorbent assay (ELISA). Anti-inflammatory effects were evaluated in interleukin-1 beta (IL-1β)-stimulated chondrocytes using quantitative reverse transcription polymerase chain reaction (qRT-PCR) arrays targeting 84 inflammation-related genes.
Results
BMAC showed higher concentrations of white blood cells (213×), erythrocytes (49×), and platelets (25×), while MSC numbers were comparable between products (~1000 cells/mL). A total of 121 soluble factors were detected in all samples, with 88 more abundant in BMAC and one in SVF. Gene ontology analysis indicated that the most concentrated molecules were mainly growth factors and/or involved in differentiation processes. Both orthobiologics reduced inflammation in the *in vitro* chondrocyte model, with BMAC showing greater efficacy.
Conclusions
Using specific commercial systems, both orthobiologics demonstrated anti-inflammatory effects *in vitro*. BMAC exhibited higher blood cell and growth factor content than SVF, associated with greater efficacy. However, variability among commercial devices limits generalisability, and further studies are required when different systems are used.

P.53

Culture-driven selection of SSEA3^{+} pluripotent MUSE-like cells from human mesenchymal stem cells

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Mesenchymal stem cells (MSCs) are widely used in regenerative medicine for their strong differentiation potential, yet their quality is strongly influenced by culture conditions. Although embryonic stem cells (ESCs) remain the reference standard for pluripotency, MSCs share several functional traits with them, suggesting a possible functional hierarchy. While standard media often rely on fetal bovine serum (FBS), Human Plasma provides a more natural environment. This study examines whether plasma-based media enriched with FGF-2 or Platelet Lysate (PL) can drive the selection and maintenance of the pluripotent SSEA-3+ MUSE-like subpopulation, preserving a reservoir of primitive cells within the adult bone marrow. MSCs derived from femoral bone marrow were cultured in media containing 5% plasma and 1% PL (P+PL) or 5% plasma and FGF-2 (1ng/ml) (P+F). At passage 1 and 3, both populations were analysed for their clonogenic potential (CFU-f assay), cell proliferation (doublings), OCT4, SOX-2 and BMP-2 gene expression (qPCR) as stem cell and lineage commitment markers, respectively, MSC surface markers, and cell membrane presence of Stage-Specific Embryonic Antigen 3 (SSEA-3) (pluripotency marker) by flow cytometry.MSCs cultured in the P+PL medium induced a highly proliferative phenotype characterized by enhanced osteogenic and adipogenic differentiation, resulting in greater matrix and lipid deposition compared to P+FGF. In contrast, P+F maintained cells in a quiescent, slow-cycling state. Crucially, this condition proved superior in preserving stemness, as shown by higher expression of OCT4 and SOX-2. Most notably, flow cytometry revealed that P+F actively supports the persistence of the SSEA-3+ MUSE-like subpopulation, which was significantly reduced in P+PL. The identity of these cells was confirmed by cell sorting: the isolated SSEA-3+ fraction demonstrated the unique capacity to form pluripotent-like clusters in suspension, validating the role of the Plasma/FGF-2 environment in selecting this primitive reservoir.MSC quality is strongly influenced by culture conditions. In the presence of PL, MSCs undergo a differentiation pathway, while FGF-2 is crucial in maintaining the cells in a stage closer to the one of true ESCs. Our data confirm that this specific physiological environment is required to select and preserve the SSEA-3+ MUSE-like subpopulation, identifying the optimal strategy to obtain high-potency progenitors for regenerative medicine.

P.54

Induced chondrocyte-derived small extracellular vesicles as a cell-free regenerative strategy for osteoarthritis

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Objective
Osteoarthritis (OA) is a degenerative joint disease with progressive cartilage loss and chronic inflammation. Current treatments are mainly symptomatic, highlighting the need for regenerative approaches. In previous studies, small extracellular vesicles (sEVs) from induced mesenchymal stromal cells (iMSCs) showed anti-inflammatory effects in vitro, supporting their potential as cell-free therapeutics. Building on this, we investigated sEVs from induced chondrocytes (iCHOs), hypothesizing that their chondrogenic origin enhances cartilage-specific and anti-inflammatory activity.
Materials and Methods
sEVs were isolated from conditioned media of induced chondrocytes in chemically defined, serum-free conditions using differential ultracentrifugation. Preparations were quantified using Nanoparticle Tracking Analysis to determine particle size and concentration, and bicinchoninic acid assay to estimate total protein content, providing complementary measures of vesicle abundance. Vesicle identity was confirmed by Western blot for CD63, CD81, flotillin and syntenin. Biological activity was evaluated in an in vitro OA model based on primary human chondrocytes stimulated with interleukin-1α, and modulation of pro-inflammatory mediators was assessed by quantitative gene expression analysis.
Results
iCHO-derived sEVs displayed a size distribution of 50–150 nm and expressed canonical markers. Preliminary results indicate moderate but consistent anti-inflammatory activity in IL-1α-stimulated chondrocytes, with downregulation of IL-6, IL-8 and COX-2. These effects were more pronounced than those previously observed with iMSC-derived sEVs.
Conclusions
iCHO-sEVs demonstate significant anti-inflammatory effects in vitro, supporting their potential as cell-free, cartilage-targeted therapeutic agents. Their enhanced efficacy may reflect the closer phenotypic resemblance of iCHO to native articular chondrocytes and the absence of limitations associated with in vitro culture expansion. Collectively, these findings suggest that iCHO-derived sEVs carry a more cartilage-specific molecular cargo, enabling more precise modulation of inflammatory signalling pathways. Ongoing studies using 3D OA models aim to further validate and translate their biological activity, while parallel investigations address antioxidant properties and modulation of reactive oxygen species-driven responses.

P.55

Mesenchymal stem cells shape vascularization and mineralization in the osteosarcoma tumor microenvironment

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Background and Objectives
The tumor microenvironment (TME) is a complex and dynamic ecosystem. In osteosarcoma (OS) it is composed of cellular and acellular components, including mesenchymal stem cells (MSCs), vasculature, extracellular matrix, and mineral components such as hydroxyapatite. Rather than acting as passive bystanders, MSCs actively influence tumor growth, and therapeutic response. To investigate the contribution of MSCs to the OS TME, we employed a three-dimensional multicellular model combining OS cells and MSCs implanted onto the chick chorioallantoic membrane (CAM). Using this approach, we evaluated how MSCs influence angiogenesis and mineral niche formation within the OS TME. A deeper understanding of MSC role within the TME is therefore essential for elucidating OS biology and identifying more effective, targeted, and durable treatments.
Materials and Methods
Multicellular co-coltures were generated by assembly of the OS cell lines SaOS-2 alone or in combination with MSCs. Cells were implanted onto the CAM on day 8 of embryonal development (ED). Tumor tissues were harvested on ED 14, formalin-fixed, and paraffin-embedded. Synchrotron-radiation phase-contrast micro-computed tomography (SRμCT) was performed on the entire sample volume at a pixels size of 0.9x0.9 μm^2, followed by histological analyses to validate tissue architecture and mineralization.
Results
SaOS-2 cells efficiently infiltrated the vascularized mesodermal layer, resulting in robust host-derived vascularization of the tumor tissue. SRμCT enabled the three-dimensional visualization of tumor organization and mineral distribution at submicron resolution. Tomographic data were correlated with histological analyses, validating mineralized regions and tissue architecture at the cellular level. Tumors generated from SaOS-2 cells alone showed limited and spatially disorganized calcium deposition. In contrast, tumors formed by SaOS-2 cells co-cultured with MSCs exhibited structured mineralized tissue, which was readily detected by SRμCT and confirmed by Alizarin Red histological staining.
Conclusions
These findings highlight the critical role of MSCs in shaping the OS tumor microenvironment, particularly with respect to mineralization and vascularization. MSC-containing 3D CAM models represent a powerful platform for studying stromal–tumor interactions under in vivo–like conditions and may support the development of more targeted therapeutic strategies for OS.

P.56

Rejuvenation of amniotic epithelial cells by epigenetic and antioxidant modulation

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Objective
Human amniotic epithelial cells (hAECs) are perinatal stem cells obtained from the innermost layer of the term placenta. They have recently gained attention in regenerative medicine due to their numerous advantages including minimal ethical concerns, non-tumorigenic properties and low immunogenicity making them safer and more suitable for clinical applications than embryonic stem cells and induced pluripotent stem cells. However, a major limitation for their clinical translation is the rapid decline in viability and proliferative potential after a few passages in culture. For this reason, the present study aims to evaluate the rejuvenating effect of epigenetic and antioxidant compounds.
Materials and Methods
hAECs have been isolated from term placenta and frozen to induce further stress. After thawing, cells were seeded at the second passage and treated with combinations of the epigenetic modulator valproic acid and the antioxidants L-carnitine, N-acetylcysteine and 2-Phospho-L-Ascorbic acid. Based on the analysis of single doses and paired compound combinations, three different combinations were identified, referred to as doses A, B, and C. Proliferation rate, metabolic activity, oxidative stress, senescence, apoptosis and expression of pluripotent markers were evaluated after a 72 hours treatment.
Results
None of the three formulations exhibited cytotoxic effects on hAECs. Notably, combination C demonstrated the most pronounced biological effects, enhancing cellular responsiveness to oxidative stimuli and significantly increasing the expression of pluripotency-associated markers across all donors. Apoptosis and necrosis levels remained largely unchanged, showing with minor donor-dependent variability. Furthermore, combination C also reduced the senescence rate, a hallmark of hAEC cultures at passage 2.
Conclusions
The combined use of epigenetic modulation and antioxidant supplementation represents a promising strategy to improve the functional stability and regenerative potential of hAECs in vitro. These findings warrant further investigation of this rejuvenation approach to enhance differentiation competence. Moreover, testing the early incorporation of these compounds during cell isolation and expansion may extend the clinical applicability of perinatal epithelial stem cells.

P.57

Pro-differentiation conditions reduce pathogenic features of lung cancer cells *in vitro*

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Objective
Among strategies investigated to reduce the aggressiveness, chemoresistance and metastatic potential of tumour cells, approaches based on differentiation have shown promising results in the case of leukaemia and in some other cancer models, offering a possible route to enhancing current anti-cancer therapies. Here, non-small cell lung cancer (NSCLC) cells were treated with pro-differentiation medium to measure the effect on pathogenic parameters.
Materials and Methods
The human NSCLC line A549 was exposed to a serum-containing medium supplemented with pro-differentiation factors (Differentiation Medium, 'DM'), and effects on the cells' proliferation, migration and adhesion properties were assessed in vitro, alongside cancer stem cell marker expression analysed after treatment in 2D or 3D culture conditions.
Results
NSCLC cells (A549) treated with DM exhibited notable morphological changes, with significant increase in cellular footprint and intracellular vesicle accumulation. These phenotypic alterations coincided with significant inhibition of cell proliferation and migration, whereas cell adhesion properties increased, as did alkaline phosphatase activity. DM treatment also caused a significant reduction in clonogenic ability, in anchorage-independent colony formation and spheroid growth, alongside a reduced expression of stemness markers Sox2, Nanog, CD44 and ABCG2, and a decrease in ALDH activity and aquaporin function.
Conclusions
These observations indicate a significant decrease in NSCLC cells' pathogenic features after DM exposure, suggesting that pro-differentiation treatment may represent a valuable option for further preclinical testing.

P.58

MatRad-OIDS: an open-source tool for segmenting microscopy 3D images and extracting IBSI-compliant radiomic features.

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Objective
Radiomic analysis of 3D multicellular cultures, like spheroids and organoids, is emerging as a powerful tool for quantitative characterization of complex cellular models, including cultures derived from mesenchymal stem cells. The list of features that can be extracted from 3D images, alongside their specific mathematical equations, has been validated since 2016 by the *International Image Biomarker Standardisation Initiative* (IBSI) commission to ensure comparability between different analysis. The aim of this work is to create user-friendly, open-source software for radiomic analysis of 3D cell culture images tailored to support both experts and non-experts' users in the whole analysis framework, from segmentation to classification models following the IBSI standard.
Materials and Methods
MatRad-OIDS is a MATLAB-based, open-source tool created to analyse z-stack images of 3D cell cultures acquired with a confocal or light-sheet fluorescence microscope. The software allows to visualize TIF/TIFF multilayer images, to segment them with manual, semi-automatic and automatic methods, extract and reduce IBSI-compliant features and train classification models based on the features extracted from the 3D cultures.
Results
MatRad-OIDS has been validated on two different datasets of microscopy images composed, respectively, of 3D spheroids and mesenchymal single cells z-stacks. Both the segmentation performances and the extracted features' compliance with the IBSI initiative have been tested. The results showed high goodness in the segmentation (with an average JI of 0.8 ± 01) and complete conformity with the IBSI standard. The output of the analysis is automatically saved in a designated folder where the segmentation masks, the reduced and extracted features, the classification models and the user parameters are stored to guarantee complete repeatability of the radiomic workflows.
Conclusions
MatRad-OIDS offers a comprehensive and easily accessible platform for radiomic analysis of 3D multicellular cultures of different types, improving the integration between acquisition, segmentation, feature extraction and classification. *MatRad-OIDS MATLAB* source code, standalone *Windows*, *MacOS* and *Linux* applications, video tutorial, user documentation, and sample datasets are publicly available at: *https://github.com/research-giovap/MatRad-OIDS/*.

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Isolation of mesenchymal stromal cells derived from human arteries: a focus on their regenerative features and cell to cell communication

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Objective
The main aim of this study was to isolate, characterize and explore cell communication of Mesenchymal Stromal Cells (MSCs) derived from human vascular wall of arteries (hVW-MSCs) in 2D method and in 3D cell culture environment as spheroids. MSCs were discovered in multiple human source, including arterial wall. Among the peculiar characteristics of MSC as self-renewal and differentiative potential in different cell types, hVW-MSCs possess a singular cell communication system.
Materials and Methods
Resident MSCs were enzymatically isolated from cryopreserved arteries, including postmortem vascular tissues stored for at least 5 years, and characterized with phenotypic, morphological, molecular and metabolomic approaches. To generate spheroids, hVW-MSCs were cultured using low attachment plates or hanging drops methods. Cell communications were investigated in both cell culture approaches using light microscopy, as well as Transmission and Scanning Electron Microscopy (TEM and SEM).
Results
hVW-MSCs isolated from different arteries showed a fibroblast-like morphology with bipolar cytoplasmic extensions, expressing mesenchymal (CD44, CD90 and CD105) markers and differentiative potential in multiple cell lineages (adipo-, osteo-, and chondro-cytes) under appropriate stimuli. When cultured in 3D, hVW-MSCs spontaneously aggregated in floating spheroids, exhibiting mesenchymal markers, stemness properties and a quiescent status for the low percentage of cells expressing ki-67 proliferation marker and a slowed metabolism for the increased accumulation of intermediate metabolites. In 2D cell culture approach, SEM analysis demonstrated the presence of a 3D complex and articulated network of micro- and nano-tubular connections. It is formed by extremely thin and very long sinusoidal cytoplasmic projections used by hVW-MSCs to connect specific cells at exceedingly long distance consenting long-range cell to cell communication. TEM highlighted the presence of mitochondria and actin microfilament collections inside these microtubular projections. Similar trend was observed also in spheroids derived from hVW-MSCs.
Conclusions
These data proved that hVW-MSCs derived from vascular wall arterial segments could represent promising candidates in the field of regenerative medicine. The dynamic cell-cell interactions, even at considerable distances through a tubular network, represent a fascinating issue to further investigate for future translational applications.

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MicroRNA-31- engineered extracellular vesicles as a target therapy for inflammatory bowel disease

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Objective
The aim of this project is to demonstrate the role of microRNA-31-carrying extracellular vesicles (EVs) in inflammatory pathological conditions, such as Inflammatory Bowel Disease (IBD). IBD is a chronic gastrointestinal disorder characterised by impaired epithelial regeneration and barrier dysfunction. Current treatments primarily target inflammation and do not directly promote mucosal healing. MicroRNA-31 (miR31) regulates epithelial regeneration and inflammatory pathways, but its therapeutic potential in IBD remains insufficiently explored. Bone marrow mesenchymal stromal cell (BM-MSC) -derived EVs represent promising delivery vehicles due to their low immunogenicity and barrier-crossing ability.
Materials and Methods
Murine colon organoids were obtained from an already established protocol. EVs were isolated from BM-MSCs and loaded with miR31 using electroporation or passive loading. EVs were characterised by tunable resistive pulse sensing and flow cytometry. EV uptake was confirmed by confocal microscopy. Therapeutic effects were assessed *ex vivo* in an IBD-like mouse colon organoid model. Outcomes included organoid viability, metabolic activity evaluation, and surface area measurements. RT-qPCR was used to analyse the expression of inflammatory (*Tnfa, Il6st*) and regenerative (*Ccnb1, Lats2*) genes.
Results
Both loading methods successfully incorporated miR31 into EVs, with passive loading better preserving EV surface markers. miR31-loaded EVs enhanced viability, metabolic activity, and growth of IBD-like organoids. Gene expression analysis showed downregulation of inflammatory markers (*Tnfa, Il6st*) and *Lats2*, together with the upregulation of *Ccnb1*, indicating suppression of inflammation and activation of Wnt/β-catenin signalling. Although electroporation induced stronger gene modulation, passive loading achieved comparable functional outcomes with minimal impact on EV phenotype.
Conclusion
miR31-loaded BM-MSC EVs exhibit regenerative and anti-inflammatory effects in *ex vivo* IBD models, supporting their potential as a cell-free therapeutic strategy. Passive loading is the preferred approach for translational development due to its superior preservation of EV integrity.

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Celector label-free microfluidic technology enables quality control and mesenchymal enrichment of fetal membrane stem cell subpopulations

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Mesenchymal stem cells (MSCs) from fetal membranes (FM-MSCs) represent promising ATMP candidates due to their regenerative and immunomodulatory properties. However, their clinical translation is hindered by cellular heterogeneity, donor variability, and lack of standardised quality control (QC) for freshly isolated cells.
We employed the Celector system, based on patented NEEGA-DF technology in dual-mode operation, for label-free analysis and sorting of FM-MSCs based on size, density, and membrane complexity. This time-resolved macrofluidic system generates chromatograph profiles (fractograms) serving as QC readouts while enabling physical separation of subpopulations without altering native cell properties. Freshly isolated FM-MSCs were analyzed and fractionated into three eluting populations: F1 (1:30-3:00 min; dead cells/debris/aggregates), F2 (3:00-4:45 min; mixed), and F3 (4:45-13:00 min; single cells). Celector QC identified poor-quality samples (cells eluting <4 min) with low adhesion and culture failure, achieving 45% isolation success prediction.
Immunofluorescence revealed F3 enrichment in mesenchymal identity: Vimentin$^+$ cells increased (F1: 46% → F3: 83%), while epithelial Pan-Cytokeratin$^+$ cells decreased (F1: 47% → F3: 31%). Classical MSC markers showed universal CD73 expression (100%) across fractions, with donor-specific variation CD44/CD34/SSEA4. Notably, CD90 expression varied: one donor showed F1→F3 enrichment (55→100%), another F2>F3 (80→56%), revealing sample-specific mesenchymal maturation patterns. Proliferation assessment (Ki67, p1) confirmed F3 superiority (25% vs ~5% in F1/F2), correlating with culture success. During culture expansion, epithelial cells were selectively lost in early passages, yielding mesenchymal-pure populations.
Celector provides rapid QC for FM-MSC production, predicting culture outcomes and enabling label-free enrichment of subpopulations. F3 fractions yield mesenchymal-pure, proliferative cells suitable for ATMP manufacturing. Donor heterogeneity underscores the need for personalized QC, with Celector addressing this critical bottleneck in clinical-grade FM-MSC production.

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