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IPLASS and CTESS Joint International Conference:

New Horizons in Cell and Tissue Engineering and Perinatal Derivatives

Book of abstracts

September 19-20, 2024
Izola, Slovenia

Editors: Mateja Erdani Kreft, Samo Hudoklin

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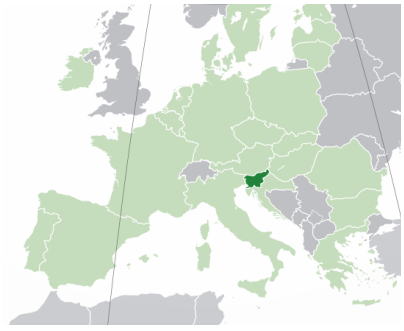

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THURSDAY, September 19, 2024

8:00-8:50 Registration and poster set-up

8:50-9:00 **OFFICIAL WELCOME AND OPENING** from

Congress President (Mateja Erdani Kreft)
President of the International Placenta Stem Cell Society (Antonietta Silini)
President of Cell and Tissue Engineering Society of Slovenia (Matej Drobnič)

SESSION 1: **Elucidating the mechanisms of action of perinatal derivatives**
Chairs: *Fabio Marongiu, Sveva Bollini*

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9:50-10:15 **Francisco J. Nicolas** (Spain) – Assessing the contribution of the different populations of AM-cells to the AM wound-healing beneficial effect

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THURSDAY, September 19, 2024

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14:20-14:45 **Fabio Marongiu** (Italy) – Investigating the Potential Application of the Amniotic Membrane and Amniotic Epithelial Cells in Ophthalmology: a focus on Retinal Regeneration

14:45-15:10 **Jessica Schiavi-Tritz** (France) – Bioproduction of secretome by Wharton Jelly Mesenchymal Stromal Cells – a mechanobiological approach

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16:40-16:55 **Enzo Calarco** (Italy, Sweden) – Intact cells or secretome in support of immune system re-education, tissue regeneration, or oncological treatment?

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9:45-10:10 **Ana Flores** (Spain) – Role of perinatal mesenchymal mesenchymal stromal cells of the decidua in the repair of pelvic floor tissue damage

10:10-10:35 **Džihan Abazović** and **Dušan Marić** (Serbia) – Advanced Strategies in Cell Therapy for Cartilage Repair: Innovations and Clinical Applications

10:35-11:00 **Coffee break and poster viewing**

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11:25-11:50 **Matej Drobnič** (Slovenia) – Restoration of articular cartilage by using the tissue engineering procedures

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Dear Colleagues,

On behalf of the International Placenta Stem Cell Society (IPLASS) and the Cell and Tissue Engineering Society of Slovenia (CTESS), we are delighted to extend a warm welcome to all participants attending the upcoming international congress in Izola, Slovenia.

We look forward to bringing together global experts in the fields of placenta research, stem cell research, cell therapy and tissue engineering. This meeting promises to be an enriching experience, providing a platform for the exchange of knowledge, ideas and innovations that will shape the future of regenerative medicine.

Our collective efforts aim to foster collaboration among academic researchers, clinicians, and industry professionals, facilitate interdisciplinary discussions and drive advances that will lead to breakthroughs in healthcare. Together we will explore the latest developments, share best practises and forge partnerships across borders and disciplines.

We look forward to our shared journey towards a future of wellbeing through science and collaboration.

With Cordial Regards,

Mateja Erdani Kreft, Congress President

Antonietta R. Silini, IPLASS President

Matej Drobnič, CTESS President

Unlocking the Potential of Amniotic Epithelial Cells: Past Achievements and Future Prospects

Toshio Miki*

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This presentation provides a comprehensive overview of our research on amniotic epithelial cells, sharing both our past achievements and ongoing projects.

Our work has demonstrated the immense potential of amniotic epithelial cells for clinical applications, underpinned by their stem cell characteristics, non-tumorigenic nature, immunotolerance, and remarkable immunomodulatory properties. Notably, we've showcased their ability to differentiate into functional cells with metabolic functions, such as hepatocytes, and have validated their therapeutic efficacy in animal models of congenital metabolic disorders.

More recently, our research has unveiled two novel facets of amniotic epithelial cells. First, we've discovered their role in exosome secretion and their involvement in liver fibrosis. Second, we are investigating their capacity for intercellular mitochondrial horizontal transfer.

Furthermore, employing cutting-edge single-cell RNA sequencing analysis through bioinformatics, we've unveiled the previously elusive potential of amniotic epithelial cells to virtually replicate the placental function during pregnancy, a groundbreaking revelation.

By sharing these updates, I hope to accelerate the progress of the amniotic epithelial cell research.

Placental macrophages – their ontogeny and function

Naomi McGovern*

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The human placenta is a major organ that regulates the health of both the mother and fetus during pregnancy. Macrophages, termed Hofbauer cells (HBC) are the major immune cells found in a healthy human placenta. Our previous work has shown that these macrophages are generated from placenta-resident erytho-myeloid progenitors, independently of a monocyte intermediate. Ongoing work in the lab has sought to further understand the functional role of these macrophages in health and disease.

Listeria monocytogenes is a bacterium that can be found in foods such as deli meats, soft cheeses, and certain raw vegetables. It is major transplacental pathogen; infection during pregnancy can lead to miscarriage, stillbirth or life-threatening infections in the newborn.

Through the development of Hofbauer cell culture assays in our lab, we provide new insight into how these macrophages act to protect the placenta from *Listeria monocytogenes* infection, at this important barrier site.

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Immune regulatory and modulatory effects offered by intact hAEC and soluble mediators

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Despite outstanding progress in pharmacological treatments, host immune response to cellular therapies remains a challenge that could fundamentally impede clinical adoption and desirable outcomes. Systemic immunosuppression through immunosuppressant drugs is routinely used to reduce rejection. However, systemic immunosuppression is undesirable due to increased risks of infections, cancers, and organ damage. The convergence of bioengineering innovations with advances in immunology has substantially expanded the landscape of cellular therapies. Immunosuppressive agents are administered to target both innate and adaptive immune cells, key to immune rejection. Immunotherapy is a novel approach to the treatment of diseases by manipulating a patient's immune system. A modulatory and reeducative approach not preventing immune response against infections. Cellular therapies are poised to transform the field of medicine not only by restoring dysfunctional tissues or reversing chronic conditions.

Different somatic and perinatal cell products have been implemented to treat various diseases in a dynamic manner not achievable by conventional pharmaceutics. Beyond the differentiation ability and stemness properties of the plethora of cell, approaches developed and described during the past 30 years, multi-functional and multi-modal cells have been extracted from full-term human placentae. Perinatal cells like the embryo-derived amnion epithelial cells (AEC) are axiomatic to provide an immuno-modulatory and re-educative effect on adult and competent immune systems.

We intend to offer a glimpse, an overview of the recent results and evidence in such a constitutive capacity that these perinatal cells can provide. We will review current research and reported observations supporting immunomodulatory strategies embedded in human AEC, capable of mitigating immune rejection and promoting immune tolerance, even long after pregnancy is over and in heterotopic contexts. Like other, widely described cells (e.g., MSC), human AEC have been described as capable of modulating rather than suppressing the xenogenic/allogenic immune system. Such multipotent perinatal cells secrete cytokines, chemokines, and growth factors responsible for tuning inflammation and immune responses.

Further, critical considerations for the translation of cellular therapies include reproducibility, large-scale production, and standardization and quality control protocols. Ex vivo, immuno-specific potency assays are under development and standardization to validate cell batches before implantation or infusion.

Mechanisms of action of hAM in addressing cystitis and bladder cancer

Mateja Erdani Kreft*

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Keywords: human amniotic membrane (hAM), antimicrobial properties, biofilm formation, interstitial cystitis, bladder cancer, biomimetic *in vitro* models

Bacterial cystitis, interstitial cystitis/bladder pain syndrome (IC/BPS), and bladder cancer represent a major clinical challenge due to the high recurrence rate and complex treatment requirements. Antimicrobial-resistant bacteria complicate the treatment of bacterial cystitis, IC/BPS remains difficult to treat, and bladder cancer is costly and resistant to therapy. The human amniotic membrane (hAM) offers promise due to its antimicrobial, anti-inflammatory, and anticancer properties.

Various hAM preparations demonstrated broad-spectrum antimicrobial activity against uropathogenic strains, including *E. coli (UPEC), S. aureus, S. saprophyticus, P. mirabilis, K. pneumoniae, Morganella morganii, Providencia rettgeri*, and *Enterobacter spp*., though not against *Serratia marcescens*. However, hAM patches exhibited limited antimicrobial activity [1]. hAM homogenate also reduced biofilm formation in three out of four UPEC strains tested, indicating a potential benefit in the treatment of bacterial cystitis.
In IC/BPS, TNFα-stimulated urothelial cells recapitulate key pathological features, validating them as an *in vitro* model for understanding IC/BPS. TNFα-upregulated genes, such as *SAA*, complement component *C3*, and *IFNGR1*, were identified as potential therapeutic targets. Treatment of urothelial cells with cannabidiol (CBD) significantly decreased TNFα-upregulated mRNA and protein expression of IL1α, IL8, CXCL1, and CXCL10, as well as attenuated NFκB phosphorylation [2]. In addition, CBD decreased TNFα-induced ROS formation by upregulating Nrf2 and antioxidant enzymes, including superoxide dismutase 1 and 2, and heme oxygenase 1. Further studies on the role of hAM in inflammation and oxidative stress in IC/BPS are therefore warranted [3].
In bladder cancer, hAM significantly impaired cancer cell migration and altered cell morphology by targeting proteins involved in cytoskeletal reorganisation without affecting normal urothelial cells. hAM homogenate inhibited the expression of the epithelial-mesenchymal transition markers N-cadherin and MMP-2 in bladder cancer cells while increasing TIMP-2 secretion. hAM also downregulated focal adhesion kinase (FAK), cortactin, and small RhoGTPases involved in actin reorganization. In muscle-invasive bladder cancer cells, hAM suppressed the PI3K/Akt/mTOR signalling pathway, a key driver of cancer progression [4]. Ongoing transcriptomic analyses show significant changes in gene expression profiles in both *in vitro* bladder cancer cells and in a preclinical mouse model.

By using biomimetic models of normal, non-invasive papilloma and muscle-invasive cancer urothelial cells, we highlight the mechanisms of action of hAM and its therapeutic potential in bacterial cystitis, IC/BPS and bladder cancer, warranting further research for future clinical translation in the field of urological regenerative medicine.

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2 - Kuret T, Peskar D, Kreft ME, Erman A, Veranič P. Comprehensive transcriptome profiling of urothelial cells following TNFα stimulation in an in vitro interstitial cystitis/bladder pain syndrome model. Front Immunol. 2022 Aug 15;13:960667.
3 - Kuret T, Kreft ME, Romih R, Veranič P. Cannabidiol as a Promising Therapeutic Option in IC/BPS: In Vitro Evaluation of Its Protective Effects against Inflammation and Oxidative Stress. Int J Mol Sci. 2023 Mar 6;24(5):5055
4 - Janev A, Ramuta TŽ, Jerman UD, Obradović H, Kamenšek U, Čemažar M, Kreft ME. Human amniotic membrane inhibits migration and invasion of muscle-invasive bladder cancer urothelial cells by downregulating the FAK/PI3K/Akt/mTOR signalling pathway. Sci Rep. 2023 Nov 6;13(1):19227.

Assessing the contribution of different populations of AM-cells to the AM wound-healing beneficial effect

Silvia Alcaraz Romero[1,2], Isabel Hernández Mármol[2], José M Puente Cuadrado[2], Francisco J Nicolás[2]*

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Keywords: chronic wound healing; hAECs, hAMSCs, HaCaT chronified model, conditioned media

INTRODUCTION The application of Amniotic Membrane (AM) at wounds halted at the wound healing process (e.g., chronification, diabetic footulcer) has proven very successful at restarting wound healing, in particular re-epithelialization. AM is participated for two kind of cells: human amniotic epithelial cells (hAECs) and human amniotic mesenchymal stromal cells (hAMSCs). We wanted to investigate which of these two cell types were behind the therapeutic effect of AM.

EXPERIMENTAL We have compared the effect of hAECs and hAMSC into a battery of experimental cell systems that aim to recapitulate different processes occurring during the wound healing process, from cell migration to formation of new vessels, including entry into proliferation of quiescent cells. To compare the effect of the two cell types, we have produced conditioned media from both type of cells using different approaches.

RESULTS AND DISCUSSION The results obtained for the AM´s isolated-cells showed that hAECs produce similar effect to AM regarding cell migration and production of different proteins critical for migration. However, the outcome effect of this phenomena was strongly potentiated by the mesenchymal conditioned media, that surpassed the effect of AM itself. Similarly, the effect of hAMSC conditioned media on proliferation and TGFß cell cycle arrest antagonism was similar to that from AM, however, it was absent from epithelial cells conditioned medium. Cell senescence was also tested with only a positive response from hAMSCs or AM but not from hAECs conditioned media.

CONCLUSIONS The use of the cells components of the AM shows an important role of the mesenchymal cells in the effects that the AM has on the reactivation of chronic wounds and ulcers, however we cannot discard that the epithelial cells could have an important role in aspects that we have not studied yet such as endothelial management, immune-modulation aspects or control of excessive fibrosis.

Muscle regeneration in translational research

Michela Pozzobon[1,2*]

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The need of new biomaterials to replenish the loss of muscle mass is currently a challenge. Indeed, after congenital malformations, trauma or tumor surgery the volume mass loss can be filled with synthetic materials already used in the clinical practice but the regain of function is still very difficult to reach. Nowadays the decellularization of tissues allows the obtainment of the highest biocompatible scaffold without the genetic material, such as the extracellular matrix (ECM). This biomaterial retains the biomechanical properties, proteins and biochemical factors that characterized the native tissue. ECM obtained removing the cellular components from the native tissue by means of decellularization, represents the optimal 3D support for cell culture since the *in vivo* microenvironment is recapitulated. We and other already demonstrated that engineered ECM actively integrate by inducing vascularization, cell recruitment and ECM production. While adverse events such as foreign body response is prevented, fibrosis can be a drawback to solve. In this context, the new approaches that are applied to counteract fibrosis will be presented, from cells to native nanoparticles called extracellular vesicles.

Deciphering the immunoregulatory role of MSCs and their potential application in inflammatory diseases

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Keywords: Mesenchymal Stromal Cells, Immunoregulation, Wharton's Jelly, Bone Marrow, Growth Factors, HLA-G

INTRODUCTION Mesenchymal Stromal Cells (MSCs), a mesodermal stem cell population, that originated from different tissue sources, exhibit remarkable immunoregulatory properties and thus could serve as an alternative treatment in inflammatory human disorders. A potential dysregulation of the inflammatory responses, due to various factors, could result in detrimental effects for the patients and in the development of a vast array of immune-related diseases (e.g. inflammatory and autoimmune disorders). Recently, the COVID-19 pandemic showed us the need to design and develop more effective agents for the toleration of severe immune responses. In the context of immunomodulation, MSCs have shown their privilege to attenuate acute immune responses through direct cell-cell contact or through the paracrine effect. In addition, interferon-γ (IFN-γ) primed MSCs, can process and present antigenic epitopes, through the extracellular increase of Human Leukocyte Antigens (HLA) class II, to dendritic cells. However, priming of MSCs is considered a crucial step for the production of anti-inflammatory cytokines such as interleukin (IL)-10, IL-12, IL-23, growth factors including TGF-B1, FGF, EGF, HGF and other molecules such as Galectins, PGE2, e.t.c. Taking into account the above, this study aimed to the evaluation of the MSCs' immunomodulatory properties, regulating the activated immune responses.

EXPERIMENTAL For this purpose, MSCs were obtained from fetal (Wharton's Jelly -WJ, n=50) and adult (Bone Marrow aspirates- BM, n=50) from healthy donors. Trilineage differentiation and immunophenotypic characterization of MSCs were performed, according to the criteria for well-defining MSCs outlined by the International Society for Cell and Gene Therapies (ISCT). MSCs from both sources stimulated with IFN-γ and the produced biomolecules, were determined using commercial ELISA kits. The expression of HLA-G1, G5, and G7 was also evaluated in WJ and BM-MSCs. WJ and BM-MSCs were co-cultured in 96 transwell plates with M0 macrophages, to evaluate their immunoregulatory properties, through the polarization effect. The determination of the HLA alleles of the MSCs was performed using Next Generation Sequencing (HLA Holotype 11 loci, Omixon Inc., MiSeq, Illumina). The frequencies of the HLA alleles were estimated using the machine learning algorithms in R language.

RESULTS AND DISCUSSION Both WJ and BM-MSCs fulfilled successfully the criteria of ISCT, confirming their well-defining condition. Stimulated WJ secreted higher levels of IL-1Ra, IL-6, IL-10, IL-13, TGF-β1, VEGF-a, FGF, PDGF, IDO, NO and PGE, compared to BM-MSCs. On the other hand, only WJ-MSCs successfully expressed the HLA-G1, G5 and G7 isoforms, in contrast to BM-MSCs, which are characterized by low expression of HLA-G1. Moreover, both MSCs lead successfully to M2 macrophage polarization. The results presented herein showed that MSCs exerted key immunoregulatory properties, thus making them ideal candidates for therapeutic protocols in inflammatory disorders.

CONCLUSION Fetal-derived MSCs characterized by greater immunoregulatory properties compared to adult MSCs, considering them as potential stem cell populations for the development of advanced therapeutic medicinal products (ATMPs). The application of the latter to patients are governed by strict European legislation, which should be followed, to serve better the purposes of personalized medicine.

Investigating the Potential Application of the Amniotic Membrane and Amniotic Epithelial Cells in Ophthalmology: a focus on Retinal Regeneration

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The human amniotic membrane (hAM) has been a cornerstone in ophthalmology for many years, particularly in corneal treatments, due to its remarkable regenerative properties. Recent studies have explored the potential of both cryopreserved and dehydrated hAM for retinal repair. This presentation will highlight the latest promising results in cases such as refractory macular holes, retinal tears, and age-related macular degeneration. The advantages of hAM lie in its ability to retain and release bioactive factors, making it well-tolerated by the host's immune system. Moreover, it can serve as a scaffold for the regeneration of native retinal pigment epithelium (RPE), as well as for culturing RPE derived from embryonic stem cells (ES) and induced pluripotent stem cells (iPS). While in these studies the presence and role of amniotic cells have been largely overlooked or considered limited to the release of regenerative factors, we propose that human amniotic epithelial cells (hAECs) themselves can be induced to differentiate into RPE cells and could be exploited for the replacement of damaged RPE.

Bioproduction of secretome by Wharton Jelly Mesenchymal Stromal Cells – a mechanobiological approach

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Keywords: Secretome, bioprocess, mesenchymal stromal cells, Wharton Jelly, mechanobiology

INTRODUCTION One of the most interesting cell for biotherapies is the Mesenchymal Stromal Cells (MSCs) which are currently tested in several clinical trials to cure diseases or improve symptomes.1,2 However, the lack of donors, the biocompatibility and the potential differentiation of adult MSCs limit their availability which lead to the use of Wharton Jelly MSCs. Within the culture media, cells release not only waste but also a secretome that contains extracellular vesicles (EVs) that may have the same therapeutical potential as their producing cells. It is well-known that the cell microenvironment influences phenotype and characteristics of the cells, 3,4 but not fully understood how it affects EVs production by MSCs. Furthermore, the scale-up and the standardization of the production process remain major challenges. This work aimed to compare the influence of the cell culture conditions (2D vs 3D) and mechanical cues on the bioprocess to improve production yield and obtain different therapeutic properties of the particles.

MATERIAL AND METHODS Cell culture: Human MSC from Wharton Jelly were obtained from the Unit of Cell and Tissue Therapy (CHRU Brabois Nancy, France) and cultured up to passage 3 in complete medium (αMEM supplemented with 3% human Platelet Lysate (hPL), 2 mM of L-glutamine and 1% of Antibiotic Antimycotic solution). Cells were seeded at 3,000 cells/cm² in T75 or 4,000 cells/cm² on microcarriers cultured in Ambr Bioreactors (Sartorius). Mechanical stimulations: At D1, half of flasks were placed on a see-saw rocking shaker at 50 rpm with an angle of 10° for 1h/day, for 3 days. According to Zhou et al5, an oscillatory fluid shear stress of 0.73 Pa was applied. Cells in the bioreactors were cultured for 7 days at 100 rpm. EVs production, purification and characterization: At D2 of culture in flask and D8 in bioreactors, cells were washed with PBS and media without hPL was added in all conditions. After collecting conditioned media, EVs were purified by centrifugations with Amicon Tubes. Size Exclusion Chromatography (SEC, Izon) was performed to obtain 13 fractions of 2 mL each. Nanoparticle Tracker Analysis (NTA, Nanosight) measured EVs size and concentration, and BCA assays quantified the protein content in each fraction.

RESULTS Particles from pure SEC fraction were analysed and showed that in static, fewer particles were produced compared to mechanical stimulation and dynamic culture (4.63×108 vs 1.70×1010 and 7.1×1010 particles/mL, respectively). Similarly, the production by cell was lower in static (6.07×102 particles/cell) compared to mechanical stimulation and dynamic culture (2.19×104 and 8.6×104 particles/cell). Also, particle sizes were larger in static (156.5 nm) compared to mechanical stimulation and dynamic culture (104.4 nm and 102.7 nm).

DISCUSSION AND CONCLUSION Here we show that the culture of MSCs under mechanical cues (mechanical stimulation and dynamic) seems to increase the production of particles compared to static. Furthermore, distinct particles were obtained suggesting that mechanical stress produced smaller particles. Further studies are required to determine the therapeutic properties of the particle with bioactivity tests to understand the key-role of mechanical microenvironment in the particle production with MSCs.

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Successful use of hAM in oral surgery and ophthalmology - role of its immunogenicity

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Keywords: human amniotic membrane, allograft, oral mucosa, immunogenicity, Luminex / MAIPA

INTRODUCTION The human amniotic membrane (hAM) possesses a low immunogenicity and regenerative properties. We conducted two clinical trials to investigate: 1) its therapeutic effect in medication-related osteonecrosis of the jaw (MRONJ) treatment, 2) its humoral immune response after transplantation on the ocular surface.

EXPERIMENTAL Clinical trials were conducted at the University Hospital of Besançon (France) in collaboration with the French Blood transfusion centers.
Study 1: Prospective 6-month follow-up study for eight patients with stage 2 or 3 MRONJ treated with cryopreserved hAM on a compassionate use basis in three French hospitals between November 2020 and April 2021. Visits on days 7 and 14 and at months 1, 2, 3, and 6. Radiological and clinical evaluations.
Study 2: Prospective 3-months follow-up study for five patients requiring cryopreserved hAM transplantation onto the ocular surface in a pilot study between August and December 2022. Visits at months 1 and 3. Anti-HLA class I and II Ab were assessed by flow cytometry using the Luminex® technique from the serum collected from the recipient pre- and post-graft (M1 and M3). Primary endpoint: Presence and identification of anti-HLA class I and II Ab at 3 months after hAM transplantation. The threshold for positivity was a mean fluorescence intensity (MFI) equal to 500. In the event of post-transplant positivity, the serum at M0 was screened to establish whether the Ab was pre-existing at the transplant.

RESULTS AND DISCUSSION Study 1 (1): Three patients had complete closure of the surgical site with proper epithelialization at 2 weeks, and two of them maintained it until the last follow-up. All patients remained asymptomatic with excellent immediate significant pain relief, no infections, and a truly positive impact on the patients' quality of life. At 6 months: 80% of lesions had complete or partial wound healing (30 and 50%, respectively), while 62.5% of patients were in stage 3. Radiological evaluations found that 85.7% of patients had stable bone lesions (n = 5) or new bone formation (n = 1). A multicentric randomized clinical study (PHRC-I 2020 French funding) on 57 patients has started in 2023 in four French hospitals (ClinicalTrials.gov ID NCT05664815). Last patients included in 2024 will be also presented.
Study 2: In four patients (P2,3,4,5), at least one reactivity directed against HLA class I and/or II antigens was found in serum collected at M3 post transplantation. Nevertheless, the MFI at M3 was not significantly higher than at M0 (<1.35 M0) except for one patient, for whom the specificities found were considered artifactual. No major inflammatory reaction or clinical rejection was observed. These results, combined to anti-HLA-G detection, will be consolidated on 18 additional patients already included.

CONCLUSIONS hAM is a promising alternative for MRONJ surgical treatment. To our knowledge, we reported the first study to investigate class I and II anti-HLA Abs after cryopreserved hAM transplantation onto ocular surface.

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Regulation of mammalian cells for therapeutic applications

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Synthetic biology tools have the potential to solve many of the challenges that impede wider application of cell and gene-based therapies through introduction of new modalities of regulating biological processes. Several of the recently introduced strategies of regulating mammalian cells response will be presented. Translation to therapeutic applications has been limited due to unfavorable ligand characteristics or the non-human origin of protein domains that may trigger an immune response *in vivo*. A strategy for engineering inducible split protein regulators based on human proteins (INSPIRE) was devised, where they are regulated by cognate physiological ligand or clinically approved drug. We show that the INSPIRE platform can be used for the dynamic, orthogonal, and multiplex control of gene expression in mammalian cells and demonstrate the functionality of a glucocorticoid responsive INSPIRE platform *in vivo*. Further, a generally applicable regulation platform INSRTR (inserted peptide structure regulator), is based on regulation of selected protein activity by the addition of a peptide that forms a rigid coiled-coil dimer with an inserted peptide allosterically disrupts the protein function. This platform was developed to enable the construction of ON/OFF protein switches, their regulation by small molecules, and logic functions with a rapid response in mammalian cells. INSRTR was demonstrated on ten different proteins with diverse biological activities including enzymes, signaling mediators, DNA binders/transcriptional regulators, fluorescent proteins, and antibodies implemented as a sensing domain of anticancer chimeric antigen receptors on T cells. INSRTR platform presents extraordinary potentials for regulating biological systems and therapeutic applications.

Personalized Dendritic Cell-Based Immunohybridoma Vaccines to Treat Solid Cancers

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Keywords: dendritic cells, cancer treatment vaccine, immunohybridoma, autologous, solid tumors, clinical trial

Immunotherapy (IT) for cancer treatment is a rapidly growing field in cancer management strategies. This applies to various forms of treatments with IT, whether in finding the best ways to implement IT into an already established treatment doctrine, to improve the effectiveness of some already well-defined IT, and to develop new strategies. The primary goal of all IT in cancer treatment is to regain the control of the immune system by triggering specific immune reactions similar to those that occur during spontaneous tumor rejection.

We developed personalized IT based on dendritic cells (DC), professional antigen-presenting cells that are activated upon encountering a foreign or mutated antigen. After DC activation, these antigens are presented to lymphocytes, especially T lymphocytes, which then proliferate and are able to recognize and attack these antigens. DCs can be prepared ex-vivo and specifically activated with tumor antigens and then returned to the patient. We prepared and activated dendritic cells by fusing them with the patient's tumor cells, thereby introducing all known and as yet unknown tumor antigens that are specific to the patient. The obtained autologous immunohybridoma cells (aHyC) were administered to patients in a clinical study of the treatment of patients with castration-resistant prostate cancer (CRPC). There were no serious side effects recorded and quality of life was maintained with aHyC treatment. In the treatment group, the natural killer cell subpopulation (NK CD56brightCD16−) remained at basal levels, but increased in the placebo group ($P = 0.004$). The change in CD56brightCD16− NK cells at the end of the trial was negatively correlated with the survival time of the deceased patients ($r = -0.80$, 95% CI, 0.95–0.34, $P = 0.005$), which suggests a mechanism of action of aHyC therapy also through NK cells. The median overall survival of all patients who received aHyC was 58.5 months (95% CI, 38.8–78.2) from the first application of aHyC. Treatment has also been shown to be more effective in earlier stages of the disease1-3. Since aHyC is a completely autologous drug, this type of treatment can be developed and used for other forms of solid tumors as well. Thus, preparations are underway for the treatment of patients with triple-negative breast cancer (TNBC) with aHyC in a clinical trial. TNBC is an aggressive subtype of breast cancer defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER-2) expression. It is estimated to account for 15-20% of all forms of BC4.

In conclusion, aHyC treatment is safe and effective, modulates the immune system and may represent a new therapeutic option for patients with solid tumors, which will also be tested in TNBC patients.

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Role of perinatal mesenchymal mesenchymal stromal cells of the decidua in the repair of pelvic floor tissue damage

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The pelvic floor is a structure of muscles, ligaments and connective tissue that provides support to the pelvic and abdominal organs, helping them to function properly and preventing them from falling out. Pelvic floor disorders are a group of conditions that affect the pelvic floor tissues and include pelvic organ prolapse (POP), urinary incontinence (UI) and fecal incontinence. UI is the inability to control urination and affects around 300 million people worldwide. UI is a common condition and can range from a minor problem to greatly affecting the quality of daily life. Stress urinary incontinence (SUI) is the most common type of UI among women and occurs when the tissues that support the urethra weaken, with parity being the main risk factor. There are several surgical and non-surgical treatment options for mild to moderate SUI, such as pelvic floor muscle training, vaginal pessary, urethral bulking agents and surgery. Surgical intervention is the recommended treatment option for severe SUI, although patients suffer postoperative complications. The underlying cause of SUI is the weakening of pelvic floor tissues, although the cellular and molecular mechanisms of the pathology are not yet fully understood. Therefore, the search for and development of less invasive therapies for the treatment of SUI remains an important issue and stem cell-based therapy may be an important option to treat this disorder. Mesenchymal stem cells (MSC) are adult stem cells and one of the most attractive sources for stem cell research and therapy. MSC from the maternal side of the placenta, i.e. from the decidua (DMSC, decidual mesenchymal stromal cells), could be an important therapeutic option due to their regenerative potential. The aim of our work is to study whether DMSCs could be used for the treatment of SUI using in vitro and *in vivo* models of the disease. The *in vivo* model is an animal model of SUI caused by parturition, for which vaginal distension was used in female Sprague-Dawley rats simulating maternal birth injuries. Animal experiments were performed with the approval of the Animal Experimentation Ethics Committee. For the in vitro model, cells isolated from SUI patients were used to assess the possible mechanism involved in the regenerative potential of DMSC.

Our results show that DMSC transplantation was effective in improving continence in treated animals by recovering pelvic tissue integrity. In vitro experiments show that the regenerative potential of DMSCs is due to a paracrine interaction resulting in DMSC migration, proliferation of pelvic tissue fibroblasts, and modulation of the pro-inflammatory and ECM-degrading microenvironment characteristic of senescence. In conclusion, DMSC could be an alternative therapeutic option for SUI by counteracting the effects of senescence in damaged pelvic tissue. The results of this research indicate that the application of perinatal mesenchymal stromal cells could be a more effective therapeutic alternative with fewer side effects for patients with SUI compared to the treatments currently used.

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Advanced Strategies in Cell Therapy for Cartilage Repair: Innovations and Clinical Applications

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Keywords: cartilage repair, cell therapy, regenerative medicine, stem cells

Cartilage repair remains a significant challenge in orthopedic medicine due to the limited regenerative capacity of cartilage tissue. In response, advanced cell therapy techniques are emerging as transformative solutions for cartilage repair. This presentation explores cutting-edge developments in regenerative medicine with a focus on cartilage restoration.

The presentation will cover various cell therapy strategies, including the use of stem cells (e.g., mesenchymal stem cells and induced pluripotent stem cells), chondrocyte transplantation, and the application of growth factors. These approaches aim to enhance cartilage regeneration and repair by leveraging the body's inherent healing potential. Recent advancements in biomaterials, scaffolding technologies, and 3D bioprinting are also discussed, as they are integral to improving cell delivery and integration in damaged cartilage.

Additionally, the presentation will highlight ongoing clinical trials and the translation of research into practical clinical applications. Case studies and outcomes from recent trials will be reviewed, showcasing how these advanced strategies have led to improved patient outcomes in both sports injuries and degenerative cartilage conditions, such as osteoarthritis.

Despite the significant progress made, challenges such as cell sourcing, scalability, and regulatory hurdles remain. The presentation will explore future directions in research and development, emphasizing the need for more robust clinical evidence to support widespread adoption of these therapies.

Breaking Barriers: Intranasal Application of Extracellular Vesicles for Brain Health

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Neurodegenerative diseases present significant therapeutic challenges due to the restrictive nature of the blood-brain barrier (BBB). This presentation explores the innovative use of extracellular vesicles (EVs) for the treatment of these conditions via intranasal application (INA). MSCs, known for their multipotent capabilities and ease of harvest, provide therapeutic benefits through paracrine secretion of EVs. Exosomes, the smallest type of EV, offer similar advantages in tissue repair and immune modulation without the associated side effects of MSCs. The intranasal route, established by William Frey in 1989, provides a non-invasive, rapid, and effective method to deliver these therapeutic agents directly to the brain, bypassing the BBB. Despite volume and absorption challenges, INA holds promise for treating neurological disorders by inhibiting inflammation and decreasing neuronal damage. This presentation delves into INA's mechanisms, benefits, and challenges, highlighting its potential to revolutionize treatment paradigms for neurodegenerative diseases.

Amniotic fluid derivatives for cardiac repair: hype or hope?

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Myocardial dysfunction and heart failure as consequence of ischemic disease and/or drug-related cardiotoxic injury still represent a worldwide major threat and socio-economic burden. Thus, translational research has been focused on developing innovative approaches to preserve the contractile cardiac tissue by either optimizing cardioprotective (by counteracting cardiomyocyte cell death and antagonizing oxidative stress) and/or boosting myocardial renewal (by inducing cardiomyocyte cell cycle re-entry and proliferation). In such perspective, stem cell-based paracrine therapy has attracted increasing attention to resurge rescue mechanisms from within the heart.

We have previously characterized the paracrine effects that the human amniotic fluid-derived stem cell (hAFSC) secretome can exert to provide cardioprotection and enhance cardiac repair and regeneration in preclinical models of myocardial ischemia and cardiotoxicity from oncological treatment. Specifically, the extracellular vesicles (EVs) we separated and concentrated from the hAFSC secretome (as for the in vitro hAFSC-conditioned medium) have revealed promising potential by providing long term preservation of viable cardiomyocytes with relevant rescue of cardiac function and transient myocardial renewal in several preclinical models of cardiac dysfunction. Nevertheless, some critical aspects need to be addressed and implemented to optimize human amniotic fluid derivatives as therapeutic candidates for future clinical translation in the field of cardiac regenerative medicine.

Restoration of articular cartilage by using the tissue enfineering procedures

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Articular cartilage is a simple, avascular and aneural, but durable tissue that enables gliding between two surfaces with minimal friction. It is a simple tissue with a thickness of 2-5 mm, consisting of a rich matrix and a single set of cells (chondrocytes). Acute injury, chronic mechanical overload or systematic diseases of the joint can cause gaps in the surface (cartilage lesions). The intrinsic healing potential of such lesions is limited, which is why they cause persistent symptoms but can also lead to early osteoarthritis.

Extrinsic intervention appears to be necessary to initiate or promote cartilage healing. Over the last three decades, various combinations of biomaterials, cells and a stimulating environment have been investigated and some of these combinations have even been used in routine clinical work. We are very proud of the fact that our university hospital jumped on the bandwagon of regenerative cartilage therapies very early on (in the late 1990) with autologous chondrocyte implantation (ACI). The first generation of ACI was later refined and combined with 2D or 3D scaffolds and later replaced by the filtered MSC source from bone marrow, which is still used today. This pioneering approach in regenerative orthopedics has allowed us to closely observe and analyze different treatment groups, collaborate with basic scientists, contribute to the development of scaffold-specific surgical techniques and instruments, simulate clinical situations on cadaveric models, etc. Although our clinical work as orthopedic surgeons focused mainly on focal cartilage lesions (defects), related research also included studies on the chondrotoxicity of radioisotopes and local anesthetics, as well as preclinical research to investigate better cell sources. More recently, we have been involved in a joint project on synovial biomarkers and clinical responses following intra-articular injection of allogeneic cord-derived MSCs in knee osteoarthritis.

There were also some side projects on cartilage that were only indirectly involved in regeneration: determining the time of death by counting live/dead cartilage cells after death (forensic topic) and studying the behavior of cartilage/bone after prolonged inactivity in a bed rest space-flight study (supported by ESA).

The aim of the lecture is to present our activities in the field of articular cartilage and to compare our results with international data in the literature.

Use of Wharton's jelly mesenchymal stromal cells and extracellular vesicles in innovative therapies

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Keywords: advanced therapy medicinal products (ATMPs), Wharton's jelly-derived mesenchymal stromal cells (MSC,WJ), extracellular vesicles (EV), good manufacturing practices (GMP), regenerative medicine, tissue engineering products (TEPs)

INTRODUCTION Success of novel advanced therapy medicinal products (ATMPs) requires the development of versatile, off-the-shelf solutions based on allogeneic substances of human origin (SoHOs) that must be generated in line with specific regulatory pathways for reducing manufacturing time and improving production sustainability(1). In this context, Wharton's jelly-derived mesenchymal stromal cells (MSC,WJ) have emerged as an attractive starting material in the production of ATMPs due to their straightforward sourcing and primitive stem and immunological features. Active involvement of our institution in clinical trials with advanced therapies is the result of a successful production and maintenance of a cell bank of MSC,WJ in compliance with Good Manufacturing Practices (GMP)(2).

EXPERIMENTAL MSC,WJ-based products developed and manufactured in our facilities have been clinically evaluated in diverse compassionate treatments in the management of posthematopoietic stem cell transplant complications (namely, Graft versus Host Disease, GvHD) and in three clinical trials in patients with chronic traumatic spinal cord injury (SCI) (2015-005786-23)(3), respiratory distress due to COVID19 (2020-001505-22) and myocardial infarction (MI) (2018-001964-49)(4).

RESULTS AND DISCUSSIONSafety of MSC,WJ-based treatments was confirmed as no adverse effects were found in any of the patients enrolled in the trials. In terms of efficacy, a higher degree of variability was observed. As an example, a single intratecal infusion of a 10x106 ex vivo expanded MSC,WJ in patients with chronic traumatic SCI induced significant improvement in the segments adjacent to the injury site. By contrast, patients with MI who received a tissue engineering product (TEP) combining 7-15x106 MSC,WJ with an allogeneic human decellularised pericardium scaffold, namely PeriCord, showed no clinical relevant differences in cardiac volumes or function when comparing with the control group. However, the TEP demonstrated excellent biocompatibility, was successfully integrated into the myocardium, and showed potent anti-inflammatory properties and capability of modulating systemic inflammation.

CONCLUSIONS MSC,WJ-based ATMPs constitute a safe and promising tool in regenerative medicine. Nevertheless, to succeed in the clinical translation of innovative investigational therapies, more investigation is needed focused on identifying markers that could reliably predict the clinical efficacy of a specific MSC-based treatment in a particular group of patients. In our institution, we are currently involved in different projects aimed to demonstrate safety and efficacy of MSC,WJ and its derivatives, mainly extracellular vesicles, as active ingredients for the regeneration of several tissues, including bone, cartilage and heart.

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Developing mucoadhesive films with human amniotic membrane (hAM) homogenate for application in oral wound healing

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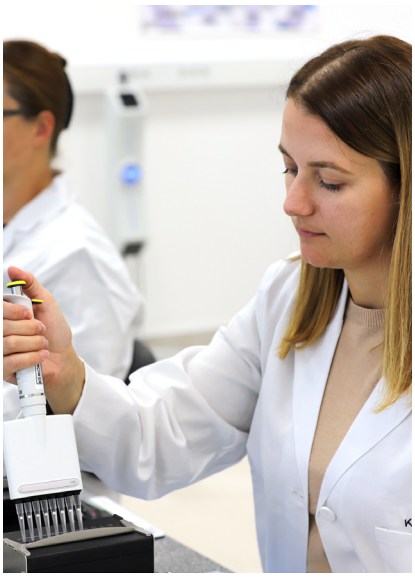
INTRODUCTION Human amniotic membrane (hAM) has been used as an available biocompatible extracellular matrix possessing anti-inflammatory and regenerative properties for post-traumatic skin defects, genital and ophthalmologic disorders but favorable effects were only observed in short-term outcome, possibly because of hAM degradation after few weeks. Since the incidence of the oral wounds depend on the aethiology and the outcomes of currently available wound healing treatment of oral tissues approaches are expensive and not predictable, the main objective and novelty of this study is to develop a technological formulation of hAM homogenate that will be used for oral tissues regeneration.

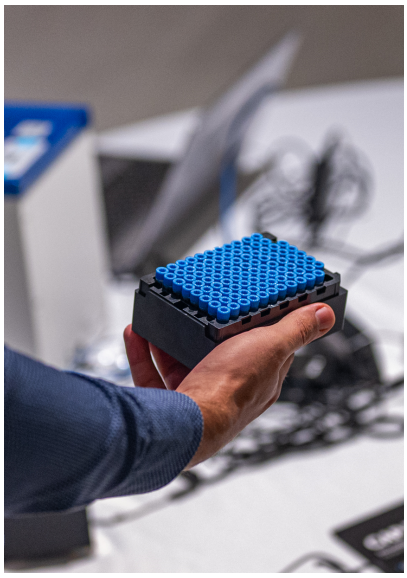
For that purpose, periodontal ligament SCs (PDLSCs) playing a crucial role in oral tissue homeostasis were used to test a functional model of new hAM homogenate formulation. Namely, after incubating with different portion of hAM homogenate PDLSCs metabolic activity and migration potential were observed by MTT test after 24 and 48h and by scratch assay after 24h. For application in the actual setting of oral wounds, different cellink and polycaprolactone (PCL) based mucoadhesive films of various portion of hAM homogenates are planned to be developed by Cellink BIOX 3D printer. For the beginning, active 3D bioprinted film systems containing different contents of the amniotic homogenate were left to dry and let the dissolvent evaporate for 24h before obtained 'delivery systems' of hAM were tested by culturing PDLSCs.

Our results revealed that the hAM homogenate concentration of 12.5% positively affected both metabolic activity and migration of PDLSCs indicating that hAM in concentration of 12.5% might be conceived and used for preparing mucoadhesive polimers which can further be used in terms of oral wound healing. Also, the preliminary results show that PDLSCs were successfully attached on new mucoadhesive hAM films after 48h.

CONCLUSIONS The new hAM mucoadhesive biofilm is low-cost and ecologically sustainable respectively and provides valuable findings for preparing printable biomaterial based on hAM homogenate to be used in oral tissue regeneration.

THE SCIENCE CORNER





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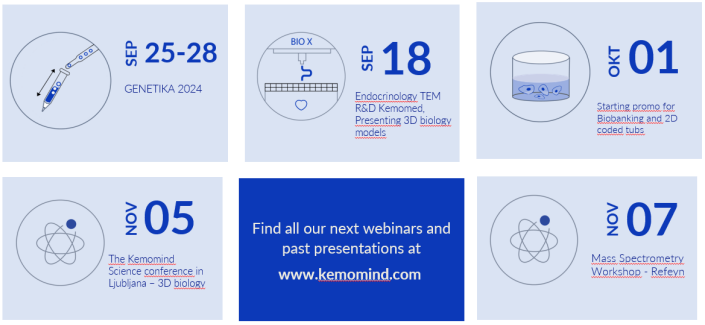

Pro-regenerative miRNAs from human amniotic membrane as a potential source for tissue regeneration

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Keywords: miRNA, extracellular vesicles, cell therapy

The human amniotic membrane (hAM) has been recognized as a promising source of therapeutic cells in tissue regeneration for over a century. The cells have been attributed with stem cell characteristics. However, procedures such as cell isolation and in vitro expansion may lead to genomic instability and loss of intrinsic properties. A promising alternative to the isolated cells, is the usage of cell-derived bioactive factors released directly from the viable native hAM. Regarding hAM derived miRNAs, one major group of molecules that are crucial in cell communication in health and disease, only little information is available. Therefore, the aim of this project is to characterize pro-regenerative miRNAs in the hAM secretome.

In order to study EV-contained and protein-bound miRNA, supernatants from hAM biopsy punches of placental amnion (P) and reflected amnion (RA) were collected after 72 h and subjected to size exclusion chromatography (SEC). EV fractions were characterized for particle concentration and size distribution by nanoparticle tracking analysis (NTA) and fluorescence-triggered flow cytometry (FT-FC) for EV content, size, and composition. Surface markers were confirmed with Transmission Electron Microscopy (TEM) and Western blot (WB). On the other hand, protein fractions were characterized for particle concentration and size distribution by NTA, and the protein concentration was measured by microBCA assay. Tissue and supernatants were analyzed by RNA sequencing.

For both P and RA, FT-FC and NTA confirmed the presence of particles smaller than 200 nm (75 % of EVs). CD81 was prominent in all samples (24 %, n = 2, biological replicates). WB and TEM confirmed the presence of CD81 (n = 3, biological replicates). Our results showed three main clusters of miRNAs differentiating tissue miRNAs, EV-contained miRNA and protein-bound miRNA. Subclusters of miRNA correlating with the hAM subregions (P and RA), both in the secretome and the tissues. Differentially expressed miRNA between hAM subregions and between tissue, EV-contained and protein-bound miRNA were also identified. Pathway enrichment analysis of these differentially expressed miRNA revealed statistically significant pathways related to skin and bone regeneration, including osteoblast regeneration, wound healing, and immunomodulation among others.

In our next step, we want to further investigate the secretome related to the regenerative potential of hAM using in vitro models pursuing a potential therapeutic approach to support tissue regeneration.

Combining Urine Derived Renal Epithelial Cells and Wharton's Jelly Mesenchymal Stem Cells into a spheroid: structural insights and immunomodulatory properties

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Keywords: perinatal cells; urine derived renal epithelial cells; spheroids; immunomodulation

INTRODUCTION Kidney transplant is a lifesaving procedure for patients with end stage kidney disease. Organs from cardiac, or brain death donors are highly exposed to ischaemia-reperfusion events, leading to an increase in kidney cell turnover to replace damaged cells. Damaged cells lining kidney tubules are then exfoliated and voided in urine; these cells are known as Urine-derived Renal Epithelial Cells (URECs)1,2. We previously observed that URECs from kidney transplanted patients expressed typical renal epithelial cell markers as Cytokeratin and CD13, and were able to suppress the proliferation of CD4 and CD8 T Lymphocytes, increasing the T regulatory cell subset, and reducing apoptosis, during coculture with Peripheral Blood Mononuclear Cells (PBMCs)3. In addition to the promising results obtained in 2D culture, the set-up of 3D more reliable model will be interesting to investigate the complex interaction between stromal cells and epithelial cells and even increase immunomodulatory capacity for future clinical application. Since URECs do not successfully constitute spheroids alone, the aim of the project was to combine URECs with Wharton's Jelly Mesenchymal Stromal Cells (WJ-MSCs) to generate spheroids. and evaluating the immunomodulatory potential of UREC+WJ-MSC spheroids during the cococulture of spheroids with PBMCs.

EXPERIMENTAL URECs and WJ-MSCs were combined in 1:1 ratio. Spheroids section of 1:1 ratio were stained with hematoxylin and eosin and immunofluorescence searching for epithelial and mesenchymal markers was performed. Cell membrane binding dies were used to stain differently the two cell populations and observe their time-lapse aggregation by Incucyte instrument. The spheroids were co-cultured with anti-CD3 and anti-CD28 activated PBMCs for 72 hours. Flow cytometry was used to evaluate the activation marker CD69 in both CD4 and CD8 T cells and their proliferation rate, along with the analysis of regulatory T cells.

RESULTS AND DISCUSSION URECs and WJ-MSCs aggregated within 24 hours after seeding, with WJ located in the outer zone of the spheroids, while URECs in the core. WJ-MSC+UREC spheroids presented a heterogeneous histology, related with the presence of two different cell populations. The expression of Vimentin mesenchymal marker was observed in the outer layer of the spheroids, while Cytokeratin epithelial marker was observed in the core of the spheroids. WJ-MSC+UREC reduced the proliferation and activation of CD4 and CD8 T cell, moreover the percentage of regulatory T cells (Treg) was significantly increased, compared with control PBMCs and to Wj-MSCs spheroids.

CONCLUSION the combination of URECs and WJ-MSCs supports the formation of functional 3D cell aggregates. Our 3D model shows interesting immunomodulatory properties, promoting an increase in Treg subset. These properties could have promising applications in cell therapy approaches and need to be further elucidated by extending the analysis panel for the immune cell population involved, including B lymphocytes and monocytes.

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Harnessing the Potential of Amniotic Membrane Homogenate: A Novel Bioink for 3D Bioprinting in Breast Reconstruction

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Keywords: amniotic membrane; biomaterial; 3D bioprinting; soft tissue engineering

INTRODUCTION Despite their safety and feasibility for patients, existing breast reconstruction techniques post mastectomy carry the risk of potential complications, and do not significantly alter the likelihood of disease recurrence compared to mastectomy alone. The field of tissue engineering has been intensively growing primarily focusing on crafting diverse scaffolds that act as templates for tissue regeneration. Human amniotic membrane (hAM) of the placenta has been utilized in skin and cornea regeneration, and numerous studies have demonstrated its antitumor, antifibrotic, anti-inflammatory, and immunomodulatory properties. Here we aim to develop the scaffold biomaterial for 3D bioprinting using human AM homogenate (hAM-h) that will preserve and exhibit its regenerative and antitumor effects over an extended period when applied.

MATERIAL AND METHODS Placentas were collected from healthy donors after the caesarean section and hAM was separated from the chorion and homogenized according to previously established protocol [1]. Homogenates were analysed for cytokine composition with flow cytometry, and different rheological parameters were determined by tensiometer, viscometer and zetasizer. Alginates of different viscosities were mixed with hAM-h to make a biomaterial for extrusion 3D bioprinting on Cellink BioX bioprinter. Printed bioscaffolds were crosslinked with CaCl2 and then characterized for their compressive strength and swelling ability.

RESULTS AND DISCUSSION Results showed that hAM-h samples consisted of high levels of growth factors such as Angiopoietin-2, EGF, FGF, HGF and other cytokines. As regards physical properties, no significant difference in density, surface tension and viscosity was noticed among different hAM-h samples. The average particle size in the samples was 192.1±24.95 nm in diameter, with polydispersity index values > 0.5 meaning the samples had heterogeneous particle size distribution. Regarding zeta potential, all hAM-h samples possessed a negative surface charge < -30 mV which indicates a solution with moderate stability. According to the force-strain curves of 3D-printed bioscaffolds, the compression profiles exhibited a trend with a greater force required to achieve a higher deformation percentage. However, the force required to compress the highALG-hAM-h bioscaffolds to 70% of original diameter was higher than force needed for lowALG-hAM-h bioscaffolds under the same deformation conditions. It was noticed also that the weight of highALG-hAM-h bioscaffolds increased over time unlike that of lowALG-hAM-h which decreased.

CONCLUSIONS This research provides valuable findings for preparing printable biomaterial based on hAM homogenate showing its potential suitability for use in soft tissue engineering applications.

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Engineering a Biodigital Placenta for Advanced Drug Testing

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Keywords: placenta; biodigital twin; feto-maternal interfaces, fetal membranes.

OBJECTIVE The overall objective of LIFESAVER is to emulate drugs and chemicals transport through the first trimester placenta by creating a new, digitally cloned in vitro system ("biodigital twin"). This system mimics the uterine/placental interface, combining physical (in vitro) and digital (in silico) parts. The in vitro construct comprises a biomechanical system representative of the amnion-chorion membrane (referred to as RACE). This component is developed using 3D bioprinting technology, with live cells and selected bioinks. Given that the optimal bioink requires a balance between printing resolution and supporting cell viability (1), we investigated the compatibility of ECM (extra cellular matrix) emulator bioinks with cells from amniotic membrane, and more specifically on both amniotic mesenchymal stromal cells (hAMSC) and amniotic epithelial cells (hAEC).

MATERIALS AND METHODS To identify the most favorable bioink to be used for RACE bioprinting, 5 different bioinks for each cell type were selected and their effects were tested on hAMSC and hAEC at passage 1. The bioinks containing laminin (GeIXA LAMININK 521), RGD peptide (CELLINK RGD) and collagen type I (Lifeink 200) were tested with hAEC, while GelXA and CELLINK Bioink were used as controls. For hAMSC, bioinks containing fibrinogen (GelMA FIBRIN and CELLINK FIBRIN) and collagen type I (Lifeink 200) were tested along with GelMA Bioink and CELLINK Bioink as controls. The cells were seeded in complete RPMI in presence or absence (control) of each bioink, dispensed as discs in 96-well plates. At different timepoints (1, 5, 8 days for hAMSC; 1, 3, 7 days for hAEC), cell viability and bioink cytotoxicity were assessed using the Celltiter-Glo kit, Alamar Blue and MTT assays.

RESULTS Bioinks possess specific characteristics to support high 3D printing fidelity, cell adhesion, and proliferation in 3D environment. The selected bioinks were initially selected based on their physical (rheological) and chemical (photocrosslinking) properties, but biocompatibility, or bioink-cell interaction, remains a fundamental criterium in 3D bioprinting. Both amniotic cell types were seeded on bioink discs and analyzed for growth and viability over a period of time. The results obtained suggest the promising potential use of bioinks containing biologically active components (CELLINK RGD, Lifeink 200, GelMA FIBRIN and CELLINK FIBRIN), based on the consistent cell proliferation profile observed in all analyzed assays.

CONCLUSIONS Our results suggest that selected bioinks for each cell types could be potentially optimal for 3D bioprinting of the LIFESAVER model, due to combination of their rheological, chemical and biological properties. Further studies will be performed to test the feasibility of the 3D embedded cell culture with the 3 selected bioinks under dynamic conditions in the in vitro system.

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Towards exploring the role of RPGR isoforms for retinitis pigmentosa and cone/cone-rod dystrophy in human retinal organoid

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Keywords: Retinitis Pigmentosa GTPase regulator, Retinitis Pigmentosa, Cone/Cone-Rod Dystrophy, Retina Organoids

INTRODUCTION Retinal dystrophies are one of the leading causes of blindness among working-age adults. They are characterized by irreversible loss of photoreceptors, caused by genetic variants in various genes. One of those is Retinitis Pigmentosa GTPase Regulator (RPGR), expressed in connecting cilium of the photoreceptors. While pathogenic variants in RPGR are known to primarily affect either rods or cones, the mechanism behind this polarity remaining elusive (1). Recent studies on patient-derived fibroblasts have shown that the ratio of the retina-specific RPGR isoforms plays a role in determining the connecting cilia length. However, it is not known how the isoform ratio affects cilia length in photoreceptors and whether this effect is different in distinct photoreceptor types. Studying the isoforms on RNA or protein level requires access to living retinal tissue which is difficult to obtain. Human retinal organoids represent a promising in vitro model for studying retinal diseases.

EXPERIMENTAL METHODS To characterize the relationship between connecting cilia length in rods and cones and RPGR isoform ratios we aim to establish human retinal organoids (hROs) as an RPGR disease model. We will modulate isoform expression via short hairpin RNA (shRNA) specific to each of the three RPGR isoforms. To deliver shRNA to retinal organoid cells, we use adeno-associated viral vectors (AAVs) and to assess the transduction efficiency we utilize confocal microscopy and deep learning-based segmentation tools (2). For the photoreceptor morphology imaging we combine live or antibody staining with high-resolution microscopy. We will measure retinal function by light-sheet microscopy and Calcium indicator integrated into the neuronal network (3). The RNA isoform ratio we will assess using qPCR analysis.

EXPECTED RESULTS Within this project we will establish an RPGR disease model which puts us in the position to disentangle the impact of the three RPGR isoforms on disease phenotypes. We expect to find differences in the isoform ratio between rods and cones and record their impact on the photoreceptor morphology and consequently different disease phenotypes. Our in vitro model can guide the development of therapeutics modulating the isoform expression ratios and thus opens a complementary path in retinal precision medicine.

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Amniotic membrane therapeutical efect in wound healing is preserved 48 hours after thawing

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Keywords: chronic wound healing; HaCaT chronified model; AM preservation for clinical application

INTRODUCTION During wound healing, the migration of keratinocytes aims to finalize the healing process. The application of Amniotic Membrane (AM) at wounds halted at the wound healing process (e.g., chronification, diabetic foot ulcer) has proven very successful at restarting wound healing, in particular re-epithelialization. However, the use of AM for clinical practice has several restrain when applied to the patients, and one of the more important one sis AM stability between thawing of the tissue and the its application into the wound of the patient.

EXPERIMENTAL AM previously criopreserved with 10% DMSO and TC199 medium was thawed at 37ºC in water bath, washed and resuspended in physiological saline medium with or without human albumin (4%). The AM was then stored at different temperatures (room temperature or 4°C) for either 24 or 48 h. Keratinocytes and TGFß-chronified keratinocytes were used to assess several parameters related to wound healing: migration, cell cycle arrest reversal, proliferation and migration markers at the wound edge related to migration and proliferation.

RESULTS AND DISCUSSION Our findings indicate that AM stored for 24 or 48 hours at 4°C performed excellently compared to AM that was immediately thawed. Amniotic membrane cells were study for viability and we observed an average of 70% of cells alive after freeze-thawing cycle in the different times of pre-incubation and independently of the storing media. Additionally, all parameters measured: cell migration, cell proliferation, antagonism of TGFß signalling and different genes related to survival, inflammation and senescence had the same response in all cases, independently of the after-thawing kept time.

CONCLUSIONS This data It suggests that the preservation method at 4°C is effective in maintaining the therapeutic properties of AM. So, preserving AM in saline at 4°C does not negatively impact its therapeutic properties on cells. This implies that AM can be stored more conveniently and distributed easily in health-care settings, which could be particularly beneficial for wound care treatments performed at far away from tissue-bank centers, either hospitals or health centers.

Extracellular Vesicles from Second Trimester Human Amniotic Fluid as Novel Therapeutics for Skeletal and Cardiac Muscle Injury

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Keywords: extracellular vesicles; human amniotic fluid; paracrine effects; oxidative stress; cardiotoxicity

INTRODUCTION We previously demonstrated that the human amniotic fluid (hAF) from II trimester of gestation is a feasible source of stromal progenitors (human amniotic fluid stem cells, hAFSC), with significant paracrine potential for regenerative medicine. Extracellular vesicles (EVs) concentrated from hAFSC secretome can deliver proliferative, anti-fibrotic and cardioprotective effects in preclinical models of skeletal and cardiac muscle injury. While hAFSC-EV isolation can be significantly influenced by in vitro cell culture, here we profiled EVs directly concentrated from hAF as alternative option and investigated their paracrine potential in vitro.

EXPERIMENTAL II trimester hAF was obtained as leftover material from prenatal diagnostic amniocentesis following written informed consent. EVs were separated by size exclusion chromatography and concentrated by ultracentrifugation. hAF-EVs were profiled by nanoparticle tracking analysis, transmission electron microscopy, Western Blot, and flow cytometry; their metabolic activity was evaluated by biochemical assays and their cargo assessed by proteomics and RNAseq. hAF-EVs paracrine potential was tested in preclinical models of oxidative stress on murine C2C12 cells and 3D human cardiac microtissue (hMT) and of doxorubicin-induced cardiotoxicity on murine cardiomyocytes (mNCMs) and human iPSC-derived cardiomyocytes (iCMs).

RESULTS AND DISCUSSION Our protocol resulted in a yield of 6.31 ± 0.98x109 EVs/hAF ml with round cup-shaped morphology and 209.63 ± 6.10 nm average size, with expression of CD81, CD63 and CD9 markers. hAF-EVs were enriched in CD133/1, CD326, CD24, CD29, and SSEA4 and able to produce ATP by oxygen consumption. While oxidative stress significantly reduced C2C12 viability, hAF-EV priming counteracted such effect with notable recovery of ATP synthesis and reduction of lipid peroxidation activity. hMT treated with hAF-EVs and experiencing H2O2 stress and TGFβ stimulation showed improved survival with remarkable decrease in the onset of fibrosis. Doxorubicin-derived cardiotoxicity affected mNCMs survival and triggered iCMs premature senescence in vitro, with hAF-EV treatment significantly antagonizing cardiomyocyte alteration and damage.

CONCLUSIONS Our results suggest that II trimester hAF can represent a feasible source of EVs to counteract oxidative damage on target cells, thus offering a novel candidate therapeutic option to counteract skeletal and cardiac muscle injury.

Human amnion epithelial cell secretome may drive the generation of NK cells with lower proliferative capability and higher effector functions

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Keywords: NK cells, human amnion epithelial cells, NK cell receptors, NK cytotoxicity

INTRODUCTION In the last years, we reported several immune regulatory properties of human amnion epithelial cells (hAEC), including inhibition of NK cell proliferation(1, 2). Here, we investigated the expression of activating receptors and immune check-points and cytotoxicity of NK cells when exposed to hAEC secretome (hAES).

EXPERIMENTAL NK cells were isolated from 4 normal donors and expanded with IL-2, in the presence or absence of hAES generated by 5 donors. Fresh medium±hAES was replaced every 3 days and NK cells cultured for 9 days before analyses. Expression of NK receptors and immune checkpoints was analyzed by flow cytometry. Cytotoxicity of NK cells was performed using K562 cell line as target in terms of degranulation (CD107a) on NK cells and viability (7AAD) on target cells.

RESULTS AND DISCUSSION The final number of NK cells was reduced in the presence of hAES. The expression of CD57, NKG2C, CD16 and TIGIT was downregulated, whereas the expression of NKG2A, NKp30, NKp46 and NKp44 was increased in the presence of hAES. Degranulation of NK cells was similar in the presence or absence of hAES (median CD107a, 5.7% and 7.1%, respectively) and increased in the presence of target (12.9% and 27.8%, respectively). Accordingly, cytotoxicity in target cells (basal level 4.4%) presented a 3-fold increment in presence of NK cells exposed to hAES (73.1%), compared to control (26.9%). The cytotoxic granule released by NK cells as well as lysis of K562 was higher when pre-activated by hAES. However, in the first 4 donors tested, no statistical significance was reached.

CONCLUSIONS Our preliminary results propose the secretome of hAEC as a critical solicitor for the generation of NK cells with increased cytotoxic receptor expression (NKG2A, NKp30, NKp44 and NKp46) and activity (against leukemia). hAES-conditioned NK cells decreased in proliferative ability and expression of senescence/exhaustion markers (CD57, NKG2C, and TIGIT)(3, 4). Further studies will assess whether these effects are constitutive for every hAEC donor, accountable to hAEC-derived soluble factors and/or extracellular vesicles.

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Intact cells or secretome in support of immune system re-education, tissue regeneration, or oncological treatment?

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Keywords: secretome; extracellular vesicle; mesenchymal stromal cells; amniotic epithelial cells; potency

INTRODUCTION Human amniotic epithelial cells (AEC) are a non-controversial source of advanced medicinal therapeutic products. They have been reported to boost the capacity of regeneration, modulate the immune response, nurture surrounding cells, and correct congenital disorders. Such therapeutic effect is not mediated by intact AEC only, but supported by AEC secretome, and vesicles in particular (AEV). EVs are secreted by all perinatal cells, including Wharton Jelly-MSC, but protein and nucleic acid cargo varies significantly according to cell of origin. We profiled and tested both intact AEC and secreted AEV in comparison with WJ-MSC.

EXPERIMENTAL We coupled profiling of surface molecules and enzymes on intact AEC and AEV, with cargo analysis. Cells were isolated according to cGMP procedures, and flow cytometric evaluation validated both AEC, WJ-MSC, and related EVs. Finally, we screened soluble factors (by Luminex and OLINK technologies).

RESULTS AND DISCUSSION We identified mediators and enzymes on the surface of intact AEC instrumental for cell-mediated activities. Such molecules were found transferred to AEVs. Both AEC and WJ-MSC modulated immune cells, in a dose-dependent matter. AEC/AEV purinergic mediators modulated immune effector cells (T-, B- and NK-cells), and induced the macrophage switch from M1 to M2. We qualified and sequenced biomolecules contained in small and large AEV, revealing anti-fibrotic effects as well as a potential role in oncological treatment. We measured the constitutive presence of membrane-bound non-polymorphic HLA molecules on AEC and AEV, in addition to soluble isoforms.

CONCLUSIONS The updated paradigm is that AECs do not necessarily need to mature into adult cells, but they can rescue native parenchymal cells via indirect paracrine effects. The ability to treat most common (chronic/congenital) diseases with allogeneic stem cells without the administration of immunosuppressive drugs will greatly expand the number of patients who could benefit from cellular therapy. Immune evasive capacity could be a "game changer", and the modulation, rather than suppression, of innate and adaptive immune cells may result in enhanced cell treatments for regenerative purposes, autoimmune disorders, and tumors treated with augmented immune response.


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Biocompatible Polyphenol Cross-Linked Wharton Jelly hydrogel : New Multifunctionnal Hydrogel For Bone Tissue Engineering

P1

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Keywords: Wharton Jelly, Tannic acid, antioxidant, antibacterial, bone regeneration
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INTRODUCTION New multifunctional hydrogels with antioxidant and antibacterial properties are today needed to meet the modern challenges in tissue regeneration. One popular approach to generate bioactive hydrogels that try to mimic these tissues' characteristics is to use decellularized tissues. Wharton Jelly (WJ) is a perinatal tissue with antibacterial and immunomodulatory properties interesting for tissue regeneration.1 But hydrogels from the decellularized WJ matrix with weak mechanically properties and unstable against reactive oxygen species are challenging to handle, limiting their application in regenerative applications. Tannic acid (TA), a naturally occurring polyphenol, can engage in hydrogen bonding with a wide range of macromolecules including collagen. By taking advantage of the polyphenol chemistry, we herein used a dual cross-link strategy to design a new family of WJ-derived hydrogel.

EXPERIMENTAL WJ-derived macromolecules were extracted from decellularized Wharton Jelly according to Freytes method2. The self-standing hydrogel was obtained following the re-equilibration of the pH and the salt concentration. The hydrogels were soaked in TA solution for 24h then periodate for 2h. After extensive washing, the hydrogels were freeze-dried to obtain 3D porous scaffold. The infrared spectroscopy, nanotomography, tensile test as well as the antibacterial, antioxidant, anticancer were carried out. Finally, the osteogenic properties of the hydrogels were evaluated in critical (i.e. 8 mm) parietal bone defect.

RESULTS AND DISCUSSION With a sheet-like structure, the TA cross-linked WJ (TA-WJ) freeze-dried scaffold was more robust and easier to handle compared to the un-crosslinked WJ scaffolds which has a loose structure. Infrared showed peaks at 1325 and 1195 cm−1, attributed to the presence of TA. TA-WJ scaffold was more resistant to the collagenase action compared to WJ scaffold. In addition, in comparison with the WJ scaffold, the swelling capacity of the TA-WJ scaffold as well as its total porosity significantly decreased, suggesting that the presence of TA limits the water interaction with the macromolecules. The presence of TA increased the strain and tensile strength of the WJ scaffold, suggesting that the TA cross-link is beneficial for the stretchability and stiffness of the as-scaffold. Besides the physical and mechanical capabilities of TA, it was also shown that TA can be released from the TA-WJ scaffold, limiting the bacteria (S. aureus and P. aeruginosa) proliferation, the excessive accumulation of oxygen reactive species in oxidative stress-stimulated fibroblasts and the secretion of the proinflammatory mediators by LPS stimulated macrophages. An increase in the osteosarcoma (SaOS2) apoptosis was observed in the presence of TA-WJ scaffold. When implanted in a critical bone calvaria defect, the TA-WJ scaffold induced a de novo bone with a significant increase in bone volume and mechanical performance.

CONCLUSIONS These data shed light on the great potential of TA-WJ scaffold as a versatile scaffold, in particular to support bone tissue regeneration.

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P2 The initial conservation conditions of the human umbilical cord influence the biobanking of perinatal derivatives

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Keywords: umbilical cord; tissue preservation; Wharton Jelly; cell banking; biomaterial

INTRODUCTION The umbilical cord (UC) contains a stem cell-rich gelatinous stromal tissue called Wharton's jelly (WJ) and vascular structures providing nourishment to the fetus. The WJ is a unique reservoir of mesenchymal stromal cells (MSC), growth factors (GF), and extracellular matrix (ECM) components such as collagen, hyaluronic acid, and glycosaminoglycan. Due to its immuno-privileged status, UC can be considered an abundant natural medical waste biomaterial for tissue engineering applications. The decellularization process for UC could provide an ideal substrate for the colonization of stromal cells while ideally preserving biomechanical advantageous properties. As its counterpart, i.e. cord blood, the WJ trophic and biophysical functions should be preserved by the maintenance in a defined immersing solution. In this way, the solution should maintain the survival of resident stem cells and avoid GF degradation during the first 24 hours between the newborn delivery and the laboratory manipulation of the UC, allowing the primary cell isolation or the use as a biomaterial source.

EXPERIMENTAL The aim is to characterize suitable storage conditions and help in identifying an easy optimal solution for storage of UC until tissue manipulation in the laboratory. Here the collected UC tissues were rinsed with saline supplemented with antibiotics, then incubated overnight (O/N) at 4°C in low-glucose (LG) DMEM or in DPBS, both including antibiotics. The morning after the tissue fragments have been incubated with Resazurin (Prestoblue™), for checking metabolic activity, and WJ-MSCs have been isolated by explant-based migration method. To evaluate the contribution of the different tissues existing in UC to the bulk effect resulting in Prestoblue assay, an MTT assay has been performed on the UC fragments to locate distinct metabolic activity. Senescence assay and histology on the fragments have been also evaluated by imaging.

RESULTS AND DISCUSSION Both DMEM and DPBS storage maintained the metabolic activity of the tissue resident cells. The O/N storage in DMEM allowed a higher yield of isolated primary cells (e.g. WJ-MSC cumulus cells) in respect to DPBS. The MTT assay did not show differences between the two storage solutions, however a faster metabolic rate in vessels rather than in WJ was noticed. Alpha-MEM (for different antioxidant supply) and high-glucose DMEM (for higher nutrient supply) should be included as alternative solutions in future comparing experiments. Endothelial cells (HUVEC) isolation from UC stored fragments will appropriately expand the investigation.

CONCLUSIONS Novel reliable methods for WJ-MSC isolation are demanded for harnessing their therapeutic potential and moving towards the consistent scale-up of this perinatal stem cells. We found that low glucose DMEM can be preferable as a cost-effective storage solution in comparison with traditional DPBS for O/N storage before primary cell isolation, while for the best solution before the derivation of the decellularized biomaterial from UC, further data must be obtained. This study will contribute to a better understanding of storage conditions ahead of assaying for WJ-MSC potency and immunomodulating effects essential for their GMP manufacturing.

1 - Chu et al. Stem Cell Research & Therapy (2024) 15:131

Two-in-One Therapy: Insulin-Producing and Immune-Modulating Perinatal Cell Spheroids for Type 1 Diabetes

P3

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Keywords: Type 1 Diabetes; perinatal stem cells; 3D culture; immunomodulation; cell therapy

INTRODUCTION Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by the immune system's destruction of pancreatic beta cells, leading to insulin deficiency. Conventional treatments focus on insulin replacement, which does not address the underlying immune attack against beta cells. This study is focused on developing aa cellular therapy for T1DM by using perinatal cells to create spheroids that can release insulin1 and mitigate the autoimmune responses2. This dual approach seeks to restore insulin production while protecting the new beta cells from autoimmune destruction.

EXPERIMENTAL First the immunomodulatory capacity of two types of undifferentiated perinatal spheroids was evaluated. The spheroids made of Wharton's jelly mesenchymal stem cells (WJ-MSCs) from the umbilical cord and those of WJ-MSCs mixed with amniotic epithelial cells (AECs) were compared. The assay is based on a co-culture of perinatal spheroids with activated peripheral blood mononuclear cells (PBMCs) from healthy donors. We assessed the activation status and the percentages of anti-inflammatory and pro-inflammatory cell populations. Subsequently, we induced the endocrine differentiation of AECs into insulin-producing cells, confirming pancreatic differentiation by immunofluorescence for specific markers. These differentiated cells were then combined into a spheroid by mixing with undifferentiated WJ-MSCs, which have strong immunomodulatory properties3.

RESULTS AND DISCUSSION The undifferentiated perinatal cell spheroids demonstrated significant immunomodulatory properties, reducing the activation of pro-inflammatory cells while promoting anti-inflammatory responses. The spheroids including differentiated cells successfully formed cohesive spheroids4 and showed potential for insulin production. Further studies are planned to evaluate the therapeutic antidiabetic efficacy of these perinatal cell spheroids in modulating immune responses and restoring insulin production.

CONCLUSIONS Perinatal spheroids represent a promising dual approach for T1DM treatment. Their ability to produce insulin and modulate the immune system may address the symptoms and underlying causes of T1DM, offering a comprehensive cellular therapy beyond traditional insulin replacement.

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P4 Environment-friendly supercritical carbon dioxide for long term preservation of perinatal tissues

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KEYWORDS: Amniotic membrane, supercritical carbon dioxide, sterilization, conditioning

INTRODUCTION Perinatal tissues are highly advantageous as allografts for tissue healing because they are immune-naïve with a limited rejection by host.1 Used since the 1st World War to treat deep wounds, interest in perinatal tissues continues to grow, particularly in the ocular and dermal fields.2 To date, methods of preserving the structural, biological as well as mechanical properties of the perinatal tissues have failed.3 Supercritical carbon dioxide (CO2sc), obtained by heating CO2 to 31 °C, was used for long-term storage of bone grafts.4 The aim of this study is to investigate the impact of CO2sc on the long-term preservation of amniotic membrane, umbilical cord amniotic membrane and Wharton's jelly.

EXPERIMENTAL Placenta and Umbilical cords, provided from the Reims Maternity hospital (authorizations from the "Cellule de Bioéthique" DC-2014-2262) are washed several times with phosphate Buffer (PBS 1x) to remove blood contaminants. The vein and arteries are gently removed from the umbilical cord and the Wharton's jelly (WJ) is separated from the amniotic cord membrane (AMc) using clip-on forceps. The fetal membranes are extracted from the placental disc using a scalpel and the chorion is separated from the amniotic membrane (AM). All samples were packed in double Tyvek® bags before CO2sc treatment. The Tyvek® bags were introduced in a bioreactor in which CO2sc is circulated at 150 bar, 40°C for 48 hours as previously described.4 The structural (i.e. DAPI labelling, Scanning Electronic Microscopy and H&S histological staining), physico-chemical (liquid displacement, porosity test and degree of cross-linking) and biological (cytotoxicity and hemocompatibility) characterizations of the treated and untreated (control) samples were performed. The experiments were performed with a minimum of four samples per experiment and the statistical analysis was conducted using GraphPad Prism.

RESULT AND DISCUSSION The presence of nuclei (HES-stained histological sections and DAPI labelling) suggests that CO2sc provides devitalized tissues. SEM observations did not show matrices destruction of tissues following the CO2sc treatment. However, the liquid displacement with water and absolute ethanol indicated respectively a decrease in ECM swelling rate (3 times less than the controls; $p < 0.0001$) and porosity (1.3 times less than the controls; $p < 0.0001$) after CO2sc treatment, while no modification of the collagen chemistry was noticed. Taken together these results suggest that the decrease in the ECM swelling could be attributed to the decrease in the amount of glycosaminoglycans. Finally, the preliminary biological assays revealed that CO2sc treatment did not affect both cytotoxicity and hemocompatibility of the perinatal tissues.

CONCLUSIONS Supercritical fluid is a versatile treatment for perinatal tissues preservation and conditioning, opening a new route of allograft preservation with environment-friendly supply. A deeper biological and mechanical characterization of the tissues are ongoing.

1 - Deus et al., Perinatal Tissues and Cells in Tissue Engineering and Regenerative Medicine
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3 - Fairbairn et al., The Clinical Applications of Human Amnion in Plastic Surgery
4 - Mitton, Rappeneau, et Bardonnet, Effect of a Supercritical CO2 Based Treatment on Mechanical Properties of Human Cancellous Bone

Scanning electron microscopy of the effects of preservation methods on morphology of the human amniotic membrane sub-regions

P5

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Keywords: amniotic membrane, placental amnion, reflected amnion, scanning electron microscopy, preservation

INTRODUCTION Amniotic membrane (AM) has attracted attention as a biomaterial for regenerative medicine due to its biological and mechanical properties, including anti-inflammatory, immunomodulatory, antifibrotic, epithelialization, stability, resistance and flexibility. These properties are attributed to their morphological characteristics and the soluble and insoluble bioactive factors produced by their cells and present in the membrane stroma, making it essential to preserve its integrity, so that the membrane can exert its therapeutic effects. Different membrane preservation methods have been proposed in the literature including glycerol preservation, hypothermic (4ºC), freeze-drying, dehydrated and cryopreservation (-80ºC), each of them presenting advantages and disadvantages, and must be carefully explored to guarantee the quality of the membrane as a biomaterial in different clinical applications. Furthermore, due to the thickness and complexity of AM's multiple structural layers, each method affects its structural integrity differently. In this context, the objective of this study was to evaluate the morphology of AM in placental amnion (PA) and reflected amnion (RA), and on its sides, epithelial and mesenchymal, after different preservation methods, by means of scanning electron microscopy (SEM).

MATERIAL AND METHODS This study was approved by the Research Ethics Committee of the University of Vale do Paraiba under the protocol number 5.172.755. Immediately after processing with penicillin/streptomycin and amphotericin B, the membrane was divided into 4 sub-regions according to its position relative to the umbilical cord: Central (R1), Intermediate (R2), Peripheral (R3) and Reflected amnion (R4). In the control group, the AM fragments were immersed in fixative; in the fresh group, they were immersed in DMEM at 24°C for 18h; in the cryopreserved group, they were immersed in DMEM/glycerol 1:1 medium at -80°C for 30 days. After the preservation time in each experimental group, the AM fragments from each of the regions were processed for SEM.

RESULTS AND DISCUSSION In the control group, the central region (R1) and reflected amnion (R4), showed a very similar morphology among all groups. On the epithelial side, the surface was uniform/homogeneous, with polygonal/hexagonal shaped cells, slightly globose for R1 and flattened for R4, mosaic pattern, well-defined intercellular junction, plump, dense and clustered microvilli in the apical region and edges of the cells. The R2 and R3 regions showed cells with a more circular shape, increased intercellular junction, and flattened and collapsed microvilli to the cells. In the fresh group, all sub-regions demonstrated cells in different sizes, loss of definition of intercellular junctions and microvilli, with the R3 showing the greatest alterations and the R4 the smallest ones. However, the cryopreserved group presented morphological aspects similar to the control group. On the mesenchymal side of AM, variations in microscopic aspects were smaller, demonstrating little differences between the four sub-regions. All groups demonstrated a dense and compact collagenous network, with a slightly variation only in the conformation of the surface, both wavy and smooth surface, which is considered normal aspect of the membrane structure.

CONCLUSION The cryopreservation using DMEM/glycerol was found optimal for preserving the epithelial and mesenchymal morphological integrity in all sub-regions of AM indicating the feasibility of this method in banking AM for its use in regenerative medicine.

P6 Human hepatocarcinoma cells undergo apoptosis process after amniotic membrane conditioned medium treatment

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Keywords: Human amniotic membrane, amnion stem cells, apoptosis, hepatocarcinoma, antitumoral properties

INTRODUCTION Recently, the placental stem cells have been positioned as a central tool for the regenerative and reparative medicine. Their therapeutic potential to treat different diseases, including cancer, has been highly reported. There is plenty evidence about the anti-tumoral effects of the human amniotic membrane given by their antiproliferative, antiangiogenic and propoptotic properties (1). Liver cancer is the fifth cause of cancer in the world, with a poor prognosis and survival. Alternative treatments to radio- or chemotherapy have been searched (2). We and other groups demonstrated the antitumoral effects of the amniotic membrane and their stem cells (3, 4), but there is still a great lack of knowledge about the molecular and cellular mechanisms involved. We have previously showed that the amniotic membrane conditioned medium (AM-CM) inhibits hepatocarcinoma cells proliferation and survival, and promotes HepG2 cells apoptosis. In this work, we aimed to analyse the AM-CM propoptotic effect in a more aggressive hepatocarcinoma cellular model, Huh-7.

MATERIALS AND METHODS The human membrane conditioned medium was obtained as we previously described (1). HepG2 and HuH-7 cells were cultured in 10 cm plates in DMEM-F12 media with 10% FBS. After 24 h, the medium was removed and diluted AM-CM (1/2 and 1/4) was added. DMEM 10% FBS and DMEM 0% FBS were used as control treatments. Microscopy, MTT, Western blot (WB), immunofluorescence (IF) and qRT-PCR assays were used.

RESULTS First, we observed cell morphology changes by bright field microscopy. Hepatocarcinoma HepG2 and Huh-7 cells shrinked and cytoplasm density increased after AM-CM treatment. We have also analysed the diversity of AM-CM effects on cell viability, since they are obtained from different human placentas with intrinsic variability. MTT viability assay confirmed this variability. Considering these results, we have treated Huh-7 cells with the more effective AM-CM media. We determined a significant increase in Bax/Bcl-2 ratio expression measured by qRT-PCR and WB. We also observed by IF, that AIF, a proapoptotic mitochondrial protein, migrates to nucleus after AM-CM treatment. Cytochrome-c expression also increased, measured by WB. Finally, we have found by IF that there is a significant increment in Caspase-3 cleavage.

CONCLUSION The AM-CM induces apoptosis in hepatocarcinoma cells. Our results position amnion derived cells as emerging candidate for alternative antitumoral treatments.

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Isolation and differentiation of human amniotic mesenchymal stem cells into corneal endothelial cells

P7

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INTRODUCTION The amnion of the human placenta is a source of multipotent and pluripotent stem cells, including human amniotic mesenchymal stem cells (hAMSCs). HAMSCs possess immunomodulatory, anti-fibrotic, and anti-inflammatory properties [1], as well as differentiation potential [2]. Moreover, they express embryonic and pluripotency markers, do not express telomerase, and are non-tumorigenic [3]. These properties make hAMSCs a valuable and non-controversial tool for application in tissue engineering. Corneal diseases are the fourth principal cause of blindness worldwide, such as corneal endothelial dysfunction. However, the available treatments have several obstacles. Recently, mesenchymal stem cells (MSC) have been spotlighted as an alternative corneal endothelial cell source because of their origin in the walnut crest [4]. The aim of this work was to isolate and differentiate hAMSCs into corneal endothelial-like cells.

MATERIALS AND METHODS Corneal endothelial cells (CEC) differentiation was assayed by a specific CEC-induction medium. Western blot (WB), qRT-PCR, immunofluorescence (IF), and MTT assays were performed.

RESULTS AND DISCUSSION First, we successfully implemented an isolation protocol using trypsin-collagenase digestion. This process yielded approximately 1,5×107 cells with 70% viability, as determined by Trypan Blue staining. The phenotypic characteristics of isolated cells were analysed by flow cytometry. The results showed that 90% of the cells were positive for MSC markers (CD73, CD90, CD105) and negative for epithelial (CD326) and hematopoietic markers (CD45, Gly A). Then, we have analysed the expression of some CEC-specific markers, after differentiation treatment. We observed a significant increase in ZO-1, Na+K+/ATPase α1, collagen VIII α1, and PITX2 expression, measured by qRT-PCR and WB. We also found a significant increment in apical localization of ZO-1 and Na+K+/ATPase α1 during hAMSCs differentiation, evaluated by IF. In addition, differentiated hAMSCs presented typical CEC morphology, with hexagonal/polygonal-like cells. Moreover, the CEC-induction medium increased cell viability for 16 days, measured by MTT assay.

CONCLUSIONS Our results suggest that hAMSCs are able to differentiate into corneal endothelial-like cells by applying a specific CEC-induction protocol. These findings will allow us to investigate their application in the ophthalmological health field.

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P8 Unmasking the Threat: Microplastics-Derived BPA and its Impact on Mesenchymal Stromal Cells from the Amniotic Membrane

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INTRODUCTION Concerns about microplastics pollution have risen due to their environmental and health risks, with particular attention to Bisphenol A (BPA), a toxic by-product of microplastic degradation that affects multiple organs. Microplastics, which are smaller than 1 mm1, can enter the human body through ingestion, inhalation, skin contact, and even maternal-fetal transmission2. Indeed, recent studies have detected microplastics in both the placenta and breast milk, underscoring their potential to affect human health from the earliest stages of development3. During pregnancy, the placenta is essential for fetal development, and the amniotic membrane, which surrounds the baby in the amniotic cavity, protects against harmful substances and pollutants to which the mother may be exposed4. Based on the known harmful effects of microplastics, and on the evidence that they are found in the human placenta, our study aimed to investigate how BPA can impact on properties and functions of mesenchymal stromal cells isolated from the amniotic membrane (hAMSC) of the human term placenta.

MATERIALS AND METHODS Mesenchymal stromal cells were isolated from the amniotic membrane of human placentae, collected from healthy women following vaginal delivery or caesarean section at term. hAMSC were treated with increasing BPA concentrations (0.05, 0.1, 0.2, 0.3, 0.35, and 0.4 μM) and functional evaluations were performed using different experimental assays.

RESULTS Our findings revealed that increasing BPA concentrations reduced cellular viability in a dose-dependent manner and triggered the production of reactive oxygen species (ROS) at mitochondrial level. This increased oxidative stress resulted in the stabilization of p53 and the activation of both senescent and apoptotic pathways in hAMSC.

CONCLUSION This research sheds light on the mechanism of action of BPA and provides new insights into the potential implications of BPA exposure on the functionality of the amniotic membrane during fetal development.

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Role of Amniotic membrane mesenchymal stromal cells in wound healing

P9

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Keywords: Conditioned media; wound healing; chronified model; mesenchymal cells

INTRODUCTION During wound healing, migration of keratinocytes onto the newly regenerated extracellular matrix is crucial for complete healing. Generally speaking, alteration of transforming growth factor (TGF-ß) can lead to chronic wounds, hindering natural healing. The application of amniotic membrane (AM) at wounds halted at the wound healing process (e.g., chronification, diabetic foot ulcer) has proven very successful at restarting wound healing, in particular re-epithelialization. AM has two types of cells: human amniotic epithelial cells (hAECs) and human amniotic mesenchymal stromal cells (hAMSCs). Identifying key cells responsible for AM's effects could lead to improved wound healing strategies, potentially reducing dependence on donor availability or clean room facilities.

EXPERIMENTAL We isolated and purified hAMSCs cells from placenta donations. We tested the conditioned media from those on a chronified model of HaCaT keratinocyte (SSTC-HaCaT)(1) that reproduce the physiological environment found in a chronic wound. Cells were analysed for the expression of a panel of mesenchymal markers: CD73, CD90, CD105; and epitelial markers: epCAM, CD166 and SSEA-4. Cells were culture in the recommended media, and when confluence was reached, growing media was removed and a different non-specific media was used for cell conditioning. Then, conditioned media were used to stimulate SSTC-HaCaT.

RESULTS AND DISCUSSION The expression of genes related to cell cycle arrest CDKN1A (p21) and CDKN2B (p15); and inflammation (IL6); were attenuated by hAMSCs conditioned media also producing the overexpression of cycline A2 (CYCA2). Moreover, we have shown that HaCaT under serum starved condition or TFGβ stimulation overcome cell cycle arrest when stimulated with hAMSCs conditioned media, correlating this results with the gene expression response found (2).

CONCLUSIONS We have observed that conditioned media from hAMSc reproduce some effects of AM on HaCaT cells. Our findings point out that the secretome of hAMSc is capable of inducing cell cycle re-start of HaCaT, together with a strong cell migration and activation of critical cell-proliferation genes for. All these data suggest that the effect of AM on difficult healing wounds could be mainly due to the contribution of human amniotic mesenchymal stromal cells.

1 - https://doi.org/10.3390/ijms24076210
2 - https://doi.org/10.1371/journal.pone.0135324

P10 Conditioned medium from human amniotic mesenchymal stromal cells inhibits LPS-induced inflammasome activation in macrophages

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Keywords: Human amniotic mesenchymal stromal cells, Conditioned medium, LPS, Macrophages, Inflammation, Inflammasome

INTRODUCTION We have demonstrated that conditioned medium derived from the culture of human amniotic membrane mesenchymal stromal cells (CM-hAMSC) acts on myeloid cells, in particular decreasing pro-inflammatory M1 macrophage and promoting the induction of anti-inflammatory M2 macrophage phenotype [1]. It is known that macrophages respond to harmful and inflammatory stimuli, such as lipopolysaccharides (LPS) from Gram-negative bacteria, by triggering inflammasome activation. Inflammasome activation is recognized to lead M1 macrophage polarization by inducing metabolic reprogramming, the generation of reactive oxygen species (ROS), and the release of pro-inflammatory cytokines [2]. Among different types of inflammasomes, the Nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome is the best characterized. In the canonical activation, it requires two activation signals which induce the oligomerization of the apoptosis-associated speck-like protein containing a caspase activation and recruitment domain [CARD] (ASC) with NLRP3 and caspase-1. This complex drives the activation of the inflammatory protease, IL-18 and IL-1β production, as well as pyroptotic cell death [3]. Hence, considering the role of inflammasome activation in leading M1 polarization, the aim of this study is to investigate the ability of CM-hAMSC to affect LPS-induced inflammasome activation.

MATERIALS AND METHODS M1 macrophages were generated in vitro by culturing monocytes with GM-CSF for 5 days. Then, inflammasome activation was achieved by stimulating the macrophages with LPS plus adenosine triphosphate, in absence or presence of CM-hAMSC. Five hours after treatment, inflammasome activation was evaluated as aggregation of the ASC protein into a multiprotein complex known as the "ASC speck", the production of inflammatory cytokine IL-1β, and the mitochondrial ROS formation in macrophages through flow cytometry, ELISA, and fluorescence microscopy analyses.

RESULTS AND DISCUSSION Preliminary findings indicate that CM-hAMSC inhibits inflammasome activation, affecting IL-1β release, and reducing the accumulation of ROS in macrophages. CM-hAMSC does not seem to modulate ASC speck formation, suggesting that IL-1β reduction induced by CM-hAMSC is downstream of the inflammasome complex, but further investigations are needed.

CONCLUSIONS We assessed whether CM-hAMSC could suppress inflammasome activation in M1 macrophages clarifying the anti-inflammatory effects of CM-hAMSC on M1 macrophages. Nonetheless, additional studies are needed to fully understand the mechanisms by which CM-hAMSC could modulate inflammasome activation in macrophages.

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Conditioned medium from amniotic membrane affects ovarian cancer cell viability and migration

P11

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Keywords: ovarian cancer, migration, human amniotic membrane, conditioned medium

INTRODUCTION Ovarian cancer accounts for more deaths than any other cancer of the female reproductive system. Nowadays surgical resection followed by chemotherapy is the standard of care. However, a number of patients are faced with recurrence due to tumor dissemination and acquired chemoresistance. Therefore, novel alternative approaches are urgently needed. The human amniotic membrane (hAM) has been proposed for various clinical applications. The hAM consists of two cell populations, hAMSC, and hAEC. In our previous research, we showed that hAMSC can effectively inhibit tumor cell proliferation, while other studies have highlighted the anti-tumor effects of hAEC. In this study, we aimed to explore the potential anti-tumor strategy using the intact hAM, eliminating the need for cell isolation. Given the growing understanding that the effects attributed to cells from the hAM are largely mediated by bioactive factors released from these cells, our investigation utilized the conditioned medium derived from hAM (hAM-CM) on ovarian cancer cells in vitro in 2D and 3D models.

MATERIALS AND METHODS The effect of hAM-CM on ovarian cancer cell line (HEY, SKOV-3, OV-90) viability was tested with CyQUANT, clonogenic assays in 2D and spheroid growth and ATP content in 3D. Apoptosis was determined using PI/Annexin V staining and flow cytometry analysis. Cell migration was assessed in 2D by wound healing, transwell assay and time lapse video-microscopy. Invasion was assessed in 3D by embedded spheroid in 3D matrix. Finally, involvement of integrins was evaluated in 2D by adhesion assay and CD24 expression by flow cytometry.

RESULTS Our results indicate that hAM-CM exhibited a dose-dependent inhibition of cancer cell viability in both 2D and 3D models, accompanied by a decrease of p-P70 S6 kinase and increase in apoptosis. Furthermore, hAM-CM inhibits ovarian cancer cell migration and modulates adhesion to fibronectin and collagen in 2D, even though some differences were observed depending on the ovarian cancer cell lines. Interestingly, hAM-CM inhibits CD24 expression, a membrane protein associated with the aggressiveness of human ovarian cancer. Finally, we observed that hAM-CM pretreatment decreases spheroid invasion in geltrex matrix.

CONCLUSIONS These findings suggest that hAM-CM may inhibit the growth of ovarian cancer cell lines both in 2D and 3D models. Moreover, hAM-CM exerted inhibitory effects on migration and invasion, possibly by inhibiting CD24 expression, that, in turn, affects activation of β1 integrin and, as consequence, cell adhesion to extracellular matrix proteins. However, further experiments are required to: i) elucidate different effects of hAM-CM on ovarian cancer cell lines: possibly correlate the results on different ovarian cancer "subtypes" depending on the specific cell line; ii) better understand the mechanisms involved in the axis β1 Integrin/CD24 as anti-tumor effects of hAM-CM

P12 lablife - Changing the concept of cell culture training

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Keywords: reproducibility, standardization, cell culture technique, education

INTRODUCTION One of the biggest global challenges in the life sciences is the reproducibility crisis (1). According to OECD guidance and a number of statement publications, one of the most effective strategies to combat this crisis is to have a special focus on adequate practical laboratory training of students and laboratory staff. Every newcomer to the laboratory has to undergo a one-on-one training until that person is able to carry out tasks in the laboratory correctly and independently. The cell culture laboratory is the most common laboratory for the life sciences. However, preparing students and technicians for high quality research according to OECD guidance is highly time-consuming. This is hard to manage for most universities and research institutions in today's fast-paced and competitive scientific world.

METHODS We developed an educational product which addresses this urgent need. We provide almost 100 high-quality videos focusing on practical work in great detail according to the highest standards of Good Cell Culture Practice (2). The videos cover all important basic skills of the cell culture laboratory, such as good practices, tips and hacks and common mistakes. In addition, we provide training material for download such as protocols and checklists.
To test the effectiveness of the lablife video programme, we compared newcomers to the laboratory who underwent the programme to those who did not watch the videos prior to their one-on-one training sessions.

RESULTS We could show that subjects who underwent the lablife video programme needed 57 % less training time. Furthermore, the error rate was reduced considerably, and, simultaneously, the awareness of doing mistakes increased dramatically. Altogether the level of knowledge of newcomers to the laboratory increased significantly.

CONCLUSION With the lablife video programme, a great amount of time and cost is reduced, and, at the same time, the level of knowledge on practical "how-to" increases significantly. Most importantly, the lablife video programme helps to enhance reproducibility and standardization in life science research.

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Determining the potential of placental tissue derivatives for use in post-mastectomy breast reconstruction: development of a 3D-printed bioscaffold - AmnioPrintCare project

P13

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Keywords: human amniotic membrane; breast cancer; breast reconstruction; 3D bioprinting, biomaterials

INTRODUCTION Breast cancer (BC) is, according to the data from 2020, the cancer with the highest estimated age-standardized incidence rates among women, ranking Serbia the 1st in Europe when it comes to the mortality rate. Current surgical procedures for patients going under the treatment of BC involve mastectomy and breast conserving surgeries. Although considered safe and viable for patients, current breast reconstruction techniques following mastectomy still pose potential complications and do not substantially affect the probability of disease recurrence compared to mastectomy alone. The field of tissue engineering has been intensively growing, with special attention given to the development of different scaffolds to serve as a template for tissue regeneration by providing structural support and biochemical and biophysical cues to guide cell growth and tissue formation. Perinatal tissues including placental membranes have been gaining attention in the field of regenerative medicine as the sources of different derivatives with the potential for use in tissue engineering and therapy. The amniotic membrane (AM) from the placenta has been used in the regeneration of skin and cornea, with numerous studies showcasing its promising therapeutic properties. AmnioPrintCare aims to develop the scaffold biomaterial using human AM homogenate (hAM-h) that could provide breast tissue repair and simultaneously hinder the disease recurrence, given the anti-tumour, regenerative, and anti-scarring effects of hAM. By establishing advanced 3D bioprinting techniques for scaffold fabrication, AmnioPrintCare will enable more accessible and personalized regenerative medicine practices in breast reconstruction.

MATERIAL AND METHODS This project is organized around the following key concepts: 1) to establish 3D printed bioscaffold based on hAM-h; 2) to confirm its biocompatibility with the breast tissue and regenerative potential in vitro and *in vivo*; 3) to confirm its antitumor effect towards BC in vitro and *in vivo*, structured in four working packages. To achieve these goals, we will use a) different technological methods and advanced 3D software and bioprinter for establishment of 3D printed bioscaffold based on hAM homogenate produced by already established protocol at the Institute of cell biology, Faculty of Medicine, University of Ljubljana, b) different cell lines and cell culture techniques, molecular biology analyses to follow its biocompatibility and antitumor effect and c) healthy and BC-induced female BALB/C mice and different cytological and histological methods to follow the effects after the implantation in mice.

EXPECTED RESULTS We expect to develop hAM-h based biomaterial for 3D bioprinting that will preserve and exhibit its regenerative and antitumor effects over an extended period when applied. We also anticipate confirming its biocompatibility with the breast tissue niche cells and its regenerative potential in vitro and *in vivo* related to adipogenesis and angiogenesis along with the antitumor effect towards BC. We believe that this interdisciplinary project will bring advancement in biotechnology and improvements in biomedicine as it could patent the new ‘’bioink” for commercially available bioprinters.

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P14 Identification of potential therapeutic targets by transcriptional profiling of normal and cancerous bladder urothelial cells treated with human amniotic membrane homogenate

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Keywords: urothelium; bladder cancer; human amniotic membrane; RNA-sequencing

INTRODUCTION Bladder cancer is the ninth most commonly diagnosed cancer and is typically divided into non-invasive and muscle-invasive subtypes, each with different molecular profiles (1, 2). Preclinical studies have shown that the human amniotic membrane (hAM) has numerous biological properties. However, the cellular mechanisms underlying its anticancer activity are not well understood (3, 4, 5). In the present study, we investigated the effects of hAM homogenate on normal and bladder cancer cells at the whole transcriptome level.

MATERIALS AND METHODS Bulk RNA-sequencing analysis was performed on untreated muscle-invasive bladder urothelial carcinoma models from T24 cells (n = 4), on human non-invasive bladder urothelial papilloma models from RT4 cells (n = 3) and on bladder normal urothelial models from NPU cells (n = 5), as well as on their respective counterparts treated with hAM homogenate. RNA extraction and integrity analysis, sequencing using the Illumina NovaSeq 6000 platform, and subsequent bioinformatic analysis by the Galaxy Project were conducted to identify differential gene expression profiles and enriched signalling pathways. Validation of RNA-sequencing data was performed by Western Blot analysis and enzyme-linked immunosorbent assay (ELISA).

RESULTS AND DISCUSSION Transcriptomic analysis revealed significant changes in gene expression profiles in bladder cancer cells after treatment with hAM homogenate ($p < 0.05$). Specifically, we observed 3631 and 3759 differentially expressed genes (DEGs) between control and treated T24 and RT4 cells, respectively. In bladder cancer cells, signalling pathways associated with cell cycle and immune regulation were significantly altered compared to untreated controls and normal bladder cells. In contrast, normal bladder cells treated with hAM homogenate exhibited fewer changes in gene expression (237 DEGs), suggesting a selective impact of the homogenate on cancer-associated pathways. Validation experiments are currently underway to confirm the key DEG identified from the RNA-seq data and to further clarify their functional role and therapeutic potential.

CONCLUSIONS Our study provides novel insights into the transcriptomic responses of normal and cancerous bladder urothelial cells to treatment with hAM homogenate. Furthermore, it highlights the homogenate's influence on cancer-associated pathways, paving the way for targeted therapeutic strategies in bladder cancer management.

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Role of perinatal mesenchymal mesenchymal stromal cells of the decidua in the repair of pelvic floor tissue damage

P15

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The pelvic floor is a structure of muscles, ligaments and connective tissue that provides support to the pelvic and abdominal organs, helping them to function properly and preventing them from falling out. Pelvic floor disorders are a group of conditions that affect the pelvic floor tissues and include pelvic organ prolapse (POP), urinary incontinence (UI) and fecal incontinence. UI is the inability to control urination and affects around 300 million people worldwide. UI is a common condition and can range from a minor problem to greatly affecting the quality of daily life. Stress urinary incontinence (SUI) is the most common type of UI among women and occurs when the tissues that support the urethra weaken, with parity being the main risk factor. There are several surgical and non-surgical treatment options for mild to moderate SUI, such as pelvic floor muscle training, vaginal pessary, urethral bulking agents and surgery. Surgical intervention is the recommended treatment option for severe SUI, although patients suffer postoperative complications. The underlying cause of SUI is the weakening of pelvic floor tissues, although the cellular and molecular mechanisms of the pathology are not yet fully understood. Therefore, the search for and development of less invasive therapies for the treatment of SUI remains an important issue and stem cell-based therapy may be an important option to treat this disorder. Mesenchymal stem cells (MSC) are adult stem cells and one of the most attractive sources for stem cell research and therapy. MSC from the maternal side of the placenta, i.e. from the decidua (DMSC, decidual mesenchymal stromal cells), could be an important therapeutic option due to their regenerative potential. The aim of our work is to study whether DMSCs could be used for the treatment of SUI using in vitro and *in vivo* models of the disease. The *in vivo* model is an animal model of SUI caused by parturition, for which vaginal distension was used in female Sprague-Dawley rats simulating maternal birth injuries. Animal experiments were performed with the approval of the Animal Experimentation Ethics Committee. For the in vitro model, cells isolated from SUI patients were used to assess the possible mechanism involved in the regenerative potential of DMSC.

Our results show that DMSC transplantation was effective in improving continence in treated animals by recovering pelvic tissue integrity. In vitro experiments show that the regenerative potential of DMSCs is due to a paracrine interaction resulting in DMSC migration, proliferation of pelvic tissue fibroblasts, and modulation of the pro-inflammatory and ECM-degrading microenvironment characteristic of senescence. In conclusion, DMSC could be an alternative therapeutic option for SUI by counteracting the effects of senescence in damaged pelvic tissue. The results of this research indicate that the application of perinatal mesenchymal stromal cells could be a more effective therapeutic alternative with fewer side effects for patients with SUI compared to the treatments currently used.

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P16 Umbilical cord blood, umbilical cord, amniotic membrane, and placenta - sources of stem cells

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Keywords: umbilical cord blood, umbilical cord, amniotic membrane, placenta, stem cells

INTRODUCTION Umbilical cord blood is an exceptional source of stem cells: hematopoietic, tissue-forming or mesenchymal, various transitional or progenitor cells, and embryonic-like stem cells. Due to biological and other characteristics, umbilical cord blood and cord cells are more readily and quickly available compared to bone marrow or hematopoietic stem cells from peripheral blood and carry a lower risk of transmitting infections that often affect the success of the transplant. The risk of developing graft-versus-host disease after transplantation is also lower. There is no age-related damage to the umbilical cord blood.

CONCLUSIONS The collection of umbilical cord blood is carried out without interfering with the body and is safe and painless for the mother and the newborn, although it can only be accessed after the child is born. The tissues and cells that can be collected during labor and after the birth of a newborn include umbilical cord blood, umbilical cord, amniotic membranes, and placenta. It is becoming increasingly likely that we will need stem cell therapy in the course of our lives. This is reason enough to store umbilical cord blood and tissue, which have great regenerative potential for all tissues and organs and the potential to treat many diseases.

Design and implementation of life-long learning programme for future ATMP professionals

P17

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Keywords: advance therapy medicinal products, educational programme, online courses, workshops

INTRODUCTION Advanced therapy medicinal products (ATMPs) offer great potential for providing long-term disease management. However, translating research findings into benefits for patients is fraught with challenges and requires concerted efforts from various stakeholders, including academic researchers, the biotech industry, regulators and patient advocates. To address these challenges and support the professional development of biomedical scientists entering the ATMP field, we have developed a comprehensive training programme that guides students through all major pillars of ATMP development: Science, Manufacturing, Regulatory/Clinical and Reimbursement.

MATERIALS AND METHODS The training programme takes an integrated approach which includes two complementary learning units: online courses that provide basic knowledge and face-to-face workshops to deepen practical understanding of ATMPs. Learning methods used in the programme include pre-recorded online lectures, online quizzes, live lectures with discussions, best practise presentations, interactive problem solving, case studies, project assignments with group work, as weell as independent individual work and reading. The online courses and all learning material are available to students via the Moodle platform.

RESULTS AND DISCUSSION The integrated learning model has proven successful in improving participants' knowledge and skills. Upon completion of the educational programme, participants can explain the importance of material and methods selection in ATMP development, describe key equipment and workflow requirements for ATMP production, and implement appropriate steps to comply with GMP requirements. They also can plan risk assessments and facility layouts for biosafety systems, illustrate the importance of clinical trials in the implementation of ATMPs, present business strategies for biotech companies and critically evaluate the development and clinical trial processes of approved ATMPs. Participants rated the online courses on average 8.5/10 and the workshops 9/10, confirming that the programme contributed to their professional development.

CONCLUSIONS The comprehensive curriculum of our training programme equips participants with the necessary skills to navigate the complex and interdisciplinary landscape of ATMP development. Successful implementation and positive feedback highlight the effectiveness of the programme and its potential for adaptation in other areas of biomedical education.
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P18 Immuno-endothelial co-culture cell model to study patient response to Vedolizumab therapy in Crohn's disease

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Keywords: *in vitro* model, Crohn's disease, Vedolizumab, response, non-response

INTRODUCTION Vedolizumab (VDZ) is a humanized monoclonal antibody, recognized as one of the safest and relatively new biologics for treatment of Crohn's disease (CD). It targets the α4β7 integrin and blocks leukocyte trafficking to the gut, yet its molecular mechanisms remain unclear and require further research [1]. Regardless of its efficacy in many patients, non-responders pose a significant challenge. The reasons for this varied response are not completely understood, and the number of identified predictive biomarkers for response prediction to VDZ therapy in CD is rather scarce [2]. Our study aims to elucidate the mechanisms behind response and non-response to VDZ treatment by developing an in vitro model using a transwell system, potentially upgraded with fluidic incorporation.

MATERIALS AND METHODS Our in vitro model is based on a transwell system with incorporated primary blood outgrowth endothelial cells (BOECs) from CD patients and healthy controls, seeded on a thin collagen layer in a porous well insert. During establishment of the model, human microvascular endothelial cells-1 (HMEC-1) will be used instead of BOECs. With the addition of isolated peripheral blood mononucleated cells (PBMCs) from patients, upper chamber will mimic a blood vessel. With addition of chemoattractant in the bottom chamber, PBMC transmigration will be induced, simulating the chronically inflamed gut in CD. VDZ will be used to study its effect on cell transmigration in patients and healthy controls. We will quantify transmigration using Alamar Blue, identify transmigrated cell types with flow cytometry (FC) and perform immunofluorescence microscopy (IFM). To further characterize the differences between responders and non-responders to VDZ therapy, PBMCs will be analysed at RNA and protein levels using quantitative-polymerase chain reaction (q-PCR), RNA-sequencing (RNA-seq), IFM and western blot (WB).

EXPECTED RESULTS Our goal is to establish an in vitro model that can be used to study and predict (non)response to VDZ therapy in CD. We will distinguish PBMCs from known (non)responders to lay the groundwork to study and predict therapy outcomes. This study will also potentially help to elucidate a part of CD pathogenesis itself. With the growing importance of personalized medicine, we therefore strive to elucidate the molecular mechanisms of VDZ (non)response, aiding the discovery of molecular biomarkers for personalized treatment of CD patients.

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Neureregenerative potential of amniotic membrane extract in diabetic keratopathy

P19

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Keywords: amniotic membrane extract, diabetic keratopathy, neurotrophic keratopathy, corneal innervation, corneal sensitivity, neuroregeneration

INTRODUCTION Diabetes mellitus causes structural and functional alterations to various structures in the human cornea - diabetic keratopathy (DK). DK is characterized by impaired corneal innervation and corneal sensitivity, which can cause neurotrophic keratopathy, leading to dry eye disease, persistent epithelial defects, corneal ulcers and perforation. Current management is based on promoting epithelial healing, treatments promoting neuroregeneration are, however, not available. The amniotic membrane (AM) has a known indication in the treatment of various diseases of the ocular surface. Studies support the effectiveness of AM in promoting corneal epithelial healing, it has, however, not yet been studied whether such clinical efficacy lies in promoting corneal nerve regeneration. Recently, AM extract (AME), which does not require an operative procedure, has been used in treating ocular surface disease.

MATERIALS AND METHODS Thirty patients with type 2 diabetes, symmetrical bilateral decrease in corneal sensitivity (less than 50 mm, measured by Cochet-Bonnet esthesiometer), and with no significant epithelial defects will be included in a prospective randomized controlled trial. One eye will be treated with AME eye drops twice daily for 28 days and placebo will be applied to the contralateral eye. Ocular examination, tear film analysis and evaluation of the corneal sensitivity and innervation will be carried out at the baseline, after 4 weeks and after 12 weeks from the baseline. Effectiveness of AME in corneal neuroregeneration will be defined as an increase in corneal sensitivity in the treated eye, measured by Cochet-Bonnet esthesiometer, and by an increase in corneal innervation in the treated eye, measured by In-vitro confocal microscopy of the cornea and quantified by a CCMetrics programme.

EXPECTED RESULTS We expect an increase of corneal innervation and sensitivity in the eye, treated with AME, and the effect of neuroregeneration to persists also after cessation of treatment. We also expect an improvement of the tear film parameters. Possible AME neuroregenerative potential could lead to an effective treatment of patients with decreased corneal sensitivity and innervation of different etiologies.

P20 In Vitro Assessment of Ciliotoxicity and Cytotoxicity Following Acute and Repated Exposure to Nasal Formulations: Implications for Safety Studies

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Keywords: *in vitro* testing, ciliotoxicity, cytotoxicity, nasal formulations, acute and repeated exposure

INTRODUCTION Nasal drug formulations have emerged as a promising approach for delivering therapeutic drugs either locally to the sinonasal mucosa or systemically through direct absorption into the bloodstream. The growing field of potential nasal therapies includes nasal vaccinat ion and even treatment of neurodegenerative diseases. However, it is crucial that these formulations do not harm the cilia and mucosa lining the nasal epithelium, which are essential for the respiratory tract's first line of defence through mucociliary clearance (1). One crucial factor in determining mucociliary clearance is the ciliary beat frequency (CBF) (2). In light of this, the primary objective of this study was to utilize high-speed digital imaging to accurately measure CBF in a human nasal *in vitro* model, in order to assess the safety of single and repeated dose treatments with nasal drug formulations through ciliotoxicity studies. Additionally, this study also aimed to evaluate potential cytotoxic effects of the tested formulations on the nasal epithelium in vitro (3, 4).

MATERIALS AND METHODS The measurement setup for CBF consisted of two main components: human nasal MucilAirTM *in vitro* models and high-speed phase-contrast microscopy. To validate the CBF measurements setup, it was tested with benzalkonium chloride, a commonly used preservative with cilio-inhibitory effect. Following successful validation, the nasal MucilAir™ models were treated with a single dose of the mometasone nasal spray (Mommox®/Mometasone Sandoz®), both at the undiluted concentration and at clinically relevant doses (3). In addition, the long-term effects of repeated doses of mometasone nasal spray were investigated over a period of 2 weeks, with daily treatments of the *in vitro* models with undiluted and clinically relevant doses (4). Cilio- and cytotoxicity were determined through CBF measurements and ultrastructural analysis with scanning and transmission electron microscopy, respectively (3, 4).

RESULTS AND DISCUSSION A single-dose treatment of mometasone nasal spray demonstrated a short-term cilio-stimulatory effect, and a dose-dependent long-term effect. Post-treatment analysis revealed unaltered ultrastructure in the MucilAir™ model (3). In a repeated-dose experiment, clinically relevant doses were not cytotoxic or ciliotoxic, while a dose- and time-dependent (cumulative) effect on ciliary activity and cytotoxicity was observed (4).

CONCLUSION *In vitro* assays consisting of nasal epithelium, ciliotoxicity, and cytotoxicity analyses hold immense promise for screening and evaluating the safety profile of nasal drugs. This is important as there is a growing emphasis on utilizing alternative methods to reduce the need for animal testing.

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Co-culture spheroids of normal and bladder cancer cells as a relevant 3D in vitro model

P21

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Keywords: bladder cancer, monoculture, co-culture, spheroids, microscopy

INTRODUCTION Bladder cancer remains one of the leading causes of cancer death in men worldwide (1). About 25% of BC cases proceed to the muscle-invasive form, known for its lethal metastatic relapses (2). Traditional two-dimensional cell cultures have been a common in vitro model in preclinical drug screening, but have limitations to accurately replicate the complex cellular interactions within the tumour microenvironment. However, recent three dimensional (3D) in vitro models, such as spheroids, have shown promising results not only in drug screening but also as a model to study cancer progression. However, most of the current bladder cancer spheroids consist only of monoculture of cancer cells. The aim of this study was to develop an advanced co-culture spheroid model incorporating both invasive human bladder cancer T24 cells and normal human urothelial SV-HUC-1 cells.

MATERIALS AND METHODS Monoculture and co-culture spheroids of SV-HUC-1 and T24 cells were grown on ultra-low attachment 96-well plates for 2 or 7 days. The seeding density for monoculture spheroids was 100,000 cells per well. To establish co-culture spheroids, T24 and SV-HUC-1 cells were seeded with different ratios: 1:1 (100,000 cells each), 1:4 (25,000 T24 cells vs. 100,000 SV-HUC-1 cells), and 4:1 (100,000 T24 cells vs. 25,000 SV-HUC-1 cells). Spheroids were examined using phase-contrast microscopy and scanning electron microscopy. Additionally, double immunofluorescence labelling of E- and N-cadherin on paraffin sections was performed and analysed by fluorescence microscopy.

RESULTS AND DISCUSSION The characterization of morphology and ultrastructure confirmed that both monocultures and co-cultures of cancer and normal urothelial cells successfully formed 3D spheroids irrespective of seeding ratios. After 2 days, both monoculture and co-culture spheroids were still in the process of aggregation, and after 7 days they formed compacted spheroids with a rounded shape. Normal urothelial cells were tightly connected, while bladder cancer cells were poorly connected with large intercellular spaces present, in both monoculture and co-culture spheroids. In co-culture spheroids, cells of the same type were grouped together, as revealed by the expression of N-cadherin in bladder cancer cells and E-cadherin in normal urothelial cells. The distribution of normal and cancer cells within spheroids depended on the seeding ratio and it was different in 2-day compared to 7-day spheroids.

CONCLUSION We present a novel model of co-culture spheroids consisting of human normal and bladder cancer cells, which has the potential to enhance our understanding of the mechanisms of bladder carcinogenesis and treatment for bladder cancer, ultimately leading to improved outcomes for patients.

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P22 Pathogenicity assessment of human *Escherichia coli* strains using an *in vitro* biomimetic model of porcine urothelium

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Keywords: porcine, *E. coli*, microscopy, virulence, cytokine, pathogenicity

INTRODUCTION The bacterium *Escherichia coli* (*E. coli*) is usually a commensal microorganism that lives in the lower gastrointestinal tract of humans and contributes to the health of its host. But *E. coli* can also be pathogenic. Extraintestinal uropathogenic strains (UPEC) are capable of colonizing and causing urinary tract infections in humans. Several models have been developed to study different aspects of UPEC pathogenicity but all have disadvantages. Hence, we established a biomimetic in vitro model of normal porcine urothelium to assess the pathogenicity of UPEC and other human *E. coli* strains.

MATERIALS AND METHODS Normal porcine urothelial (NPU) cells were cultured to a highly differentiated stage and subsequently infected with bacterial suspensions of *E. coli*. After a three-hour incubation, the viability of NPU cells was determined. The model was analysed by electron microscopy to assess the adhesion and invasiveness of *E. coli* strains and possible ultrastructural changes to the NPU cells due to the pathogenic action of the bacteria. Cell culture supernatants were, following infection, also analysed to determine the presence of nine cytokines secreted from NPU cells in response to infection. Genotyping of the strains (virulence-associated genes, phylogenetic groups, type of core lipid, type of lipopolysaccharide and certain serotypes) as well as determining the strains' ability to form biofilm were performed. We also analysed possible statistical correlations between strain pathogenicity and other beforementioned strain characteristics.

RESULTS AND DISCUSSION In experiments using control strains (UPEC strains J96 and 536, commensal SE15 strain and laboratory MG1655 strain), both UPEC strains significantly reduced the viability of NPU cells as opposed to strains MG1655 and SE15. The *in vitro* biomimetic model thus clearly demonstrated the ability to show a difference in the pathogenicity of the tested bacterial strains. The model was then tested using a broader range of human urinary and faecal strains. Great variety in the ability of individual strains strains to reduce NPU cells viability was demonstrated, indicating their different nature and pathogenic potential. Scanning and transmission electron microscopy results showed the presence of bacterial cells adhering to the NPU cells, changes in the normal cell ultrastructure as well as the presence of bacterial cells intracellularly as evidence of pathogenicity. The model also responded to the infection with E. coli strains by secreting inflammatory cytokines, as each strain provoked a different pattern of cytokine secretion. Statistical analysis revealed significant correlations between the group of highly pathogenic strains and the following virulence-associated genes: *cnf1, hlyA, clbAQ, papGIII, sfaDE* and *tcpC* and also a correlation between the group of strains inducing a high fold change of total cytokine synthesis and the cnf1 gene.

CONCLUSIONS By establishing a biomimetic *in vitro* model, we showed that the pathogenic potential of UPEC strains can be evaluated in a rapid, financially appropriate and ethically unquestionable way and that the model is relevant for both the medical and commercial sectors, including the pharmaceutical industry.

Epigenetic modulation of T helper cells by Decidual Mesenchymal Stromal Cells

P23

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Keywords: Decidual Mesenchymal Stromal Cells, Th lymphocytes, multiple Sclerosis, experimental autoimmune encephalomyelitis, IL-17; EZH2.

INTRODUCTION Clinical studies in Multiple Sclerosis (MS) show an increase in T helper (Th) cells Th17 within diagnosed patients. This finding correlates with Experimental Autoimmune Encephalomyelitis (EAE) model. In EAE, IL-17 (Th17 cytokine) plays a role in disease onset and progression and Th17 cells are involved in the formation and accumulation of lesions. Moreover, they are linked to neurodegeneration due to sustained inflammatory processes, which can lead to irreversible damage to myelin and axons. Conversely, the involvement of Th2 cells triggers immunoregulatory mechanisms mediated by IL-4, which alleviate the disease. Th cells display remarkable plasticity in their phenotype, exhibiting alterations in their functional pattern through regulation of cytokines and/or epigenetic factors, such as chromatin state modulation. Epigenetic modifications are mediated in part by the Trithorax group complex and the Polycomb group complex, which activate and repress transcription, respectively. EZH2, a component of Polycomb complex, catalyses trimethylation of histone 3 lysine 27 (H3K27me3) and silences expression of related genes. Our previous research has demonstrated that placental Decidual Mesenchymal Stromal Cells (DMSC) delay the onset of EAE and prevent severe symptoms. *In vivo* efficacy of DMSCs is associated with an elevated production of IL-4 and a reduction of IL-17 in CD4+ cells of treated animals. We also concluded that decrease in IL-17 was mediated by factors found in the DMSC secretome. This study proposes a mechanism by which DMSCs redirect the Th17 to Th2 response in EAE. Our results show an in vitro impact of DMSC secretome on CD4+ epigenetic marks H3K27me3 and H3K4me3 during the process of Th2 and Th17 phenotype definition.

EXPERIMENTAL DESIGN In order to detect the presence of Ezh2 at two gene loci, one expressed preferentially in Th2 (Gata-3) and the other specifically in Th17 (IL7), we carried out chromatin immunoprecipitation (ChIP) assays on Th2 and Th17-polarised murine CD4+ cultures, in the presence or absence of DMSC secretome. The cells were cross-linked, lysed and chromatin was fragmented and immunoprecipitated with αH3K4me3, αH3K27me3, αEzh2 and αH3. Chromatin fragments were amplified by qPCR using primers for the IL17 and Gata-3 loci.

RESULTS AND DISCUSSION Our analysis revealed that the level of occupancy of the IL-17 locus by EZH2 is lower in Th17 cells than in Th2 cells. This indicates that there is a greater potential for IL-17 expression in Th17 cells than in Th2 cells. In contrast, in the presence of the DMSC secretome, the Menin 1-mediated H3K4me3 positive mark is significantly increased in Th2 cells at the Gata-3 locus, whereas it is decreased at the IL-17 locus in Th17 cells.

CONCLUSIONS The observed outcomes are likely indicative of a DMSC-mediated phenotypic reorientation from Th17 to Th2. Nevertheless, further functional studies are required to provide a definitive conclusion. What is certain is that these findings provide a compelling rational for further investigation into the epigenetic mechanisms triggered by DMSCs and their potential relationship with the *in vivo* therapeutic benefits on autoimmune disease mediated by the adaptive immune system.

P24 *In vitro* Differentiation of Amniotic Membrane and Amniotic Epithelial Cells into Retinal Pigment Epithelium

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INTRODUCTION Age-related macular degeneration (AMD) is a disease that affects the outer retina, causing the irreversible atrophy of the retinal pigment epithelium (RPE). Current therapies aim at delaying the progression of the disease but fail to promote regeneration and definitive cure. Regenerative medicine strategies have been tested in which the damaged RPE was effectively replaced by autologous transplantation. However, this approach is likely applicable only to a limited number of patients. Preliminary analyses, show that human amniotic epithelial cells (hAEC), derived from term placenta, natively express some marker genes typical of RPE. Therefore, we examined the effect of specific factors that are known to play a key role during retinal embryogenesis in inducing full differentiation of hAEC into mature RPE. Moreover, we tested the possibility to differentiate either amniotic membranes fragments (AM) or isolated hAEC.

MATERIAL AND METHODS Amniotic membranes (AM) from term placentas were either cut into sections to obtain AM fragments or digested with 0.05% Trypsin-EDTA to isolate hAEC. These AM fragments and the cultured hAEC were subjected to a RPE differentiation protocol for up to 7 days. The protocol used various doses and combinations of specific factors including Sonic Hedgehog, Noggin and CHIR99021. Samples were taken at different time points for gene expression analysis using qRT-PCR, and for protein expression analysis using immunofluorescence.

RESULTS AND DISCUSSION The use of hAEC still attached to the AM seems to benefit cellular growth and their environment, compared to the isolated counterparts; in fact, intercellular interactions are well maintained, cellular stress, as assessed by morphological examination is clearly reduced, and the epithelium seems healthier and preserved throughout the protocol. Some of the selected factors, particularly when in combination, promoted an increased expression of typical RPE markers, both at gene and protein level. In particular, tight junction protein 1 (TJP1) was detected at intercellular junctions at the end of treatment; the expression of transcription factor PAX6 was upregulated at day 7, although it only showed cytoplasmic localization, suggesting the cells were not fully competent. On the other hand, transcription factor OTX2 was found to be functionally expressed at the nuclear level only in rare cells.

CONCLUSIONS The preliminary results of this study suggest that hAEC can acquire some phenotypical and functional features of RPE in vitro. Moreover, the use of intact AM fragments would be more practical for future clinical applications.

Optimization of electroporation of primary cells from different human tissues to study cell fusion in tissue regeneration

P25

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Keywords: electroporation; primary human cells, cell fusion, tissue regeneration

INTRODUCTION Tissue regeneration is a highly regulated physiological process aiming both at maintaining tissue homeostasis, as well as restoring functionality of injured tissues and even organs. Tissue resident cells, the mesenchymal stem/stromal cells are the main players in tissue regeneration (1). Following their activation, proliferation, immunomodulation and differentiation these cells repair and regrow tissues. An important mechanism of their tissue regenerating ability is cell fusion. Our study aims at optimizing the electroporation parameters for electrofusion of primary cells isolated from various human tissues, i.e. cartilage and perinatal tissues such as placenta, amniotic membrane, umbilical cord, and umbilical cord blood, setting up the basis for studying cell fusion.

EXPERIMENTAL Primary human cells of various types (chondrocytes and MSCs) from various donors (N=3) were isolated, expanded in vitro and characterized as previously described (1). The expanded chondrocytes were used at the final concentration of 1×106 cells/mL. The cells were exposed to hypotonic electroporation buffer for three minutes before being transferred into the 2 mm gap electroporation cuvettes (VWR, USA). The exposure time (2-5 min) to the hypotonic buffer has been previously described to greatly improve the yield of cell electroporation (2). Pulses (8 × 100 µs, 1 Hz) of varying electric field strengths, ranging from 0,6 kV/cm to 1,8 kV/cm, were applied to the cells using the ELECTROcell B15 HV+LV electroporator (Leroy Biotech, France). To determine cell viability, cells were transferred to 12 well-plates (TPP, Switzerland) for 72h, and stained with eBioscience™ Fixable Viability Dye eFluor™ 780 (Invitrogen, USA). The percentage of live cells was determined using the Attune NxT (Invitrogen, USA) flow cytometer. To assess the percentage of successfully electroporated cells, 10 µg/ml of propidium iodide (Molecular Probes™, USA) was added to the cell suspension prior to electroporation and the number of successfully electroporated cells was determined by flow cytometry.

RESULTS AND DISCUSSION Our results show that the minimal applied electric field needed to achieve electroporation varied slightly between donors of primary chondrocytes. For cells of the two out of three donors, we observed a high percentage of successful electroporation (86% and 90%, respectively) in the electric field of 0,8 kV/cm, while the cells from the third one could be successfully electroporated (80%) at 1,0 kV/cm. Therefore, we assumed that the electric field of 1,0 kV/cm is the threshold for successful electroporation of all tested cells. Using the cell viability assay, we showed that the cells had high viability (80% or higher) even at the highest tested electric field strength (1,8 kV/cm).

CONCLUSIONS Based on these data we can conclude that the electric field pulses in range from 1,0 to 1,8 kV/cm enable successful electroporation of primary human chondrocytes, not significantly affecting their viability. Electroporation that does not affect cell viability is necessary for successful electrofusion. Our results provide the basis for further optimization of electroporation of primary cells obtained from various human tissues to achieve their successful fusion with MSCs.

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P26 Enhancing Feasibility and Manipulation of Epithelial Cell Sheets for Tracheal Defect Restoration: A Tissue Engineering Approach

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Keywords: tissue engineering, epithelial cell sheets, porcine small intestine submucosa (SIS), degassing, tracheal regeneration

INTRODUCTION Tracheal reconstruction poses a significant challenge due to its intricate, layered architecture, which extends beyond a simple cylindrical structure. Our previous work has shown that combining a partially decellularized tracheal scaffold with epithelial cell sheets can achieve sustained patency in experimental animals. However, the fragility of cell sheets hinders their surgical manipulation and implantation. This study aims to improve the feasibility and convenience of handling epithelial cell sheets using biocompatible materials as carriers.

EXPERIMENTAL Porcine small intestine submucosa (SIS) was chosen as a biocompatible carrier for testing. Firstly, we pretreated the SIS with a customized negative vacuum system (degassed procedure) to enhance its effectiveness. We improved the function of degassing SIS by incorporating epithelial growth factor (EGF). The degassed SIS was evaluated in vitro using a cell sheet reattachment design and compared with the non-degassed SIS control. A rabbit trachea patch repair model was then used to test the *in vivo* promotion of healing by the degassed SIS compared to the control non-degassed SIS. Cell sheet viability was measured, and the surface attachment area was analysed by MTT assay. H&E staining and Masson's trichrome stain were used to assess tissue regeneration.

RESULTS AND DISCUSSION The degassed SIS demonstrated improved cell sheet reattachment in vitro compared to the non-degassed SIS control. In the cell sheet reattachment model, the reattached cell sheet coverage was significantly higher in the degassed SIS group than in the non-degassed group. Cell sheet viability was also significantly higher in the SIS group than in the control group. *In vivo* studies showed that the tracheal defect repaired by the degassed SIS patch showed enhanced healing and reductions in fibrosis and luminal stenosis compared to the control group, with the thickness of the transplanted grafts in the degassed SIS group significantly lower than those in the control group. SIS degassed with EGF enhanced tracheal wound healing, reduced stenosis, and restored tracheal epithelium. The tracheal lumens in the EGF group were covered by epithelial cells, achieving the morphology of pseudostratified ciliated columnar epithelium with goblet cells.

CONCLUSIONS Our study demonstrates that pre-treating porcine small intestine submucosa (SIS) with Epithelial Growth Factor (EGF) by a customized negative vacuum system enhances its effectiveness as a carrier for epithelial cell sheets, promotes cell sheet reattachment and supports tracheal regeneration. Further research is needed to optimize this approach and explore its clinical applications in tracheal reconstruction.

Long-term cryogenic preservation of perinatal stem cells in DMSO-supplemented and DMSO-free GMP-grade formulations

P27

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Keywords: cryopreservation; DMSO; mesenchymal stromal cells; amniotic epithelial cells

INTRODUCTION Advanced therapeutic medicinal products (ATMP) represent biological formulations (viable cells) with a limited shelf life. Cryopreservation ensures the immediate availability of the medicinal cells and allows time for quality tests and donor-recipient match. However, challenges have been reported in preserving viable epithelial cells as well as long-term storage of MSC . Furthermore, exposure to standard-of-care cryoprotective agents, typically dimethyl sulfoxide (DMSO), has been proven challenging for clinical use and negatively affecting cell viability and functions . Thus, we evaluate alternative formulations without DMSO.

EXPERIMENTAL We performed cryogenic storage of perinatal stem cells (AEC, amnion and Wharton Jelly MSCs) , comparing 8 commercial GMP-grade solutions (4 formulations supplemented with 10% DMSO, and 4 DMSO-free cryosolutions). We evaluated both standard cooling rate (1 C/min) and higher or lower freezing rates using a controlled rate freezer device. The thawing procedure was performed using a warming rate of 60–80 C/min, by immersion in a water bath or a GMP-grade thawing device. We measured cell viability and recovery after several months or years post-freezing. Since detrimental mitochondrial activities (e.g., reduced ATP production) have been reported at low temperatures, we coupled viability and recovery measurements with ATP quantification. We analyzed cell adhesion to the substrate upon thawing and proliferation capacity.

RESULTS AND DISCUSSION hAEC presented a superior capacity to survive and perform equally well as freshly isolated cells when exposed to DMSO-free cryosolutions (viability and recovery >90%). Conversely, MSCs had a batch-to-batch variability in terms of survival and identity in the presence of DMSO. Cell adhesion and expansion were variable, with a preference for DMSO-free conditions, and frequently resulted in prolonged gaps before duplications occurred, particularly in DMSO-free conditions. ATP content and functions were adequately preserved, with important differences in the absence of DMSO.

CONCLUSIONS Controlled-rate freezers or passive freezing devices represent valuable and efficient strategies to control the rate at which cells traverse that temperature difference. DMSO-free cryosolutions showed great efficacy in preserving hAEC for the long term, without loss of energetic characteristics and efficacy. Such a switch may not serve equally well for MSC. Post-thawing washes may be appropriate for both ATMPs to provide a stable and safe product to infuse in patients.

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P28 Spatial Transcriptomics Discoveries Of Human Pathologies Using Xenium Analyzer

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Keywords: spatial in-situ transcriptomics, single-cell analysis, Xenium

INTRODUCTION Using the Xenium Analyzer for high-throughput single-cell analysis to understand cellular diversity in tissues has opened new avenues for investigating gene expression with spatial information. Carefully pre-designed or customized curated panels and analysis tools for visualizing and studying spatial gene expression patterns allow for a deeper look into cellular mechanisms and interactions. The Xenium Analyzer instrument is designed for fully automated high-throughput analysis of cells in their tissue environment without the need for conventional sequencing.

EXPERIMENTAL Using different human tissues, we aimed to evaluate the feasibility of the Xenium platform for in-situ spatial transcriptomic analysis to distinguish different human pathologies, which is important from a treatment perspective. We analyzed the expression of 377 genes using classic and enhanced multimodal cell segmentation methods in 5 µm thick FFPE tissue sections and 10 µm thick FF tissue sections with a pre-designed human multi-tissue and cancer panel.

CONCLUSIONS The Xenium platform demonstrated great potential for in-situ spatial transcriptomic analysis of human tissue pathologies, revealing significant gene expression differences between tissue types. This approach could greatly enhance our understanding of cancer origins by dissecting cell-type differences, defining gene regulatory interactions, and deciphering spatial gene expression patterns. This is crucial for improving disease diagnosis, which can help clinicians predict disease progression and improve treatment strategies by allowing for more personalized treatments.

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