MECHANICAL PROPERTIES DESIGN OF PECTIN-COLLAGEN I BIOSCAFFOLDS FOR THE RECAPITULATION OF NATIVE SKIN PHENOTYPE TRAITS IN VITRO

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Introduction

The successful recapitulation of native tissues' phenotypes in an *in vitro* environment is at the heart of developing reliable tissue models for both clinical and scientific research. Three-dimensional (3D) cell culture has long been established as the superior way to model living tissues, when compared to flat adherent culture flasks. As a result, bioscaffolds have become simultaneously the leading tool for ex vivo cell culture and one of the most prominent topics of research in the field of Tissue Engineering. Their fundamental nature has further made them amenable to 3D bioprinting, once more expanding the boundaries of how complex tissue models can be. Bioscaffolds' mechanical properties have received sufficient attention to establish their relevance in skin tissue engineering. However, limitations in 3D research platforms have meant that in-depth quantitative analysis on how they can be used to prioritise the expression of gene clusters associated with discrete parts of skin has been lacking. We have previously shown pectin-collagen I hydrogels offer a promising platform for creating complex, 3D bioprinted multi-layered bioscaffolds which can also maintain cell viability for extended periods of time. In the present work, we explore a wide range of compositions to assess the range of phenotypes we can recapitulate through modifying the mechanical properties of the bioscaffold, and we also validate the relevance of parameters, specific to the biological characterisation of pectin-Collagen I bioscaffolds' mechanical properties.

Methods

The present work performs quantitative RNA-seq on human dermal fibroblasts (HDF), 3D bioprinted in pectin- Collagen I hydrogel bioscaffolds to assess the relationship between mechanical properties of the scaffold and the expression of gene clusters associated with different parts of the dermis. Correlations between the scaffold's mechanical properties and gene clusters associated with wound healing and fibrotic tissue formation are also assessed. Quantification of the bioscaffolds' mechanical properties is performed through bulk compression testing, utilising conventional measures, such as Young's modulus and ultimate strength, as well as less ubiquitous parameters, such as the span of the elastic region and the material properties between the points of ultimate strength and of fracture of the material. The relevance of the bulk mechanical properties of the hydrogel bioscaffolds for the mechanical environment that the cells experience has been validated through application of dynamic mechanical analysis data to existing theoretical models from the field of material science.

Results and Discussion

Changes in the bulk stiffness of the material showed the ability to shift HDFs' expression profile from healthy skin-like profile to a fibrotic- or wound-like behaviour, in line with data from in-vivo models in the literature. More intricate material design – through the span of the elastic region and the material properties between the points of ultimate strength and of fracture of the material – allows the preferential recapitulation of traits reported in either the papillary or the reticular dermis, indicating opportunities for the further optimisation of in vitro skin models and their representativity.

Conclusion

The present work offers a theoretical model of how mechanical properties design of pectin-Collagen I bioscaffolds can be guided to aid the representativity of skin models. The obtained data show that stiffness and ultimate strength can be supplemented in the description of biologically relevant mechanical properties of bioscaffolds with parameters such as the span of the elastic region and the material properties between the points of ultimate strength and of fracture of the material.

DEVELOPING A 3D *IN VITRO* ADIPOCYTE MODEL TO INVESTIGATE METABOLITE-SENSING GPCR FUNCTION

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Introduction

Metabolic disorders such as type 2 diabetes are a growing healthcare challenge, however much remains unknown about their underlying biology, thus limiting the availability of curative treatments. Chronic low-level inflammation of adipose tissue is an important hallmark of these disorders, and there is growing evidence that metabolite-sensing G Protein-Coupled Receptors (GPCRs) play a fundamental role in metabolic- immune interactions [1]. However, it has been challenging to dissect these complex signalling pathways using traditional 2D cell culture or *in vivo* experimental models.

Materials and Methods

A 3D adipocyte spheroid model has been developed by seeding human-derived SGBS pre-adipocyte cells [2] into ultra-low adhesion plates and differentiating them into adipocytes. Differentiated and undifferentiated spheroids were characterised using RT-qPCR and a variety of imaging techniques. Furthermore, adipocyte functional readouts such as lipolysis and glucose uptake assays have been optimised for use with the 3D culture model.

Results and Discussion

Differentiated SGBS spheroids show increased expression of key adipogenic markers such as adiponectin and GLUT4, as well as increased expression of metabolite-sensing GPCRs like the FFA4 free fatty acid receptor. The spheroids grew in size through differentiation, and developed a bulging and irregular surface due to the formation of lipid droplets. Lipid accumulation in the spheroids as a result of differentiation was confirmed both using a LipidSpot™ fluorescent dye and Oil Red O staining. Critically, the differentiated spheroids show characteristic adipocyte functions, including increased lipolysis in response to treatment with a β-adrenoceptor agonists, and increased glucose uptake following insulin stimulation.

Conclusions

This SGBS adipocyte spheroid model therefore provides a 3D *in vitro* platform that can be used to investigate how metabolite-sensing GPCRs control adipogenesis and adipocyte function in a more physiologically relevant microenvironment than traditional 2D cell culture. Thus, this platform can allow us to further dissect the complex GPCR signalling networks within adipocytes and better understand how these receptors can be targeted to treat metabolic disease.

References

[1] Duncan, Vita *et al.*, Front. Endocrinol. 14:1197102, 2023. [2] Wabitsch *et al.*, Int. J. Obes., 25*(1)*:8-15, 2001.

Acknowledgements

We thank EPSRC and SFI for funding this work through the lifETIME Centre for Doctoral Training (EP/S02347X/1).

EMULSION TEMPLATED COMPOSITES: POROUS NERVE GUIDANCE CONDUITS FOR PERIPHERAL NERVE REGENERATION

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Introduction

Following peripheral nerve injury, nerve guidance conduits (NGCs) are implanted to bridge gaps between nerve stumps as an alternative to nerve autograft. Conventional NGCs are simple tubes, usually stiffer than the native tissue, and unable to facilitate regeneration over larger gaps. Semi-permeable NGCs are beneficial, allowing diffusion of nutrients and waste products to the regenerating nerve while impeding the migration infiltrating fibroblasts that cause scar tissue.

Materials and methods

In this study, novel emulsion templating techniques were developed using poly(glycerol sebacate)-methacrylate (PGS-M; a synthetic UV-curable biodegradable material) to fabricate composite structures with variable porosity and stiffness. NGCs with favourable and optimal mechanical properties were then manufactured. Composite structures with porous and nonporous sections were fabricated by creating a PGS- M water-in-oil high internal phase emulsion (HIPE) and then mixing slowly with PGS-M prior to UV-curing.

Results and Discussion

Compression and uniaxial tensile testing showed that the “HIPE +1G” composite demonstrated optimal stiffness for peripheral nerve repair. Scanning electron micrographs of these scaffolds showed the presence of highly interconnected pores with an average pore size of 7.38µm (± 2.26µm). NG108-15 neuronal cell proliferation increased significantly over a 7-day period on porous and nonporous scaffolds. LIVE/DEAD staining showed high cell viability. Immunocytochemistry showed that average and maximum NG108-15 neurite lengths on PGS-M conduits were not significantly different to those on TCP controls. L929 fibroblasts were unable to effectively migrate through the porous NGCs as shown by histological sections. Explanted embryonic chick dorsal root ganglia demonstrated that primary neurons can grow on the NGCs whereas Schwann cells can migrate efficiently along the lumen of the conduits. *In vivo* results showed that the conduits allowed successful regeneration of the sciatic nerve in a 3mm gap.

Acknowledgements

The authors would like to thank the EPSRC funded Centre for Doctoral Training in Advanced Biomedical Materials grant reference EP/S022201/1 for funding this work. FC also thanks the Royal Society for funding of a Royal Society Leverhulme Trust Senior Research Fellowship 2022 (SRF\R1\221053). LJ would like to thank Dr. Sara Memarpour Hobbi, Rachel Furmidge, Boyang Liu, Caitlin Jackson, Dr. Colin Sherborne, Dr. Caroline Taylor and Dr. Matthew Worsley for their help and guidance in the creation of this work. Confocal imaging work was performed at the Wolfson Light Microscopy Facility, using the ZEISS LSM880 AiryScan confocal microscope.

MAGNETIC HYDROGELS FOR BONE TISSUE ENGINEERING

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Introduction: Bone injuries are a significant healthcare burden, with over 4 million bone grafts performed annually to treat conditions such as skeletal abnormalities, malformations, fractures, critical size defects and damage caused by disease[1]. However, bone grafts can be a limited resource to preserve function at the donation site, and the additional surgery is associated with donor site morbidity and pain[1,2]. Bone tissue engineering utilises stem cells, 3D scaffolds (natural and synthetic) and biological growth factors to support bone regeneration, as an alternative to bone grafting[3]. External stimulation, such as magnetic fields, have also been applied to further stimulate bone healing. The effect of magnetic fields on the cellular and molecular mechanisms are postulated to be via mechanotransduction, but require better understanding[4].This study introduces a magnetic 3D hydrogel model, utilising an internal magnetic component alongside mesenchymal stromal cells (MSCs). The model will allow investigation into the mechanism behind magnetic field stimulation, with a view towards a potential therapeutic approach for bone tissue engineering.

Materials and Methods: Our magnetic hydrogel comprises a gelatin matrix functionalised with a photocrosslinkable methacryloyl group, and incorporated iron oxide magnetic nanoparticles (200 nm). MSCs are encapsulated within the magnetic hydrogel as both single cells or spheroids. The magnetic hydrogel was extensively characterised utilising rheology, scanning electron microscopy, water contact angle and contraction assays. Additionally, cell viability within the hydrogel was assessed with a live/dead assay over 28 days. To assess the potential osteogenic response of the MSCs to an external magnetic field, MSCs were encapsulated within the magnetic hydrogel model and exposed to a moderate static magnetic field (370mT) for 1 hour per day to promote osteogenesis. Cells were assessed at days 14 and 28, where qPCR was utilised to examine the effect of a static magnetic field on early and late osteogenic markers. Stem cell markers were also examined to determine if self-renewal of the stem cell population was occurring.

Results and Discussion: Magnetic hydrogel characterisation: The mechanical properties, pore size and water contact angle of the magnetic hydrogels were optimised to create an ideal microenvironment for cell encapsulation. The mechanical properties of the hydrogel can be adjusted by simply changing gel concentration, and/or concentration of encapsulated MNPs. MSC viability was maintained over 28 days with no significant change. Static magnetic field promotes osteogenesis: Analysis of early osteogenic markers at day 14 revealed significant upregulation of alkaline phosphatase (ALP) and Runx2 in single cells encapsulated within the magnetic hydrogel and treated with an external magnetic field, indicating accelerated MSC differentiation towards osteogenic lineages. This was mirrored with the MSC spheroids, albeit later, with a significant upregulation of ALP at day 28. Analysis of late osteogenic markers also demonstrated an increase in osteopontin at day 14 for single cells with a magnetic field, suggesting continued progression of osteogenic differentiation. Likewise, within the spheroid model, a significant upregulation of osteocalcin was identified with an external magnetic field. Interestingly, stemness markers Alcam and Nestin were also elevated at day 14 for single cells, hinting at potential self-renewal of the stem cells within the hydrogel. In summary, the magnetic hydrogel demonstrates the ability to promote osteogenesis in bone tissue engineering models. Given the significant number of bone grafts performed annually, this approach offers a promising alternative to traditional methods. Further research is being carried out to understand the cellular and molecular changes behind the MSC osteogenic response in response to a magnetic field.

References:

1. Wang, W. & Yeuang, K. W., 2017. Bone grafts and biomaterials substitutes for bone defect repair: A review. Bioactive Materials, 2(4), pp. 224-247
2. Turnbull, G. et al., 2018. 3D bioactive composite scaffolds for bone tissue engineering. Bioactive Materials, 3(3), pp. 278-314.
3. Paun, I. A. et al., 2018. 3D Biomimetic Magnetic Structures for StaticMagnetic Field Stimulation of Osteogenesis. International Journal of Molecular Sciences, 19(495).
4. Henstock, J. R. et al., 2014. Remotely Activated Mechanotransduction via Magnetic Nanoparticles Promotes Mineralization Synergistically with Bone Morphogenetic Protein 2: Applications for Injectable Cell Therapy. Stem Cells Translational Medicine, Volume 3, pp. 1363-1374

MODELLING HUMAN IMMUNE RESPONSES TO FUNCTIONALISED BIOMATERIALS

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Introduction: Bone healing remains a significant challenge for reconstructive orthoplastic surgeons, reflecting bone's slow healing time, the morbidity of currently available treatment options and the lack of available donor sites for larger defects[1]. Major advances have shown functionalised biomaterials capable of promoting osteoblast differentiation from mesenchymal stem cells (MSCs) to promote bone regeneration[2,3]. The clinical feasibility of using these MSC biomaterials for bone reconstruction depends on a thorough understanding of the interplay between potentially allogenic donor MSC biomaterials and the recipient human adaptive immune system. Humanised in *vitro* models are increasingly used in the pre-clinical trial phase of biomaterial testing. We aimed to establish a novel, in *vitro,* humanised model of T cell activation to test responses to MSC biomaterials and allow for immunomodulation experimentation.

Materials & Methods: The biomaterial comprises a poly(ethyl acrylate) coating, functionalised with fibronectin with BMP-2, with an MSC layer which is differentiated over 4 weeks to form osteoblasts. This has shown efficacy in murine models and a canine veterinary trial, achieving bone healing in critical defects[2,3]. Peripheral blood mononuclear cells were isolated from healthy human volunteers and T cells were 'primed' with aCD3/aCD28 activator. Subsequently, they were rested before co-culture with biomaterials. T cell activation was investigated using flow cytometry. Experiments were carried out in technical replicates and for multiple biological donors, including both male and female sex.

Results & Discussion: Positive and negative controls demonstrated model efficacy with appropriate T cell responses to no further stimulus or restimulation with aCD3/aCD28 activator. Utilising the model to test human T cell responses to the biomaterial showed significant CD25 activation responses by 5 days coculture with the undifferentiated MSC biomaterial ($p=0.0365$). This was seen by 3 days with the differentiated osteoblast condition ($p=0.0036$), leading to greater surface activation (CD25, ICOS, PD1). Preliminary immunomodulation approaches using material-driven methods involved changing the biomaterial protein from fibronectin to laminin. Repeat modelling demonstrated significant T cell responses to both biomaterials but with no significant difference driven by change in glycoprotein.

Conclusions: We have established a novel, in *vitro* humanised model of T cell activation that can be used to test different cellularised biomaterials and immunomodulation. Future work will characterise the T cell cytokine milieu around these biomaterials and model the impact of the proinflammatory microenvironment on response. Ultimately, we aim to use the modelling to demonstrate material-driven immunomodulation at the material-immune cell interface on the path to clinical translation.

References:

1. Chimutengwende-Gordon, M., et al., *Limb reconstruction after traumatic bone loss.* Injury, 2017. **48**(2): p. 206- 213.
2. Llopis-Hernández, V., et al., *Material-driven fibronectin assembly for high-efficiency presentation of growth factors.* Sci Adv, 2016. **2**(8): p. e1600188.
3. Cheng, Z.A., et al., *Nanoscale Coatings for Ultralow Dose BMP-2-Driven Regeneration of Critical-Sized Bone Defects.* Adv Sci (Weinh), 2019. **6**(2): p. 1800361.

OPTIMISING THE USE OF DEGRADABLE MICROCARRIERS IN STIRRED TANK BIOREACTORS FOR THE PRODUCTION OF IMMUNOMODULATORY HUMAN MESENCHYMAL STROMAL CELLS.

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Introduction: Human mesenchymal stromal cell (hMSC)-based therapies possess substantial potential for both immuno- suppressive and tissue regeneration properties. However, current clinical trials require a large number of cells (~100 million hMSCs per dose) for an effective therapy. To meet this demand, this rare population of tissue progenitors requires expansion in the laboratory. Using traditional planar expansion methodologies, the long- term culture and passaging necessary to generate these cell numbers often leads to hMSC senescence, loss of crucial immunomodulatory properties, and relies on rigid substrates that tend to promote spontaneous differentiation into osteogenic lineages. Additionally, these methods are labour-intensive and are limited by surface area-to-volume ratios during culture, restricting their production at large scale.

Stirred tank bioreactors (STR) combined with microcarriers substrates facilitate greater cell culture densities and control over physicochemical parameters necessary for optimal cell growth. However, current cell harvest methodologies from microcarriers post expansion subject hMSCs to intense mechanical and proteolytic enzymatic stress for cell detachment. This results in low recovery and reduced cell viability with a loss of immunomodulatory properties. This study optimises STR bioprocessing methodologies using degradable microcarriers that promote the expansion of naïve, immunomodulatory MSCs as well as reducing harvesting time.

Materials and Methods: Bone marrow derived hMSCs were cultured in either planar T-flasks or dynamic STR systems in complete DMEM for 10 days. hMSCs attached to microcarriers were quantified using the NucleoCounter® NC3000 system. Release of soluble factors from STR cultures were measured using ELISAs after TNF-a and IFN-g priming. Glucose, lactate and LDH concentrations were measured using an AltairTM 240 clinical analyser. hMSC functional immunomodulatory capabilities (suppression of T-cell proliferation, Treg reprogramming, viability, and susceptibility to apoptosis) and phenotyping (surface marker expression) by flow cytometry; proliferative capacity was quantified using MTT assays. Differentiation capacity was assessed after 3 weeks of culture with respective differentiation media for osteogenic and adipogenic lineage commitment by immunohistochemical staining. Colony forming potential was assessed post-STR harvest by staining 10 day- cultured cells with crystal Violet.

Results, Discussion, and Conclusion: We demonstrate that enzyme-mediated degradation of Cytodex 3 microcarriers significantly enhances the recovery of hMSCs from STR systems, without affecting viability. We observe an almost complete recovery of cells which retain a naïve, stem like phenotype, functional immunomodulatory properties as well as their lineage differentiation potential. Importantly, hMSCs from an STR can be further expanded within a subsequent STR; demonstrating the potential of this bioprocessing approach to generate large numbers of therapeutically active progenitors required for cell banks and clinical therapy. Our results provide a simplified pipeline to address some of the main downstream challenges in the large-scale expansion and isolation of therapeutically viable hMSCs

Acknowledgements.

This work was supported by Science Foundation Ireland 18/EPSRC-CDT/3583 and the Engineering and Physical Sciences Research Council EP/S02347X/1. CÚRAM is co-funded by the European Regional Development Fund and Science Foundation Ireland under Ireland's European Structural and Investment Fund Grant Number 13/RC/2073

POROUS POLY(GLYCEROL SEBACATE)-METHACRYLATE SCAFFOLDS FOR VASCULARISED ADIPOSE TISSUE ENGINEERING

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Introduction: Autologous fat grafting (AFG) is widely used for soft tissue reconstruction to treat conditions such as depressed scarring and hemifacial atrophy. In AFG, adipose tissue is harvested from a donor site via liposuction, then the lipoaspirate is reinjected at the defect site to restore tissue volume. Despite favourable short-term outcomes, AFG procedures have poor long-term volume retention, attributed to poor revascularization of the grafted lipoaspirate, resulting in necrosis and resorption. The loss of adipose tissue volume remains a key clinical problem and necessitates additional procedures to achieve the desired outcome, thereby increasing healthcare costs and subjecting patients to additional invasive procedures[1].

Inert, non-degradable fillers have been developed, such as silicone implants, but these are costly, do not promote tissue regeneration and have high revision rates[2]. An in situ tissue engineering approach combining an appropriate biodegradable biomaterial scaffold and lipoaspirate to support adipose tissue regeneration has the potential to improve surgical outcomes. Poly(glycerol sebacate) (PGS) is a biodegradable synthetic biomaterial which has previously been used for soft tissue engineering[3]. Here we aimed to develop porous PGS-methacrylate (PGS-M) scaffolds to support adipose tissue survival and promote vascularisation.

Materials and Methods: Emulsion templating of photocurable PGS-Methacrylate (PGS-M) was used to fabricate porous 3D scaffolds, which were characterised using scanning electron microscopy (SEM) and compressive mechanical testing. PGS-M scaffolds were seeded with human adipose-derived stem cells (ADSCs) to assess cell biocompatibility and adipogenic differentiation. The angiogenic potential of scaffolds seeded was assessed using the chick chorioallantoic membrane (CAM) assay.

Results and Discussion: PGS-M scaffolds were fabricated with a range of pore sizes, and displayed a high porosity (>80%), with interconnectivity between pores. These scaffolds were highly elastomeric, and exhibited mechanical properties that closely mimic native adipose tissue. ADSCs cultured on PGS-M scaffolds showed increased metabolic activity over time, indicating that the cells adhered to and proliferated on the scaffolds. When stimulated, an accumulation of lipids and an upregulation of adipogenesis-associated genes were observed, indicating successful ADSC differentiation on the scaffolds. Using the CAM assay, targeted vascular ingrowth into scaffolds was observed, both macroscopically and histologically. Notably, implanting human lipoaspirate with PGS-M scaffolds on the CAM increases vascular ingrowth compared to lipoaspirate alone.

Conclusions: These results highlight that porous PGS-M scaffolds facilitate cell growth and adipogenic differentiation of ADSCs. In addition, when implanted on the CAM, the scaffolds exhibit pronounced cellular and vascular ingrowth through interconnected pores. Finally, combining lipoaspirate with PGS-M scaffolds improved the vascular response on the CAM, suggesting that these scaffolds could enhance the vascularisation of grafted adipose tissue. Future studies will focus on developing an injectable form of these scaffolds which could be used alongside existing fat grafting techniques to support adipose tissue regeneration and improve volume restoration.

References

1. Mahoney, C.M. et al. (2018) Frontiers in Pharmacology, 9
2. Abu-Ghname, A., Perdanasari, A.T. and Reece, E.M. (2019) Seminars in Plastic Surgery, 33(03), pp. 147– 154.
3. Frydrych, M. et al. (2015) Acta Biomaterialia, 18, pp. 40–49

EXPLORING THE ROLE OF TSPAN6 IN REGULATING MACROPHAGE FUNCTION AND RECRUITMENT WITHIN THE DUCTULAR REACTION DURING CHRONIC LIVER INJURY.

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Introduction

Chronic liver disease (CLD) is a major global problem associated with the risk of liver failure and cancer. Ductular reaction (DR) is characteristic of CLD pathology, where bile epithelial cells (BEC) proliferate alongside excessive scarring and inflammation. Determining the crosstalk between DR and the immune system could help develop new regenerative medicine therapies and cancer prevention strategies. We focused on studying the tetraspanin protein family in this context as they are known to play important roles in cellular crosstalk; specifically tetraspanin (TSPAN)6 has been linked to chronic inflammatory pathologies and cancers by regulating extracellular vesicle (EV) release.

Materials and methods

To determine cross-talk between the regenerating biliary nodules and the infiltrating immune system we performed immunohistochemistry and immunofluorescent assays to visualise co-localisation. Immunocytochemistry and flow cytometry was used to determine expression levels in primary cells. Conditioned media co-culture experiments allowed us to validate any effect ductal cells had on infiltrating macrophages. Ultracentrifugation was used to deplete conditioned media from biliary epithelial cells of extra-cellular vesicles and nano-tracking analysis (NTA) was used to characterise purified EVs.

Results and discussion

Using immunohistochemistry and immunofluorescence, we confirmed TSPAN6 expression in CLD, and demonstrated its localisation in BEC of the DR. We also show a positive correlation of TSPAN6 expression with infiltration of immune cells that are critical to liver regeneration, such as monocytes and neutrophils. TSPAN6 expression was maintained in isolated primary human BEC and was upregulated in response to Lipopolysaccharide (LPS).

Modelling BEC-monocyte crosstalk *in vitro* confirmed the activation of monocytes in response to conditioned media from LPS-stimulated BEC. Depletion of EVs from the conditioned media led to an augmentation of macrophage activation. In contrast, Tspan6 knockdown in BEC led to an immunosuppressive effect on macrophages. This is in keeping with previous findings that Tspan6 is a negative regulator of EV release.

Conclusions

Our findings suggest that TSPAN6 could be a key player in the crosstalk between cells of the ductular reaction and the immune system. Future work will eludicate the exact role of TSPAN6 in EV driven cell-cell communication between BEC and monocytes and explore the potential of TSPAN6-directed therapies in CLD.

DEVELOPMENT OF A NOVEL TEAR COLLECTION DEVICE FOR POINT-OF-CARE OCULAR AND SYSTEMIC DISEASE DIAGNOSIS

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Introduction: Tears, as an easily accessible biological fluid particularly rich in proteins (7-11 g/L), continue to grow research interests as an emerging surrogate for biomarkers of both ocular and systemic diseases [1]. Collecting tear samples in a point-of-care capacity however remains a challenge with the current approaches used in clinics. Limitations of interest include: high protein variability between the approaches, non- representative biochemistry due to reflex tearing, lack of 100% retrieval of pooled proteins, and protein dilution from saline flushing [2]. Therefore, the purpose of this work is to address the need for a more reliable method for collecting tear fluid by developing and characterising a minimally invasive hydrogel capable of collecting tear proteins.

Materials and Methods: All experiments were carried out using UV-casted copolymer gels by free radical polymerisation in 20 wt% increments prepared from variations of: 2-hydroxyethyl methacrylate (HEMA); methyl methacrylate (MMA); glycidyl methacrylate (GMA); glycerol monomethacrylate (GMMA); and N,N- Dimethylacrylamide (DMA). The materials were characterised by FTIR spectroscopy to confirm copolymerisation, gravimetric analysis to investigate the swelling characteristics, wettability measurements, SEM and interferometry imaging to visualise the topographies and porosities, and UV-Vis to determine the light transmittance through the materials. With regards to material-protein interactions, two clinically relevant model tear proteins MMP-9 and IgE were used. Immunohistochemistry was performed to qualitatively assess the distribution of protein on the gels, and protein absorption affinities evaluated using a NanoOrange protein quantitation assay.

Results and Discussion: Characteristic FTIR peaks for the four monomers under investigation in each copolymer matrix were seen. Between 1 - 450% mass increases were found after 24hrs incubation, the latter of those with the largest swelling abilities containing greater DMA content due to polar amide groups. Gels containing MMA and GMA were significantly more hydrophobic but the addition of DMA in all cases improved the wettability. SEM and interferometry imaging showed the surfaces of the gels to be very smooth, and all pores larger than average sizes of the protein complexes measured by DLS (MMP-9: 159.8 ± 92.8 nm; IgE: 128.8 ± 108.7 nm), essential for protein influx. Pore densities ranged from 11.8 ± 2.1% to 38.8 ± 3.3%. Visible light transmittance through all tested materials was >90%, as required. Immunohistochemistry confirmed protein binding, with no preferential distribution of either proteins on the gels. Protein uptake across the samples ranged from 8.1% - 87.7% for MMP-9, and 9.7% - 81.6% for IgE, with significant differences found between the uptakes to the same sample.

Conclusion: The parameters assessed are all important for a device required to be comfortable in the ocular cavity whilst collecting tear biomarkers efficiently. The data collectively so far points to four promising candidates of maximum protein sorption, for a new tear collection device capable of absorbing multiple proteins. *In vitro* cell studies are currently ongoing to assess the safety and cytotoxicity of the samples according to ISO 10993-5, alongside investigating the uptake in an artificial tear fluid medium.

References:

1. Hagan S. *et al.* EPMA Journal. 7(1): 1-20, 2016.
2. Rentka A. *et al.* Annals of Clinical Biochemistry. 54(5): 521-529, 2017.

Acknowledgements: *The authors would like to thank the EPSRC funded Centre for Doctoral Training in Advanced Biomedical Materials grant reference EP/S022201/1 for funding this work, and Menicon Co., Ltd (Nagoya, Japan) for sponsoring this work.*

DESIGNING ANIMAL-FREE ORGANOIDS BASED ON ENGINEERED VEGETABLES [VegFold]

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Introduction

In Tissue Engineering (TE) one of the main challenges lies in the recreation of the microarchitecture of the Extracellular Matrix (ECM). The use of bioinspired structures such as plant tissues as biomaterial for scaffolds represents an alternative to the currently used materials, since their properties such as porosity or native vasculature network closely resemble those found in mammalian tissues such as bone and cardiac, respectively (1,2,3).

This project aims to develop plant-based scaffolds for tissue engineering by the decellularisation of tobacco and apple tissues and the seeding of stem cells for their application as a model system. Moreover, we aim to add growth factors for cell differentiation through utility of the delivery system PODS® in collaboration with Cell Guidance Systems Ltd.

Materials and Methods

Plant tissue to be used as a biomaterial for scaffold production must first be decellularised. This requires removal of the plant cells from the tissue. In this approach, *Nicotiana tabacum* leaves were decellularised by applying a protocol involving serial washes with solutions of Sodium Dodecyl Sulfate (SDS) and Sodium hypochlorite (NaClO). Apple (*Malus domestica*) tissue was also decellularised by serial washes with SDS and $CaCl_2$ solutions. The scaffolds were characterized by Scanning Electron Microscopy (SEM) and Confocal Laser Scanning Microscopy (cLSM) after staining with Propidium Iodide and DAPI for nuclear material identification. The scaffolds were then coated with either fibronectin or collagen IV to evaluate their compatibility to human Mesenchymal Stem Cells (hMSCs). To assess the cellular adhesion, hMSCs were seeded on scaffolds with or without coating and imaged by epifluorescence microscopy.

Results and Discussion

Tobacco scaffold decellularisation was confirmed by the absence of nuclear staining during the cLSM imaging. Both, tobacco, and apple scaffolds were imaged by SEM showing that they maintained their surface integrity after the decellularisation process. Moreover, epifluorescence microscopy imaging revealed that hMSCs were able to adhere to the plant-based scaffolds and their cell spreading morphology was more pronounced when an ECM protein coating was present.

Conclusions

The scaffolds generated so far are suitable for cell seeding. Hence, further evaluation of cell survival and functioning within the scaffold will be performed to further evaluate their efficacy. Subsequent studies will examine the utility of PODS® technology to assist differentiation through the delivery and release of growth factors.

References

1. Contessi Negrini N, *et al*. Front Bioeng Biotechnol. 8:723, 2020.
2. Bilirgen AC, *et al*. Biomaterials Science and Engineering. 7(3): 926- 938, 2021. 3. Gershlak JR, *et al.* Biomaterials.125:13–22. 2017

PROMOTION OF EXTRACELLULAR VESICLE PRODUCTION FROM HUMAN TENDON STEM/PROGENITOR CELLS VIA DYNAMIC CELL CULTURE

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Introduction: Tendon injuries significantly impact an individual's quality of life, driving the need for innovative solutions beyond traditional surgical approaches[1]. Extracellular Vesicles (EVs) offer a promising potential strategy for promoting tendon regeneration by delivering specific biological signals to cells[2]. This study focuses on the extraction of human Tendon Stem Progenitor cells (hTSPCs) from surgical explants[3] and the characterization of EV production under both perfused and static conditions.

Materials and Methods: hTSPCs were obtained from tendon surgery biopsies (Review Board prot./SCCE n.151, 29/10/2020), tested for stemness properties by using flow cytometry (CD14, CD34, CD73, CD90, CD105, and HLA-DR) and trilineage differentiation (adipo, osteo, and chondrogenesis) and cultured in Growth Differentiation Factor-5 (GDF-5) supplemented media to promote tenogenic differentiation. Then, hTSPCs were cultured under static and dynamic conditions using a custom perfusion bioreactor. Upon reaching 80% confluence, TSPCs were transferred to a serum-free medium for 24 hours to collect conditioned media (CM). Ultra-centrifugation was used for EVs isolation, followed by comprehensive characterization including size and concentration measurements using the Nanosight NS300 system, morphology examination via scanning electron microscopy (SEM) and transmission electron microscopy (TEM), and protein content quantification using Bradford assay, Western blot, and MACSPlex Exosome kit.

Results and Discussion: hTSPCs demonstrated stemness and multipotency, expressing CD73, CD90, and CD105, with no expression of CD14, CD34 and HLA-DR.Trilineage differentiation capacity was confirmed histologically. Pre-treatment of cells with GDF-5 supplementation upregulated the tenogenic genes SCX-A, TNMD and COL3A1, and cells showed a more elongated tenocyte-like morphology with an increased aspect ratio. Nanosight analysis revealed the presence of particles with a sub-200 nm diameter and concentration of 7x10^6 particles/mL, in both culture conditions. The dynamic EV pellet exhibited a three-fold increase in total protein concentration. Western blot analysis confirmed the presence of EV-associated proteins CD81 and TSG-101 exclusively within the dynamic EV pellet. MACSPlex analysis revealed higher expression of EV markers, particularly CD63 and CD45, in dynamic conditions, whereas CD9 showed elevated expression in static conditions.

Conclusion: Our data suggests efficient harvesting of sub-200 nm vesicles from serum-starved hTPSCs, consistent with existing EV research. Dynamic culture conditions appeared to enhance EV production, although further characterization is necessary for a comprehensive understanding of EVs' role in tendon regenerative processes. Future plan involves investigating their potential to instruct stem cells (e.g., mesenchymal stem cells) towards a tenogenic lineage. Preliminary results showed that Crude EVs supplementation helps promoting TNMD expression, but further studies are required.

References

1. Citro V, Clerici M, Boccaccini AR, et al. Tendon tissue engineering: An overview of biologics to promote tendon healing and repair. *J Tissue Eng* 2023; 14: 20417314231196275.
2. Fang WH, Agrawal DK, Thankam FG. "Smart Exosomes": A Smart Approach for Tendon Regeneration. *Tissue Engineering Part B: Reviews* 2022; 28: 613–625.
3. Ciardulli MC, Scala P, Giudice V, *et al. Cells* 2022; 11: 2545.

Acknowledgements: This project has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 955685, www.helsinki.fi/p4fit.

INCORPORATING COMPLEX ANATOMICAL FEATURES TO HEPATIC TISSUE MODELS THROUGH PµSLA

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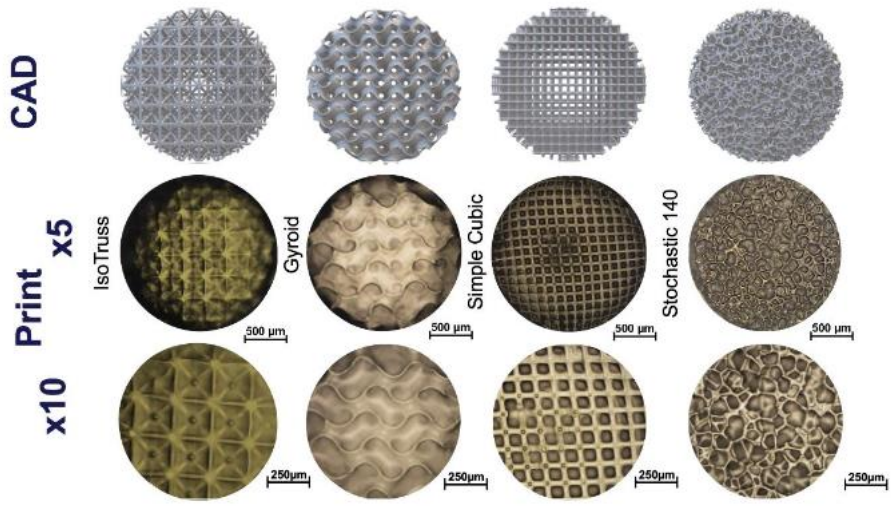
Introduction: In tissue engineering, scaffold design plays a pivotal role in mimicking the native microenvironment and guiding cellular behaviour. This study focuses on the design and analysis of stochastic scaffolds inspired by decellularized liver extracellular matrix (ECM) compared to conventional periodic lattices for hepatocyte culture. Using advanced computational tools (nTopology) in combination with cutting-edge 3D printing techniques (µSLA), stochastic scaffolds were meticulously designed and 3D printed with parameters derived from decellularized liver ECM, aiming to replicate its complex structural and biomechanical cues.

Materials and Methods: Incorporating implicit and field driven design using nTopology, stochastic scaffolds were created to replicate the natural irregular and heterogeneous arrangement of collagen fibres found in ECM, offering enhanced biomimicry and cell-scaffold interactions. The design process involved decellularised liver ECM characterization followed by the manipulation of design parameters to achieve desired structural cues found in tissue. Subsequently, the designed scaffolds and three other periodic lattices designs were fabricated using micro- stereolithography (µSLA), a high-resolution additive manufacturing technique renowned for its high resolution and precision. This project also required conducting comprehensive comparative studies to evaluate hepatic cell behaviour on stochastic scaffolds versus commonly used periodic lattices. Through a series of in vitro experiments, cell adhesion, proliferation, and functional activity are assessed. By systematically analysing these cellular responses, we seek to elucidate the impact of scaffold architecture on hepatocyte behaviour and functionality.

Results and Discussion: Fabrication of stochastic scaffolds and periodic lattices was achieved as well as the optimized post processing necessary to host hepatocytes. Employing tools such as nTopology allowed us precision and careful manipulation of parameters to achieve desired structural details and stochasticity, enhancing biomimicry, but also offering flexibility and scalability in scaffold design. Moreover, the use of decellularized liver ECM as a reference provides a biologically relevant blueprint for scaffold design. The use of µSLA enabled the accurate translation of intricate scaffold designs into physical constructs, faithfully replicating the complex structural features crucial for guiding hepatocyte behaviour effectively. This integrated approach, which combined the power of implicit design tools with µSLA fabrication, facilitated the creation of biomimetic scaffolds that closely mimic the intricate microenvironment of the native liver ECM collagen fibres.

Conclusions: In conclusion, this project presents a novel approach to scaffold design in hepatic tissue engineering, using stochasticity, decellularized ECM-inspired parameters and advanced manufacturing techniques to create microenvironments that better mimic native tissue. Through comparative studies, we anticipate gaining valuable insights into the influence of scaffold architecture on hepatic cell behaviour,

Figures and tables



CHEMOTHERAPEUTIC ASSESSMENT ON A DYNAMIC, MULTICELLULAR AND SPATIALLY SEGGREGSTED MODEL OF PANCREATIC CANCER

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Introduction: With a 5-year survival rate of only 11% in the USA[1] and less than 7% in the UK[2], pancreatic cancer (PDAC) is a deadly disease. These statistics are partly attributed to the tumour's resistance to currently available treatment arising from a complex and heterogeneous tumour microenvironment (TME). A key challenge in developing *in vitro* PDAC models is to mimic the different key features of the TME. In this work we have developed a robust, biomimetic model for therapeutic assessment *in vitro* with the goal to optimize patient specific treatment regime.

Materials & Methods: We have previously developed 3D polyurethane (PU) based polymeric scaffold assisted multicellular (cancer cells, activated stellate cells and microvascular endothelial cells) models of PDAC[3,4] with spatial complexity (scaffold compartmentalization). Two different two architectural configurations were used: (i) a single scaffold and (ii) a zonal scaffold comprised of an inner (cancer) compartment and an external (stroma) compartmen[5]. In this work, by using a perfusion bioreactor we achieved mimicry of the interstitial flow, which enables more accurate drug delivery. Chemotherapeutic assessment using Gemcitabine (GEM) was carried out to validate our model. Imaging of cellular proliferation/spatial organization, apoptosis of the different cell types and ECM secretion was carried out along with q-PCR assessment of various biomarkers.

Results & Discussion: The key observation within our static models was higher resistance to GEM within our dual scaffold model in comparison to the single scaffold[6]. Specifically, there was no reduction of cell viability in the zonal model, especially for the cancer mass, highlighting the importance of spatial cellular arrangement within complex *in vitro* 3D models. Furthermore, Collagen I distribution in the zonal scaffold was unaffected 24 hours post-treatment, in contrast to the single multicellular scaffold, re-iterating the importance of desmoplasia in relation of PDAC's chemoresistance. The introduction of dynamic flow affected the cell spatial organization, changes in biomarker expression involved with EMT and matrix remodeling, specifically upregulating genes related to mesenchymal phenotype (vimentin) and matrix remodeling (MMPs) while downregulating epithelial markers (cytokeratin 19, EpCAM). Differences in the effect of GEM was also observed in a direct comparison between static and dynamic cultures. These data highlighted the importance of fluid and its role in PDAC's resistance to chemotherapy.

Conclusion: In conclusion, we have developed a robust, biomimetic PDAC model that allows us to recapitulate multiple aspects of the pancreatic TME including cell-cell and cell-ECM interactions, interstitial fluid flow and the development of an *in vivo*-like niche by the cancer cells. Our work highlights the importance of spatiotemporal cellular arrangement and interstitial fluid flow for accurate in vitro studies of the chemoresistance for PDAC.

Acknowledgements: E.V. (PI) and P.P.M. (co-I) are grateful to the Medical Research Council UK for a New Investigator Research Grant (MR/V028553/1), which also financially supports P.G.

REFERENCE:

1. https://www.cancer.net/cancer-types/pancreatic-cancer/statistics
2. https://www.pancreaticcancer.org.uk/what-we-do/media-centre/pancreatic-cancer-statistics/ 3. Totti, S. et al. RSC Advances. 2018; 8(37)
4. Gupta, et al. RSC Advances.2019; 9 (71)
5. Gupta, et al. Frontiers in Bioengineering & Biotechnology. 2020; 8 (290).
6. Gupta et al., Adv. Biology 2024, 2300580

ENGINEERING A BONE MARROW ENDOSTEAL NICHE MODEL FOR DRUG SCREENING IN ACUTE MYELOID LEUKAEMIA

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Introduction: Acute Myeloid Leukaemia (AML) is the most common acute leukaemia in adults, accounting for <1% of all cancer cases but 2% of all UK cancer mortalities (Cancer Research UK, 2021). AML is caused by the acquisition of mutations in haematopoietic stem and progenitor cells, causing leukaemic stem cells (LSCs) to arise. Interactions between LSCs and bone marrow stroma within the endosteal niche induce LSC quiescence, protecting LSCs from chemotherapy. This leads to relapse in approximately 50% of patients. Circumventing this protective microenvironment is key to treating AML. Therefore, the aim of this project is to develop a 3D- bioprinted co-culture bone marrow model, hence providing a highly reproducible model to assess novel and current AML therapies. This 3D niche system was used to explore the efficacy of cyclin dependent kinase (CDK) 2/9 inhibitor fadraciclib, alone and in combination with current AML therapies such as venetoclax, a BCL-2 inhibitor; cytarabine, an antimetabolite; and azacytidine, a hypomethylating agent.

Materials and Methods: Alginate/gelatin hydrogels were selected for this model due to their suitable printability and biocompatibility. First, mechanical properties and microarchitecture of alginate/gelatin hydrogels were assessed using rheology and scanning electron microscopy (SEM) respectively. Following this, viability of MOLM-13 cells in monoculture and in co-culture with HS-5 spheroids were assessed in hand-cast and 3D-bioprinted hydrogels for up to seven days. Finally, the effect of fadraciclib and selected chemotherapies on MOLM-13 cell viability within alginate/gelatin hydrogels was determined using viability staining on fluorescent microscopy.

Results and Discussion: Characterisation of the 3D environment provided by alginate/gelatin hydrogels commenced with rheology to assess the stiffness and SEM to study the microarchitecture. All gel concentrations assessed (1% alginate/8% gelatin and 2% alginate/8% gelatin) were within the stiffness range to emulate bone marrow. Interestingly, increasing the concentration of the crosslinking polymer, alginate, increased the stiffness of the hydrogels. Correspondingly, the SEM revealed that higher alginate concentrations resulted in more dense polymer networks compared to lower alginate concentrations, and that the network became more porous with time in culture. 2% alginate/8% gelatin hydrogels were selected as the basis of the model due to their improved stability in longer-term culture. Cell viability was assessed within 2% alginate/8% gelatin hydrogels. Viability staining showed that MOLM-13 cells and HS5 spheroids remained viable within alginate/gelatin hydrogels for at least seven days, highlighting that this gel is suitable for the model. When repeated in 3D-bioprinted alginate/gelatin hydrogels, there were no significant differences in viability and no disruption of HS5 spheroid morphology in the 3D-bioprinted gels, revealing that the printing process does not negatively impact the cells. Finally, fadraciclib was added to MOLM-13 cells in monoculture and co-culture with HS5 spheroids. These data revealed that fadraciclib induced cell death in MOLM-13 cells cultured within alginate/gelatin hydrogels, with a decreased level of cell death witnessed in co-culture with HS-5 spheroids.

Conclusions: Here, a reproducible 3D bioprinted human bone marrow niche system was developed, which could be utilised for assessment of novel single and combination therapies. This system was used to assess fadraciclib, a novel AML chemotherapy, and revealed that fadraciclib is a potent cytotoxic inhibitor in AML. This apoptotic effect decreases when co-cultured with HS5 spheroids, simulating the protection conferred by the bone marrow niche.

LABEL FREE NANOPARTICLE TRACKING FOR EYE IN VITRO MODELS

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Introduction: Nanotechnology holds the potential to increase the biostability and bioavailability in the vitreous humour, of drugs approved to treat retinal diseases, improving their performance, and decreasing the number of intravitreal injections. To understand the pharmacokinetic profile and optimise these novel therapeutics, pre- clinical in vitro models are needed. Existing nanoparticle tracking techniques require fluorescent labels, which can impact cytotoxicity, nanoparticles' motion, and cell internalization. Here we present a novel label-free nanoparticle tracking technique[1] as an inexpensive, reproducible, accurate method to study nanoparticle diffusion through vitreous humour substitutes in vitro.

Materials and Methods: Three agar-hyaluronic acid hydrogels with different viscosities (low, medium, and high) were synthesised as vitreous humour in vitro substitutes[2]. Gold nanoparticles (AuNP) were used to understand the effect of nanoparticle charge (100 nm AuNPs positively and negatively charged) and nanoparticle size (50 nm, 100 nm, and 200 nm AuNPs) on their diffusion and distribution in the synthetic hydrogels. The nanoparticles were tracked over time using a label-free real-time technique, based on the optical phenomena of caustics, on an inverted optical microscope. The tracking data was analysed with the ImageJ TrackMate plugin to obtain experimental values of the diffusion coefficient. These values were correlated to the area of the convex hull of the nanoparticles trajectory to better characterise their distribution in the hydrogels.

Results and Discussion: The results indicated that nanoparticle surface charge presented no significant effect on the diffusive behaviour of nanoparticles in the hydrogels. More interestingly, nanoparticles' diffusion values revealed that these hydrogels are heterogeneous, exhibiting different local environments with distinct phases depending on their water content (aqueous, intermediate and gel). Nanoparticles in the aqueous phase had diffusion coefficients of the order of 10^{-12} m^2/s (in agreement with values of diffusion in water), whereas those in the gel phase had diffusion coefficients of three orders of magnitude lower (10^{-15} m^2/s). Additionally, nanoparticle size showed an effect on their distribution in the different phases of the hydrogels. Experimental data showed that 50 nm AuNPs were not present on the gel phase of the low viscous hydrogel, and 200 nm AuNPs were hardly found on the aqueous phase of the medium viscous hydrogel.

Conclusions: The use of label-free tracking revealed that the diffusion of nanoparticles through these hydrogels was heterogeneous and can be directly linked to the different phases in the hydrogel. In addition, nanoparticle size had an important role in nanoparticle distribution across the synthetic hydrogels, making it an important parameter to take in consideration in the design of novel therapeutics for the treatment of ocular diseases. This label-free nanoparticle tracking technique is presented as an advanced tool to characterise the diffusion and transport of nanoparticles in heterogeneous hydrogels. Hence, this technique has the potential to become an indispensable method in the development of in vitro pre-clinical models to optimise nanoparticle-based drug delivery systems for the treatment of retinal diseases.

References

1. Patterson, E.A. and Whelan, M.P. Small.4(10) :1703–1706, 2008.
2. Sachin S. Thakur, et al. European Journal of Pharmaceutics and Biopharmaceutics.148:118-125, 2020.

Acknowledgements: This work was supported by the Doctoral Network in Technologies for Healthy Aging, and the Engineering and Physical Sciences Research Council.

BIOENGINEERING PERIPHERAL NERVE TISSUE: FROM SCAFFOLDS TO MODELS

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Introduction: Current Nerve Guide Conduits (NGCs), used to treat peripheral nerve injuries, are not yet comparable with the gold standard treatment, autografts, due to the lack of topography, guidance cues and cellular components associated with native nerve tissue1. The addition of guidance scaffolds, such as polymer fibres to NGCs, has been shown to increase nerve regeneration distances, however parameters such as material type, fibre diameter size, filling density and additional modifications, differs in literature. Additionally, in order to test these parameters, and NGCs, animal testing still remains the gold standard, as *in vitro* models currently used use a range of cell types, static culture conditions, and are often 2D, not mimicking the *in vivo* environment. Therefore, more clinically relevant, 3D models, *in vitro*, and *ex vivo*, are required.

Materials and Methods: PHAs were produced by bacterial fermentation and characterised as per the methods by Basnett et al[1]. Polycaprolactone (80,000 g/mol) was purchased from Merck. PHAs were dissolved in chloroform, and PCL in dichloromethane, and manufactured into fibres using electrospinning and pressurised gyration. Fibres were characterised by optical microscopy quantified by scanning electron microscope for alignment and fibre diameter. Mechanical analysis and surface roughness of fibres was determined via atomic force microscopy. NG108-15 neuronal cells, and rat primary Schwann cells were cultured on core sheath fibres for 6 days. Chick Dorsal Root Ganglion (DRG) bodies were extracted and explanted whole on to the ends of a 3D *in vitro* fibre testing method[3]. DRGs were cultured statically, and in flow conditions, using a QV900 perfusion system from Kirkstall Ltd. Chick DRGs and NG108-15 neuronal cells were labelled for β III tubulin, and primary Schwann cells labelled using S100β.

Results and Discussion: PHAs, and PCL fibres, were produced via electrospinning and pressurised gyration, in a range of different fibre diameters for testing, from 1 to 13 µm. Aligned fibres were successfully threaded into 3D printed NGCs, in which fibre alignment through conduits was confirmed via microCT. Ex vivo studies confirmed the most efficient fibre diameter, promoting DRG axonal outgrowth, was 10 µm. PHA fibres significantly promoted DRG axonal outgrowth, and Schwann cell migration distances, compared to PCL fibres of the same diameter. Dynamic conditions significantly supported axon outgrowth and Schwann cell migration distances from chick DRG explants after 7 days in culture, compared to static conditions alone.

Conclusions: Aligned fibre scaffolds fibres are very promising in aiding nerve regeneration after injury. They also provide an ideal scaffold for developing nerve injury models. Pressurised gyration delivers a scalable, cost-effective aligned fibre manufacturing method compared to conventional techniques such as electrospinning. The use of PHAs, compared to FDA biopolymers such as PCL, has been shown to significantly increase neuronal, and Schwann, cell adhesion, proliferation and differentiation for nerve tissue engineering applications. They also have many advantages in tissue engineering due biocompatibility and biodegradation rates, but their green scalability. A 3D chick DRG *ex vivo* model, offers many advantages as a nerve injury model. The additional of perfusion, to the model, significantly improves the model, more representing the *in vivo* environment.

References

1. Taylor et al. ACS Biomater Sci Eng, 9(3):1472-1485, 2023.
2. Basnett et al. ACS Appl. Mater. Interfaces, 13, 28, 32624–32639, 2021.
3. Behbehani et al. Int J Bioprint, 4(1):123, 2018.

Acknowledgements: We would like to thank collaborators at The University of Liverpool and University College London, for their contributions to this work. We would also like to acknowledge Dr Bhumika Singh at Kirkstall Ltd for assistance with the Quasi vivo 900.

CONSTRUCTING A 3D IN VITRO CENTRAL NERVOUS SYSTEM LEUKAEMIA MODEL

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Introduction: Acute lymphoblastic leukaemia (ALL) is the most common malignancy in children. The disease had a dismal prognosis up until the 1970s when effective chemotherapeutic regimens were established. However, despite the 90% 5-year survival rates, 10% of children and 50% of adults still relapse. The relapses usually occur in two different areas of the body: the bone marrow (BM), or more frequently, the central nervous system (CNS) (30-40% of total relapses). CNS involvement at diagnosis is considered a risk factor for future relapse, and as it often goes undiagnosed, every patient with ALL are routinely treated for CNS involvement with intrathecal chemotherapy, which is injected directly into their cerebrospinal fluid. That treatment has significant side effects and does not always prevent the relapse. Thus, there is an urgent need for novel, targeted treatments. In this project, we aim to construct a 3D in vitro model to replicate the CNS-ALL disease to study the behaviour of these cells in the niche and facilitate testing of new drugs.

Materials and Methods: For the aim of the study, ALL cell line (SEM) and benign meningioma cell line (BenMen1) were used, as well as primary human cells, including mesenchymal stem cells (MSC, Promocell) and meningeal cells (HMC, Sciencell). Microfluidic devices (ScreenIn3D) were used for 3D cell co-culture, as well as soft hydrogels (Cellendes). The viability of cells was assessed by LIVE/DEAD staining (ThermoFisher).

Results and Discussion Previous studies have shown that meningeal cells in the CNS, as well as MSCs in the bone marrow, can support ALL cells, and possibly provide protection against treatments. 2D co-culture of meningeal cells and leukemic cells resulted in suboptimal cell viability. This was attributed to the fact that the different media of each cell type were incompatible with the other cell type. To overcome this obstacle, we used microfluidic devices (*Figure 1*) that allow for the simultaneous culture of multiple cell types in different compartments, thusensuring different cells were mainly exposed to their preferred type of media, while still being able to communicate. The microfluidic devices allowed for different set-ups; the formation of spheroids by meningeal cells and MSCs in the microwells, while an opposite seeding was also investigated, with adherent cells forming a monolayer on BME (base membrane extract)- coated connected well and leukemic cells in the microwells. Both formats resulted in extended cell viability of up to at least 28 days and increased cell proliferation of leukemic cells. To further improve cell retention and support, leukemic cells were embedded in soft hydrogels, that initially retain the cells within the wells, but over time allow the cells to proliferate and move to other compartments. Adherent cells were shown to attract leukemic cells to their compartments, as cell movement was observed.

Conclusions

Microfluidic devices were found to be an optimal tool for the simultaneous co-culture of niche-specific cell types and leukaemic cells for improved cell viability. Future experiments will include the investigation of the effects of standard of care chemotherapy drugs used to treat ALL to validate the system and subsequently the use of experimental drugs.

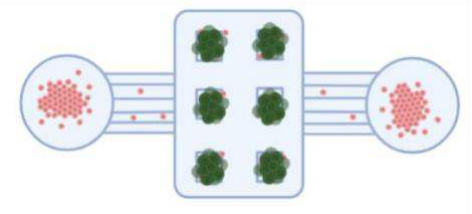

Figure 1. Schematic representation of microfluidic device containing stromal cell spheroids in microwells (middle) and leukemic cells in the extra wells).

GROWTH FACTOR-LOADED MESOPOROUS SILICA PARTICLES, ELECTROSPUN IN PCL FIBRES PROVIDE TOPOGRAPHICAL AND CHEMICAL CUES FOR MSCs TENOGENIC DIFFERENTIATION

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Introduction: Tendon injuries often result in significant pain and disability and impose severe clinical and financial burdens on society. The main aspect that still challenges the effectiveness of treatments developed over recent decades is limited tendon natural healing, attributed to low cell density and vascularization. The emergence of tissue engineering offers more hopeful outcomes in regenerating tendon-like tissues with properties akin to native tendons. Biomaterial-based strategies have emerged as a potent solution to restore tissue function by employing a blend of cells, materials, and appropriate biochemical and physicochemical elements. With this biomimetic approach it will be possible to take advantage of the synergetic effect of multiple factors to control proliferation, self-renewal, and tenogenic differentiation of mesenchymal stromal cells (MSCs).

Materials and Methods: PCL was dissolved (20% w/v) in either acetic acid (AA) or formic acid (FA):AA. The model protein bovine serum albumin (BSA) was added at 50% of the polymer weight and the solution electrospun to produce aligned fibres. Fibres physical properties were determined and BSA incorporation assessed using FTIR. As an alternative delivery system, mesoporous silica nanoparticles (MSNs) were developed. MSNs were loaded with BSA, Lysozyme and GDF7. Encapsulation efficiency (EE) and release were assessed using Bradford and ELISA assays. BSA-loaded MSNs were characterised by SEM, BET and FTIR. MSNs containing fibres were imaged with SEM and the distribution of particles confirmed by XRD analyses. The mechanical properties of fibres with and without MSNs properties were determined in static and in dynamic conditions. Fibre cytotoxicity to MSCs and tendon stem cells (TSCs) was determined with live/dead and Alamar blue assays.

Results and Discussion: PCL dissolved in acetic acid yielded fibres with a 391 ± 0.2 nm diameter, whereas FA:AA fibres were smaller at 282 ± 0.5 nm. After adding 3% BSA, fibre diameters increased significantly to 479 ± 0.2 nm (PCL) and 318 ± 0.1 nm (FA:AA). FTIR confirmed BSA incorporation but revealed structural alterations, more prominent in AA than in FA:AA fibres. MSNs with an average diameter of 118.7 ± 12.2 nm and a BET surface area of 774 m2/g were obtained by condensing TEOS at a concentration of 6% for 4 hours. The optimized particles had an EE of 30% for BSA. The EE for lysozyme and GDF7 reached 99% because of their positive charge at neutral pH, leading to enhanced electrostatic attraction with the negatively charged MSNs. This also resulted in a delayed release of the bioactive molecules in comparison to BSA, which was fully released within the initial 5 days. Up to 4% w/v of particles could be incorporated into PCL:AA before causing it to become unspinnable, leading to the formation of well-aligned composite fibres. MSN particles were spread evenly throughout the scaffold cross-section, in contrast to FA:AA fibres where particles clustered on the outer surface. The presence of MSNs in AA fibres has no impact on Young's modulus, while the formation of clusters in FA:AA fibers leads to a decrease in mechanical properties. Applying mechanical stress for 21 days caused the fibers to align in the direction of the force, resulting in a higher hardness of the scaffold, and a lower strain at break. The platform displayed strong mechanical properties, functioning as a surface for the attachment and growth of both MSCs and TSCs for 21 days.

Conclusions: This study presents a 'green electrospinning' approach employing safe solvents, incorporating loaded MSNs for drug stability. The resulting fibre scaffold, serving as an autograft substitute, features a 3D mechanical matrix for controlled cell orientation and tissue formation. Aligned fibres with high surface area and nanoporosity, in conjunction with GDF7-loaded particles, offer versatile drug delivery, enabling adjustable kinetics for guiding MSC differentiation toward tenocytes.

Acknowledgements: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: The authors acknowledge operating grant support from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 955685, www.helsinki.fi/p4fit.

PROTOTYPE OF A NOVEL BIOREACTOR FOR FINGER FLEXOR TENDON TISSUE ENGINEERING

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Introduction

Diseases of musculoskeletal finger tissues affect a large part of the population, e.g. trigger finger and Dupuytren's Disease; these conditions limit finger function. Their aetiology is unclear, but an emerging consensus suggests that mechanical influences, e.g. repetitive stress, can cause pathological changes in cell behaviour[1]. Study of the subject is limited by the need to simulate the tissue's complex biomechanical environment, which is not met by conventional in-vitro approaches. Here we present a prototype device to provide biomechanically relevant mechanical stimulation for the culture of flexor tendon digitorum constructs.

Materials and Methods

Our approach followed the principles described by Mouthuy *et al.*[2] All custom components were created in Fusion 360, 3D printed with a FormLabs 2 printer and Clear V4 resin, unless stated otherwise.

Bioreactor chamber: The bioreactor consisted of a 50µm polyurethane membrane tube, ~30cm long, with a plastic insert on both ends, sealed with epoxy resin and an external plastic clamp and bolts; the inserts carried feeding tubes to perfuse the chamber. A polyethylene terephthalate multifilament scaffold was placed inside the chamber, attached to both ends, to simulate a flexor tendon. The chamber was filled with water.

Finger model: A 3D model of a male index finger was simplified[3]. Anatomical joints were replaced by simple pivot joints. Flexor tendon pulleys were designed using existing morphometric measurements[4], 3D printed out of Elastic resin. The bioreactor was mounted at the distal phalanx and threaded underneath the pulleys, simulating the flexor tendon. A simple string simulated the extensor tendon.

Mechanisation: The model was mounted on a Zaber T-LSR300D linear actuator. A LEGO frame and pulley were used to allow antagonistic movement of the extensor while using only one actuator. A script was written to automate the actuator. A load cell was fixed to the proximal side of the actuator.

Data collection: The proximal end of the bioreactor chamber was fixed to the load cell, to measure tension in the proximal portion of the simulated flexor tendon. The actuator was mounted vertically, with the finger pointed down. The finger performed multiple flexion-extension cycles in various configurations, including no load, 20g load on distal phalanx, A1 pulley constriction (simulating trigger finger) with and without load, and others.

Results

The bioreactor chamber took ~3x less volume than the original design[2] while retaining great tensile strength and a hermetic seal. The finger model fit the bioreactor and could perform simple, automated flexion/extension. Load cell data represented resistance of finger joints to flexor force, which reflected experimental conditions.

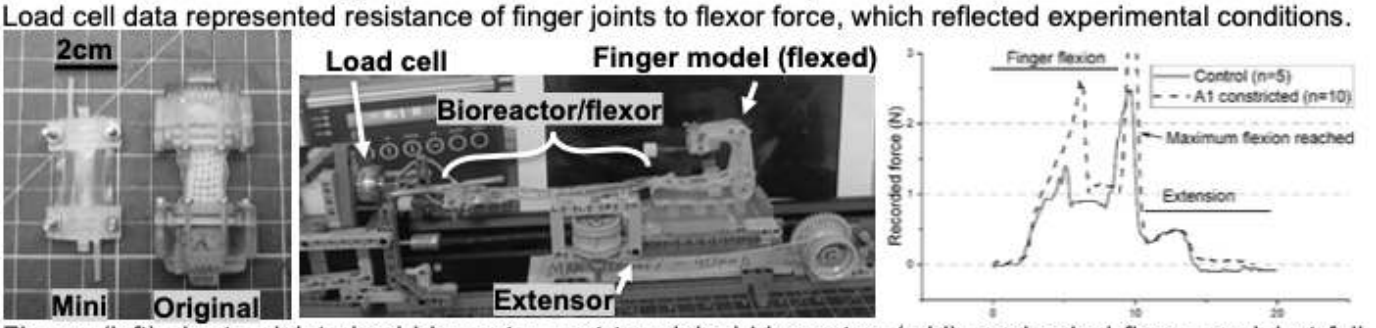

Figure: (left) short, miniaturised bioreactor next to original bioreactor; (mid) mechanical finger model at full flexion; (right) example force reading, comparing recording during control vs. with A1 pulley constricted (no load). Constricting the A1 pulley caused an increase in tension during finger flexion. Average of 5-10 cycles.

Discussion

The prototype bioreactor and mechanised model successfully achieved their design goals. The main limitation was friction between the bioreactor membrane and pulley system, and basic build quality of the platform. The model could only perform simple flexion and extension, and adding more degrees of freedom to simulate a real finger is one of the first objectives for future work. Forces and movements must be adjusted to accurately reflect the real body. Integrity of the bioreactor will need to be confirmed before using it for cell culture.

Conclusions

A successful prototype device to mechanically stress flexor tendon constructs was created. The prototype was automated and collected sensible data, but was limited by build quality and basic design, among other engineering challenges; improvements are planned. Should the platform prove suitable for cell and tissue culture, it may be used to study the interplay between finger tissues, diseases, and the forces they experience.

References

1. Mouthuy *et al.* Comms. Eng. 1: 2, 2022.
2. van de Berge *et al.* Occup. Environ. Med. 80(3): 137-145, 2023.
3. Janson R. MyMiniFactory, user "HandOT1", 2020
4. Doyle J.R. J. Hand. Surg. Am. 13(4): 473-484, 1988.

INFLUENCE OF CELL DENSITY IN AN IMPROVED 3-D BIOPRINTED ACI/MACI MODEL

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Introduction

Autologous chondrocyte implantation (ACI) is surgical procedure used for the treatment of cartilage defects in joints. Chondrocytes are harvested, grown in vitro, and then implanted into the excised cartilage defect, normally with a membrane used to cover and contain the cells. There is a range of variations of ACI, with matrix assisted approaches (known as MACI) using a hydrogel to encapsulate the cells prior to implantation. This aim of this study was to use an in vitro ACI/MACI model to assess whether the Reactive Jet Impingement (REJI) bioprinting technique could offer an enhanced approach to MACI, by delivering a bioprinted cell-filled gel with well defined and consistent cell densities to fill a cartilage defect site.

Methodology: The REJI bioprinting technique was used to print chondrocytes differentiated from the Y201 stem cell line within a collagen-alginate-fibrinogen (CAF) hydrogel. Gels were printed into 96 well plates, with two different cell densities employed: 10 million cells per mL of gel, and 50 million cells per mL of gel. Cell viability, DNA quantification, the expression of functional genes (ACAN, COL2A1, COL1A1 and SOX9), the formation of extracellular matrix (ECM; using immunocytochemical and immunohistochemical staining), and mechanical properties were monitored for up to 28 days. Additionally, the morphology of the CAF hydrogels was examined using scanning electron microscopy (SEM).

Results and Discussion: Low and high cell density loaded CAF hydrogels were successfully printed using the REJI system and were viable for 28 days. ICC and IHC showed that production of COL2A1, GAGs and ECM-like matrix increased throughout the culturing period and high cell density CAF gels showed higher ECM production rates compared to low cell density CAF gels. These results indicated maintenance of phenotype and cell-cell interaction. Live/Dead assays showed cytocompatibility of the CAF gels from the early stages of culturing.

Conclusion: The REJI system can print and maintain Y201 derived chondrocytes and support the production of matrix proteins when cultured in vivo. High cell density CAF gels showed higher COL2A1 production and enhanced production ECM-like matrix, highlighting the potential for high cell density in a biomimetic gel formulation to be used as an enhanced approach to MACI for regeneration of articular cartilage tissue.

3D IN VITRO MODEL OF HUMAN BURN WOUND

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Introduction: Burns impose significant physical, psychological, and economic burdens on millions worldwide. According to the World Health Organization (WHO), an estimated 180,000 deaths occur annually due to burns. To date, research on the recovery process of these injuries has heavily relied on animal models. Experimental models offer controlled settings to replicate the microenvironment of skin injuries, providing insights on their regeneration and the evaluation of novel treatment approaches, such as small extracellular vesicles (exosomes)[1]. The objective of this work is to develop a 3D *in vitro* burn model using human skin equivalents. This model aims to simulate the inflammatory response through the addition of macrophages and to evaluate angiogenesis by subjecting the models to testing on the chorioallantoic membrane assay (CAM)[2]. Additionally, exosomes were integrated as topical treatment to evaluate their effect on the wound healing process.

Materials and Methods: Human skin equivalents were developed using decellularised and lyophilised porcine skin scaffolds seeded with human dermal fibroblast (HDF) and human keratinocyte (HaCaT) cells. HDFs were injected directly to the dermis, while HaCaT cells were seeded onto the epidermis. After 3 incubation days, burn wounds (8 x 2 mm) were created on the skin models using a preheated flat metal wire**.** The burn models were cultured in an air- liquid interface (ALI) for 3, 5 and 7 days using 12-well hanging cell culture inserts. M1 (IFN-g and LPS) and M2 (IL-4 and IL-13) macrophages were polarised from the THP-1 cell line and incorporated individually into the model alongside the burn wound and ALI. The macrophages were pre-cultured in the same 12-well plates and used for the subsequent ALI culture. Exosomes derived from adipose-derived stem cells (ADSCs) were isolated using the ExoQuick-TC® ULTRA isolation kit and screened for exosome markers (CD63, CD81, ALIX, FLOT1, ICAM1, EpCam, ANXA5 and TSG101), as well as size and zeta potential using a Zetasizer Nano ZSP equipment. Exosomes were topically applied to the burn injuries. Histological analysis was conducted to confirm re-epithelialization of the skin scaffolds. The inflammatory response and wound healing process were monitored by measuring the IL-6 and IL-10 levels. CAM was performed for all experimental combinations.

Results and Discussion: The porcine skin (**Fig 1A**) was effectively decellularised as shown in **Fig 1B**. Recellularisation of decellularised and lyophilised skin scaffolds were confirmed via Hematoxylin and Eosin (H&E) staining (**Fig 1C**). Preliminary data suggests that air-liquid interface (ALI) culture enhances HaCaT re-epithelialization and promotes HDF proliferation over a 7-day period, both in skin equivalents and burn models (**Fig 1D).**

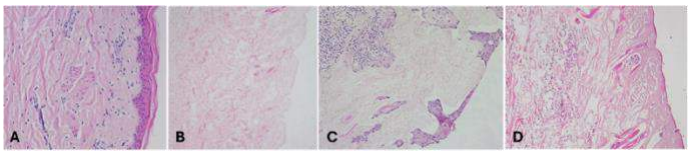

Fig 1. Histological analysis of skin. H&E staining of *ex vivo* porcine skin sections used as positive control (**A**), acellular extracellular matrix scaffold (**B**), the *in vitro* skin equivalent (**C**) and burn model (**D**).

The comparative assessment of treatments will involve the evaluation of the inflammatory response by quantifying IL-6 and IL-10 levels in burn models containing macrophages, both with and without exosomes. The successful integration of the models with CAM tissue will be evidenced by the formation of new blood vessels, demonstrating the scaffold's potential to stimulate angiogenesis[2]. Topical application of exosomes is expected to enhance re-epithelialization, reduce inflammation, and increase vascularization[1].

Conclusions: The *in vitro* model obtained in this research is expected to mimic the pathophysiology of burn injuries. The incorporation of macrophages and CAM embryo assessment allow the simulation of inflammation and angiogenesis of the burn wound healing, respectively. This 3D model provides a valuable platform for evaluating the efficacy of burn treatment, studying burn wound pathogenesis, and testing novel therapies, such as exosomes, in a controlled environment.

References

1. Casado-Diaz A *et al.* Front. Bioeng. Biotechnol., 8:143, 2020.
2. Mangir N *et al.* ACS Biomater. Sci. Eng. 5: 3190-3200, 2019.

PLATELET LYSATE-LOADED ALGINATE MICROPARTICLE HYDROGEL FOR CARTILAGE LESION REPAIR

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Introduction: It is estimated that over 8.75 million people are affected by osteoarthritis, making it a prevalent condition that significantly impacts the population's quality of life and imposes a substantial burden on healthcare systems [1]. Platelet-rich plasma (PRP) injections have emerged as a promising approach by delivering concentrated growth factors to promote chondrogenesis. However, the treatment is hindered by rapid clearance in the synovium, necessitating frequent administration [2]. To overcome this hurdle, our study introduces a novel approach involving a composite hydrogel system that encapsulates platelet lysate (PL) within alginate microparticles (MPs). This innovative system enables sustained PL release and provides a conducive, mechanically robust environment for chondrocytes to undergo chondrogenesis, while maintaining hydrogel injectability, allowing for delivery via minimally invasive arthroscopy.

Materials and Methods: Dialdehyde alginate was synthesised by oxidation of alginate with sodium periodate to render it hydrolytically degradable, followed by synthesis of PL-loaded alginate MPs through bioprinting of cell- laden alginate with an electromagnetic into a $CaCl_2$ bath. These microparticles were subsequently embedded in a hydrogel matrix composed of gelatin methacryloyl and hyaluronic acid methacryloyl. FITC was conjugated to MPs, enabling degradation profile analysis through FITC release in media and hydrogels using a fluoresce spectrophotometer. PL release rate was simultaneously determined via Bradford assay. Concurrently, the secreted extracellular matrix (ECM) from chondrocyte-laden hydrogels was evaluated through histological analysis, GAG and DNA assays, and immunofluorescence for chondrogenic markers.

Results and Discussion: MPs were found to have diameters ranging from 100-200 µm, varying based on extrusion pressure, PL incorporation, and alginate composition. Protein release assays confirmed sustained PL release over 28 days, directly correlated with the degree of alginate oxidation. A higher degree of oxidation resulted in faster PL release, but this was further controllable by incorporating non-degradable alginate, allowing for excellent tunability. Incorporating PL into MPs significantly increased chondrocyte proliferation and chondrogenesis, as evidenced by elevated GAG/DNA and collagen type-II contents within the hydrogel. This underscores the dual role of MPs, serving synergistically as sacrificial porogen and drug delivery vehicles.

Conclusions: This study reveals the potential of a composite system comprising alginate MPs encapsulating platelet lysate within a hydrogel matrix for cartilage lesion repair. By controlling the release of PL through alginate oxidation, this approach offers versatility in cartilage tissue engineering. The hydrogel system acts as a supportive matrix, facilitating chondrocyte proliferation and the formation of hyaline neotissue. Importantly, the composite system can be delivered arthroscopically to cartilage lesions, suggesting potential improvements in the clinical management of cartilage damage.

References

1. Swain S *et al*. Osteoarthritis and Cartilage. 28(6), 2020. 2. Liang Y *et al*. Cartilage. 13(3), 2022

Acknowledgments

We express our gratitude for the funding generously provided by the North Staffordshire Medical Institute 2021. Arjan Atwal acknowledges the PhD funding received from Keele University.

Nanotopography influences host-pathogen quorum sensing and selection of anti-microbial metabolites in mesenchymal stromal cells and Pseudomonas aeruginosa co-cultures

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Introduction

The two major causes of implant failure are poor osseointegration and bacterial infection post-surgery. These factors can result in revision surgery, and in some cases significant implant site morbidity and mortality. Current strategies to treat infections rely on the administration of large amounts of antibiotics to disrupt the bacterial biofilm that forms on the implant surface. However, this approach can inadvertently compromise the recruitment of an individual's cells to populate the implant–bone interface, a process that is essential for successful osseointegration. Therefore, there is a clinical need to develop biomaterials that can improve cell implant surface adhesion and osteogenic differentiation.

Materials and Methods

We present Ti surfaces with high aspect ratio nanotopographies. These topographies reduce biofilm formation by pathogenic bacterium *Pseudomonas aeruginosa*, but also reduce human mesenchymal stromal cell (hMSC) adhesion. We therefore coated the nanotopographies with plasma polymerized polyethylacrylate (PEA) that can organize fibronectin (FN) into open nanonetworks (Figure 1), facilitating hMSC adhesion and solid-phase presentation of growth factors, such as bone morphogenetic protein 2 (BMP2) to improve osteogenesis.

Additionally, as *P. aeruginosa* quorum sensing molecules (QSMs) are well characterized, we developed a panel of QSM standards for mass spectrometry analysis and evaluated hMSC and *P. aeruginosa* metabolites and secretome in response to co-culture with each other.

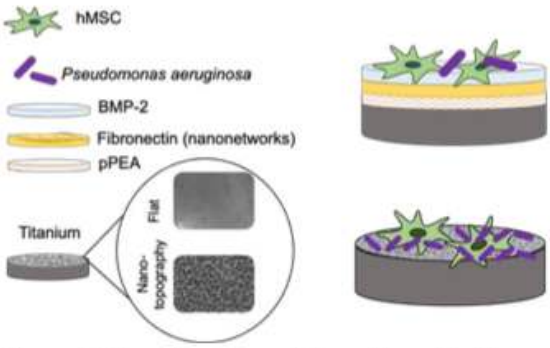

Figure 1Schematic representation of the active layers on titanium flat surface or nanotopography (left). Co-culture of hMSC and *P. aeruginosa* (right).

Results and Discussion

We demonstrate that the coating does not hinder the anti-biofilm effects of the topographies and that they enhance hMSC response, which in turn further impairs bacterial biofilm formation.

We further revealed that the better support of hMSCs using the PEA+FN+BMP2 coating means the cells themselves can suppress QSM signaling and resultant biofilm formation. Finally, we demonstrate the ability to select bioactive metabolites that can act as adjuncts to the surfaces to reduce biofilm formation and further enhance hMSC activity.

Conclusions

The changes in the hMSC metabolome and secretome in co-culture with *P. aeruginosa* allowed us to identify activity metabolites, notably citrate, that reduce biofilm formation while helping hMSC differentiation. Our results support a platform that can be used to understand hMSC-biofilm dynamics and effects on quorum sensing. It can also be utilized to identify novel bioactive metabolites that can act as antibacterial adjuncts. Further, the use of Ti topographies with pro-osteogenic, anti-biofilm properties could help develop novel orthopedic implant materials where infection is a risk.

Acknowledgements

The Medical Research Council (MRC) MR/S010343/1. Bio-TUNE, European Union's Horizon 2020 research and Marie Sklodowska-Curie no. 872869. The Wellcome Trust 204820/Z/16/Z. Enriched Stro^{+1} cells were obtained in collaboration with Prof. Richard Oreffo.

USE OF NATURALLY DERIVED CHEMICAL CROSSLINKERS TO ENHANCE MECHANICS OF BONE-ECM HYDROGELS.

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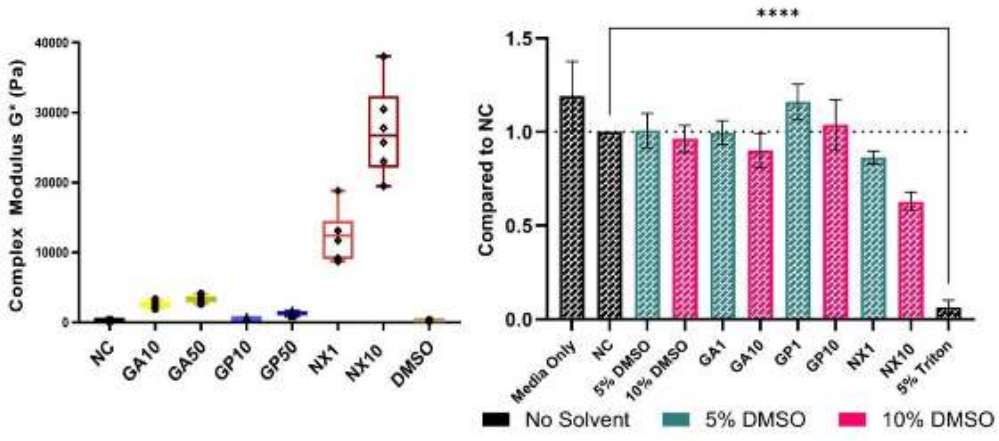
ABSTRACT THEME: Biomaterials

INTRODUCTION: Decellularized extracellular matrix (ECM) hydrogels have found applications as coatings for cell cultures, carriers for cell delivery, and, more recently, as bioinks. Despite being injectable soft materials, introducing crosslinks into the collagen network offers a means to enhance the stiffness of the ECM polymer matrix and bolster its mechanical strength. This study investigates the impact of commonly utilized glutaraldehyde (GA) compared to naturally derived genipin (GP) and another naturally derived crosslinker (NX) on ECM hydrogels derived from bone, aiming to understand their effects on gel properties.

MATERIALS & METHODS: Bovine tibiae underwent demineralization using 0.5 M hydrochloric acid and delipidation with chloroform:methanol before undergoing trypsin-based decellularization. The resulting bone extracellular matrix (bECM) was solubilized via pepsin-based digestion and neutralized to produce bECM hydrogels at a concentration of 8 mg/mL. These ECM hydrogels were immediately immersed in solutions containing GA, GP, or NX at concentrations of 1, 10, and 50 mM for a total duration of 2 hours, followed by multiple PBS washes. Evaluation of the crosslinked hydrogels was conducted using rheological analysis, swelling tests and enzymatic degradation assays. *In vitro* toxicity assessments were carried out using media conditioned by the samples and direct contact with the crosslinked hydrogels and were evaluated using PrestoBlue® and CyQuant LDH cytotoxicity assays.

RESULTS & DISCUSSION: Exposure to all crosslinkers led to significant increases in the complex shear modulus (G*). Relative to non-crosslinked (NC) gels, exposure to 50 mM NX (NX50) resulted in an 85-fold increase in G*, followed by NX10 with a 39-fold increase, GA50 with a 10-fold increase, GA10 with an 8-fold increase, GP50 with a 4-fold increase, and GP10 with a 2-fold increase. However, these increases were accompanied by significant decreases in the linear viscoelastic range of the hydrogels, indicating increased stiffening of the construct and reduced ability to withstand higher shear strains. Crosslinked hydrogels also exhibited significant decreases in swelling ratio, suggesting enhanced stability of the hydrogel structure against hydrostatic pressures post-crosslinking. Despite the strength and stability gains observed following GP exposure, no increased resistance to degradation was noted, as both GP gels degraded over the same time period as NC gels. However, the use of GA resulted in increased resistance, with GA50 gels resisting complete degradation over the 21-day test period. NX utilization resulted in minimal visual changes in the crosslinked gels following collagenase treatment, with free protein quantification confirming notably lower levels of release compared to all other gels. The addition of sample-conditioned media to SH-SY5Y neuroblastoma cells showed no significant cytotoxicity for either GA or GP reagents; however, significant reductions in cell metabolism were observed for NX-crosslinked samples. Nevertheless, upon direct contact with the sample, no significant metabolic challenges or cytotoxicity were observed for any reagent or concentration.

CONCLUSIONS: Naturally sourced crosslinking agents present feasible alternatives for adjusting the mechanical properties of bECM hydrogels. Each agent used exerted distinct effects on hydrogel mechanical attributes and resilience against degradation. Utilizing various reagents at different concentrations for crosslinking offers a pathway to tailor the mechanical and physical traits of bECM hydrogels.



3D PRINTED BACTERIAL CELLULOSE BASED HYDROGEL PATCHES FOR WOUND HEALING

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Introduction: Bacterial Cellulose (BC) is a bacteria-derived biopolymer, which is gaining interest as a potential tissue engineering scaffold material. This is due to BC showing a unique range of properties, including favourable mechanical properties, high hydrophilicity, crystallinity, and purity. BC can also be functionalised both *in-situ* and *ex-situ*, allowing properties to be added and improved, further widening the scope for BC application.

Materials and Methods: In this study, the bacterium *Komagataeibacter xylinus*, was used to produce BC via bacterial fermentation. To confirm that the polymer produced was pure BC, SEM, TGA and FTIR characterisation were performed. After verification, other characterisation techniques including water content, water absorptivity and protein absorption assays were carried out. Protein absorption was measured to compare the potential cell attachment capability of BC in three forms: wet pellicle, freeze-dried and air-dried, with Polycaprolactone (PCL) and Tissue Culture Plastic (TCP) as controls.

Finally, Alginate/BC suspensions were 3D-printed, at compositions of 25:75, 50:50 and 75:25wt% in a 2-layer woodpile structure. Gelation was induced using a calcium chloride crosslinker and initial resazurin testing was used to test cell viability.

Results and Discussion: After production was completed, productivity for the fermentation was measured, giving a yield of 0.8g for every litre of production media used. SEM characterisation was able to identify a porous, 3D fibrillar network with a similar structure to the extracellular matrix, indicating potential for high cell compatibility. FTIR analysis revealed a spectrum with expected absorption peaks for BC and TGA identified the BC thermal degradation at 282.66°C.

Protein adsorption testing results also gave positive results. Both freeze-dried and wet pellicle scaffolds, exhibited significantly higher protein adsorption values when compared to common tissue engineering material PCL. BC also showed a high-water content, (>97%), water absorption measurements confirming the hydrophilic nature of BC, also favourable for cell viability.

3D Printing of Alginate/BC blends allowed excellent printing fidelity at each composition. Resazurin assays, compared cell viability between each composition and BC in its native form, with testing confirming the cytocompatibility of these scaffolds with the HaCaT cell line (keratinocytes) confirming suitability for wound healing.

Conclusions: The production, characterisation, and processing of BC were achieved successfully. BC was compared to current biomaterials, highlighting its potential for wound healing applications. Further work will involve functionalisation of the current Alginate/BC structures, adding active agents that can induce oxygen production, give increase vascularisation and add antimicrobial properties to improve the wound healing potential in these scaffolds.

References

1. Gregory D *et al.* Materials Secience and Engineering: R: Reports 2022
2. Oz Y *et al.* Journal of Biomaterials Applications, 648-681, 2021
3. Raut M *et al.* International Journal of Molecular Sciences, 986, 2023

Acknowledgements: Elliot Amadi is a PhD Student, working in the RoyLab group in Sheffield. The author would like to thank the University of Sheffield that has funded this project via the Faculty Prize Scholarship. The author would also like to thank the RoyLab and other lab groups working in the Kroto Innovation Centre, University of Sheffield, Sheffield, UK.

MESENCHYMAL STROMAL CELL DERIVED EXTRACELLULAR VESICLE IMMOBILISATION ONTO VASCULAR GRAFTS

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Introduction: Cardiovascular diseases (CVDs) are the leading cause of death globally [1]. The use of vascular grafts has shown great promise in treating CVDs however, they are still at risk of developing acute thrombosis and intimal hyperplasia over time [2] [3]. Promotion of endothelialisation at the internal wall of the blood vessel will enhance the natural repair process and subsequently limit the formation of scar tissue and restenosis. In collaboration with Terumo Aortic, using their synthetic endovascular grafts made from Polyethylene Terephthalate (PET) for treatment of abdominal aortic aneurysms (AAAs) [4], mesenchymal stromal cell (MSC) derived extracellular vesicles (EVs) will be investigated as a biologically active graft coating.

Materials and Methods: MSCs were primed in normal (21% O_2) and hypoxic (1% and 5% O_2) culture to generate MSC-EVs with enhanced angiogenic factors [5]. MSC donor cells were analysed for cell viability, proliferation, potency, signalling capacity, and cell cycle to evaluate the effect of hypoxic culture. Subsequently, MSC-EVs were isolated (ultrafiltration and size exclusion chromatography) and characterised by size and concentration (tunable resistive pulse sensing, nano flowcytometry, nanoparticle tracking analysis, microBCA, dynamic light scattering, and transmission electron microscopy). Samples of the PET graft material were prepared with a proprietary coating to facilitate EV binding via integrins *(Figure 1)* [6]. EVs were then fluorescently stained with CFSE (green) and imaged using confocal microscopy.

Results and Discussion: MSC populations were viable with no significant difference in proliferation rate over 7 days. Hypoxia encouraged MSC osteogenesis, inhibiting adipogenesis. Normal and hypoxic MSC-EVs had a similar size and concentration. MSC-EVs successfully adhered to the vascular graft material, where the topography of the woven fabric manipulated EV distribution across the surface.

Conclusions: The analysis of normal, 1% and 5% hypoxic cultured MSCs did not show any difference regarding the cell population. To investigate any changes in MSC-EV cargo, the EVs were (i) assessed for their cytokine cargo, and (ii) assessed for potential angiogenic effects. When human umbilical vein endothelial cells (HUVECs) are cultured on Matrigel, the addition of vascular endothelial growth factor (VEGF) promotes endothelial cell tube formation, it is hypothesised that the addition of hypoxic cultured MSC-EVs will produce a similar response. HUVECs and peripheral blood mononuclear cells (PBMCs) interaction with immobilised MSC-EVs on graft material will be determined.

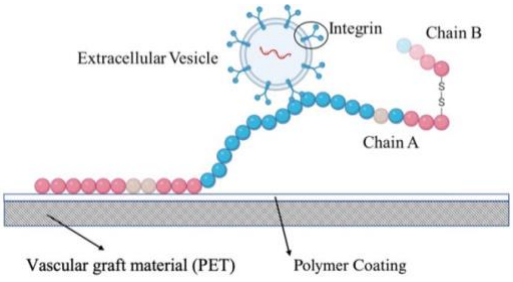

Figure 1: Schematic diagram of fibronectin bound to polymer coated graft material, exposing integrin binding sites for EV immobilisation.

References

[1] World Health Organization, "Cardiovascular diseases (CVDs)." Accessed: Nov. 28, 2022. [Online]. Available: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds)
[2] T. Pennel and P. Zilla, Tissue-Engineered Vascular Grafts, 3–34, 2020.
[3] K. Yoshizawa et al. J Neurointerventional Surgery, 2022. [4] "Terumo Aortic." Accessed: Jan. 13, 2023. [Online]. Available: https://terumoaortic.com/products/relay-pro/ [5] C. Almeria et al., Front Bioeng Biotechnol, vol. 7, Oct. 2019
[6] M. Cantini et al. Biomaterials, 2020.

DELIVERY OF ASCORBIC ACID FROM ELECTRICALLY CONDUCTIVE ELECTROSPUN FIBRES FOR CARDIAC TISSUE ENGINEERING

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Abstract theme: Biomaterials

Introduction

Accumulation of reactive oxygen species is known to contribute towards the pathophysiology of myocardial infarction and the subsequent cardiac remodelling [1]. As such, antioxidant molecules are often incorporated within tissue engineered scaffolds to alleviate oxidative stress and enhance regenerative effects [2], [3]. Electrospinning is among the popular techniques for scaffold fabrication and drug delivery systems, producing fibres with high surface areas and tailorable properties. The release of hydrophilic drugs from fibres may be further controlled through addition of nanomaterials such as multi-walled carbon nanotubes (MWCNT) [4]. Moreover, enhancing the electric conductivity of cardiac scaffolds is associated with improved electrical integration of the graft with the host tissue [5]. We have previous blended ascorbic acid (AA), a well-known antioxidant vitamin, into polycaprolactone (PCL) fibres [6]. The scaffolds were compatible with human umbilical vein endothelial cells and displayed improved antioxidant effects. In the present study, we aim to enhance PCL fibre conductivity using MWCNT and investigate the influence of MWCNT concentration on AA release kinetics.

Materials & Methods

Scaffolds were fabricated using an IME Technologies electrospinner, with polymer solutions consisting of 10% w/v PCL in hexafluoroisopropanol (HFIP), containing 3% w/w AA, and/or 0.5% w/w MWCNT, respective to PCL mass. Briefly, AA was homogenised in HFIP, PCL added, and the solution mixed on a tube roller overnight. COOH functionalised MWCNT (25 nm x 10-20 µm) and an equivalent weight of Span® 80 in HFIP were stirred overnight, sonicated, then added to the PCL/AA solution. The resultant solution was sonicated prior to electrospinning. Additional scaffolds containing AA and different concentrations of MWCNT will be fabricated. MWCNT will be characterised using dynamic light scattering and all scaffolds will be characterised using scanning electron microscopy (SEM) and ImageJ analysis, tensile testing, differential scanning calorimetry and water contact angle. Antioxidant effects and electroconductivity will be analysed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and four point probe method, respectively. AA release kinetics will be studied and fit to a range of mathematical models. Finally, we plan to assess scaffold antibacterial effects, and biocompatibility with rat H9c2 myoblast cells using the CellTiter-Blue® Cell Viability Assay.

Results & Discussion

PCL electrospun scaffolds containing 3% w/w AA and/or 0.5% w/w MWCNT were successfully fabricated (Fig 1). All fibres were smooth and randomly aligned, with the combined scaffold displaying areas of deposits likely due to the higher concentration of solids suspended within the polymer solution. Average fibre sizes were 1.25 ± 0.15, 1.22 ± 0.13 and 1.11 ± 0.11 µm for the AA, MWCNT and combined scaffolds, respectively.

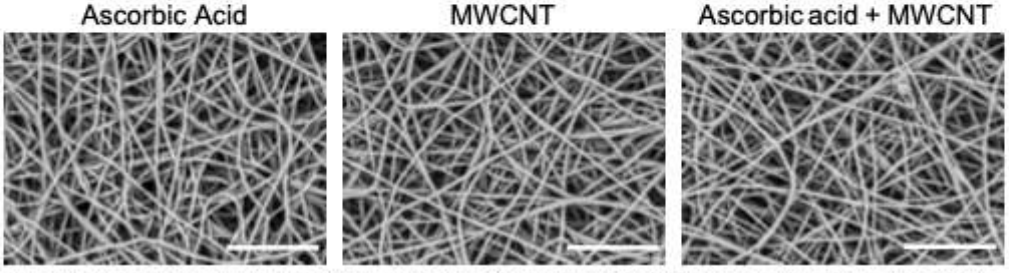

Fig 1. Images of Au-sputter coated electrospun fibres, obtained using a Hitachi TM4000 tabletop SEM. Scale bar = 30 µm.

Conclusion

AA and MWCNT were successfully added to electrospun PCL fibres, with all scaffold groups displaying similar morphologies. These fibres could have implications for future treatment of myocardial infarction.

References

[1] Kurian G *et al.* Oxid. Med. Cell. Longev. ID:1656450 2016 [2] Perez-Araluce T *et al.* Biomimetics. 9: 23, 2024 [3] Handley E *et al.* Adv. NanoBiomed Res. 2: 2200005 (2022) [4] Bulbul Y *et al.* Int. J. Pharm. 568: 118513, 2019 [5] Morsink M *et al.* Acta Biomater. 139: 141, 2022 [6] Handley E *et al.* J. Appl. Polym. Sci. 140: e54242 2023

Acknowledgements

This work is funded by an EPSRC grant (EP/T517884/1) and MRC grant (MR/L012766/1).

Towards Effective RIHT Therapies: Identifying Proteins for *In Vitro* Thyroid Scaffold Evaluation

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Introduction: Radiation-induced hypothyroidism (RIHT) affects up to 92% of head and neck cancer patients who underwent irradiation (IR) therapy, due to unintended injury to the thyroid gland (1). RIHT is characterized by a lack of thyroid hormones, which severely diminishes patients' quality of life through a wide range of symptoms. Current treatments face limitations regarding efficacy and drug interactions (2). Emerging tissue engineering approaches pose as promising alternatives for RIHT treatment, with electrospun scaffolds that mimic extracellular matrices showing great potential for tissue regeneration (2). However, there is a lack of research on specific proteins for *in vitro* thyroid scaffold evaluation. Hence, this study aims to identify proteins expressed in commonly used thyroid cell lines suitable for assessing the regenerative capabilities of thyroid scaffolds . Utilizing a previously established murine RIHT model, we identified preliminary protein candidates (3). Additionally, an electrospun scaffold, previously validated through cell proliferation and viability studies, was selected for *in vitro* protein assessment via immunofluorescent staining (4).

Materials and Methods: Thyroid glands from a murine RIHT model were excised at day 3 and 28 post-IR and 10µm cryostat sections prepared for fluorescent staining. Confocal microscopy assessed RIHT induction and protein expression of E- Cadherin (ECAD), α-smooth-muscle actin (αSMA), Fibronectin (FN), Zonula Occludens-1 (ZO-1), Collagen I (COL1) and thyroid peroxidase (TPO). An electrospun 10% (w/v) polycaprolactone scaffold was fabricated, using hexafluroisopropanol as a solvent. The scaffold was subjected to SEM imaging, tensile testing, contact angle measurement, and differential scanning calorimetry. Nthy-ori 3-1 cells were seeded onto the scaffold, and their metabolic activity, proliferation, morphology and protein expression were monitored over 14 days using various assays. Image processing and statistical analysis were performed with Fiji and R-Studio, respectively.

Results and Discussion: Immunofluorescent staining revealed reduced thyroxine at day 3 and day 28 post-IR, confirming RIHT induction. Concurrently, ECAD decreased significantly while αSMA and FN increased, suggesting fibrotic changes. Additionally, a transient reduction in COL-1 indicated excess extracellular matrix deposition and a decline in ZO-1 thyroid epithelium disruption. Notably, an upregulation of the thyroid-specific enzyme TPO, vital for hormone synthesis, was observed. SEM analysis of the scaffold indicated uniform, smooth and randomly aligned fibres with a diameter of 1.13 ± 0.02 µm and a porosity of 86.58 ± 0.93%. The water contact angle was 128.00 ± 0.05°. Mechanical tests yielded a young's modulus of 1.47 ± 0.35 MPa (<5% strain) and an ultimate tensile strength of 0.38 ± 0.07 MPa. Scaffold crystallinity was 50.32 ± 6.34%, with a 64.41 ± 0.6 °C melting temperature. Cell-seeded scaffold DNA content increased 6-fold, and metabolic activity doubled, during the study. Collagen- like fibril formation was evident in osmium images. Transient αSMA, FN and COL1 expression increases, along with a ZO-1 decrease, mirrored IR-induced tissue disruption but normalized by day 14. Both ECAD and TPO displayed increasing trends, suggesting a potential for scaffold-supported thyroid hormone production.

Conclusion: This study identified key protein markers for assessing the regenerative potential of thyroid scaffolds *in vitro*. These findings highlight the potential of this scaffold for targeted tissue repair in RIHT. Further research is warranted to explore thyroid protein regulation and their interactions with tissue-engineered scaffolds.

Acknowledgements

This work is funded by the EPSRC grant EP/T517884/1 and MRC grant MR/L012766/1

References

1. Zhou, *et al*, Journal of Cancer 12:451, 2021 2. Heim, *et al*, Front Endocrinol 13:997288, 2022 3. Heim, *et al,* EMDS Conference, 2023, Ghent, BE 4. Heim, *et al,* Materialia (SSRN Preprint), 2023

Examining Vascular Cell Behaviour on Dimpled Electrospun Fibre Topography Within a 3D Printed Millifluidic Bioreactor

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Introduction: Cardiovascular diseases (CVDs) are a major cause of mortality and morbidity [1]. Current treatment methods of CVDs, such as arterial bypass graft operations, can suffer from complications such as unfit autologous tissue and compliance mismatch [2]. A promising solution to this is the Tissue Engineered Vascular Graft (TEVG), a device which acts as a scaffold for vascular cell types. By exchanging the damaged vascular tissue for a TEVG, integration of the surrounding healthy vasculature may be promoted, thus effectively restoring the blood vessel to its former functionality [3]. A promising fabrication method of TEVGs is electrospinning, a process which yields formation of an ECM-like substrate consisting of micro- or nano-scale fibers by subjecting a polymer solution to an electrical field. Prior research has suggested that modification of the individual fiber surface can positively influence vascular cell viability and promote the expression of angiogenic-specific genes [4]. It has also been demonstrated that electrospun scaffolds embedded within a fluidic environment show variations in cellular attachment and morphology in addition to the upregulation of genes associated with cellular signalling and motility [5]. As such, this work aims to combine these environments by examining the influence of dimpled electrospun fiber topography on vascular cell behavior in a millifluidic environment.

Materials and Methods: Smooth and dimpled fibre scaffolds were both electrospun using polycaprolactone (PCL). Smooth fibers consisted of 16%w/v PCL dissolved in a 5:1 chloroform/methanol (CFM/MeOH) solution at ambient humidity (~40%), while dimpled fibers were comprised of 16% w/v PCL in a 5:1 CFM/MeOH solution, which was then supplemented with dimethyl sulfoxide (DMSO) at a ratio of 9:1 and spun at increased humidity (~78%). This yielded dimpled fibres as a result of the significantly lower vapor pressure of DMSO. Images of fiber morphology were obtained via SEM. The stereolithography files were generated in SolidWorks and 3D printed via a Way2Production SolFex 350 SLA printer with E-Shell 600 resin. Human Umbilical Vein Endothelial Cells (HUVECs) were seeded upon both scaffold types, after which the scaffolds were inserted into both static well plates and the bioreactor system, from where both cell viability and dsDNA content was assessed and compared via the CellTitre Blue Cell Viability and PicoGreen dsDNA assays respectively.

Results and Discussion: Following construction of an appropriate CFD model, simulation of the fluidic flow indicated that a flow rate of 12.23mL/h yields an average shear stress across the electrospun scaffold of 100mPa, which coincides with the shear stress experienced by vascular cells in smaller-diameter vasculature [6]. Validation of the bioreactor design via waterproof testing did not demonstrate any leakages, and appropriate dimensions for the support of a 10mm diameter electrospun scaffold were verified. SEM images of the electrospun fibers (Figures 1a and 1b) confirm both smooth and dimpled morphologies were achieved, while maintaining a relatively similar average diameter (3.9 and 4.15µm respectively, average dimple diameter 3.39±0.8µm). Cell viability of the HUVECs was maintained across a 12-day period (Figure 1c) in static culture, indicating that both scaffold types are suitable for further cellular analysis.

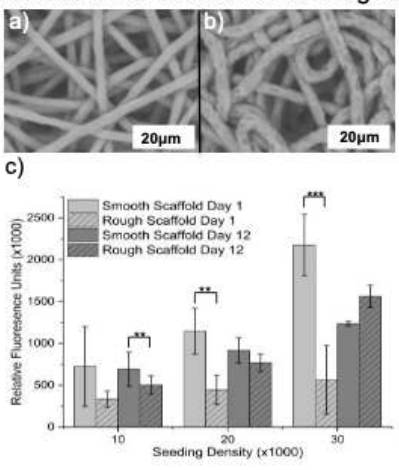

*Figure 1) SEM images of both a) smooth and b) dimpled fiber scaffolds, c) HUVEC viability in static culture across a 12-day period (n=4). Error bars represent standard deviation, with one-way ANOVA results with Tukey's Post-hoc test shown, *p < .05, **p < 0.005, ***p <0.0005.*

Conclusion: Scaffolds consisting of both smooth and dimpled fibers of similar diameters were successfully fabricated via electrospinning. A suitable bioreactor design for inclusion of these scaffolds was validated via CFD and manufactured via 3D printing, from where initial testing indicates the design to be a suitable housing for the electrospun scaffolds. Future work includes subjecting both cell-seeded scaffold types to fluidic flow within the bioreactor. Analyses of subsequent cellular behaviours will include DNA quantification, DAPI and Phalloidin staining, and RT-qPCR analysis.

References 1. Hasan et al., Acta biomaterialia; 10, 11–25, 2014, 2. Karkan et al., J Biol Eng 13, 83, 201; 3. Matsuzaki, et al., Appl Sci(Basel), 9, 1274, 2019; 4. Ren et al., Acta biomaterialia, 70, 140-153, 2018; 5. Franzoni et al., Am J Physiol Heart Circ Physiol, 49-59, 2016; 6. Kabirian et al., J Biomed Mater Res Part A 1231–1237, 2015.

Acknowledgements This work is funded by an EPSRC grant EP/W524384/1 and MRC grant MR/L012766/1.

IMMUNOMODULATORY LIVER-TARGETING MICROPARTICLES IMPACT THP-1 DIFFERENTIATED MACROPHAGE PHENOTYPE

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Introduction

Achieving successful hepatocyte engraftment is a critical challenge in liver cell therapies. The clearance of transplanted cells from the liver is predominantly driven by proinflammatory cytokines, chemokines, or receptors, either during the instant blood-mediated inflammatory reaction or upon activation of resident macrophages. Incorporating immunomodulatory cytokines, such as interleukin 10 (IL-10) or employing agents such as etanercept, a TNF blocker specifically targeting TNF-α, holds potential for mitigating inflammation and enhancing cell transplantation outcomes. Utilising microparticles (MPs) to encapsulate these agents allows for controlled release strategies, potentially boosting cell engraftment. However, conventional MPs made from commercially available polymers are not specific to liver, leading to suboptimal delivery of encapsulated molecules. We have developed methods to create galactosylated microparticles to bind to the asialoglycoprotein receptor (ASGPR) on hepatocytes in the liver.

In vivo testing of immunomodulatory microparticles requires significant use and sacrifice of mice. In line with the 3R's we sought to develop an efficient and effective *in vitro* model to assess the immunomodulatory properties of cytokines released from microparticles. We demonstrate THP-1 cells can be employed as a robust *in vitro* therapeutic model for assessing functional release of immunomodulatory cytokines from microparticle delivery systems. The measurement of functional release and subsequent impacts upon macrophage behaviour demonstrates the potential of this in vitro model to be applied prior to *in vivo* studies.

Materials and Methods

Galactosylation of poly(lactic-co-glycolic acid) (Gal-PLGA) was synthesised in-house. MP morphology and size distributions were measured using SEM and laser diffraction analysis. *In vitro* functional release was measured using ELISAs. Immunomodulatory response of MPs *in vitro* was performed using THP-1 differentiated macrophages and assessed using ELISA and qPCR. MP attachment to hepatocytes was performed using HepG2 cells.

Results and Discussion

MPs fabricated from Gal-PLGA exhibited enhanced MP retention (>85%, compared to 40% with conventional PLGA) *in vitro*. THP-1 differentiated macrophages demonstrated robust activation towards pro-inflammatory- like and pro-immunoregulatory-like phenotypes. *In vitro* functional etanercept release showed binding with TNF-α and subsequently reduced inflammation in THP-1 macrophages, observed by down-regulation of pro- inflammatory genes (CXCL2, IL-1β) and reduced release of soluble inflammatory cytokines (TNF-α, IL-6).

Conclusions

We have demonstrated innovative methods to synthesise galactosylated PLGA. Gal-PLGA microparticles were fabricated with encapsulated etanercept and suitable controlled release kinetics. We further demonstrate that THP-1 differentiated macrophages provide a robust model for immunomodulatory response *in vitro*. The controlled functional release of etanercept resulted in reduced inflammation in the *in vitro* THP-1 model.

Title: UNLOCKING THE POTENTIAL OF THERMALLY RESPONSIVE NANOPARTICLES IN PRECISION DRUG DELIVERY TO ENHANCE BONE REGENERATION

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Introduction: Nonunions, which affect 5-10% of the approximately 12 million fractures annually, persist for at least 3 months without healing. Chronic nonunions, lasting over 12 months despite interventions, pose significant challenges in fracture management. Bone is primarily composed of osteoblasts, with mesenchymal stem cells (MSCs) in the bone marrow capable of differentiating into osteoblasts. This process is governed by bioactive molecules such as insulin-like growth factors, the transforming growth factor-β (TGF-β) superfamily, fibroblast growth factors, and bone morphogenic proteins. Among these molecules, bone morphogenetic protein-2 (BMP2), which belongs to the TGF-β superfamily, plays a key role in differentiating MSCs into osteoblasts. During this process, old bone is resorbed by osteoclasts, leading to the release of BMP2 into the serum. BMP2 is also upregulated and released from the bone in response to bone damage (fracture). As BMP2 plays a major role in bone remodeling, the Food and Drug Administration approved the clinical use of recombinant human BMP2 in 2002. However, the direct use of BMP2 for therapeutic purposes has been associated with several complications, as the dosage required to achieve therapeutic effects is often higher than the normal physiological concentration. This higher dosage is necessary because BMP2 has multiple functions beyond its role in MSC differentiation. Moreover, the current challenges in BMP2 therapy underscore the importance of developing alternative delivery methods that can provide controlled and localised release of the protein. Therefore, there is a pressing need for a system that can deliver BMP2 to the target site in a precise and effective manner, minimising systemic side effects. In this context, the present study aims to investigate the potential of encapsulating BMP2 in Poly(N-isopropylmethacrylamide) (PNIPMAM) coated onto the surface of superparamagnetic iron oxide nanoparticles (SPIONs). This thermally responsive polymer coating will enable controlled release of BMP2 upon exposure to heat above the normal body temperature. By utilising the magnetic properties of SPIONs, combined with external alternating magnetic field stimulation, we aim to create a localized heating effect that triggers the release of BMP2 precisely at the desired site.

Materials and Methods: SPIONs were synthesised by thermal decomposition, and their particle size was assessed using transmission electron microscopy. Subsequently, the SPIONs were functionalised with a PNIPMAM coating. The functionalized particles were characterised using nano-differential scanning fluorimetry to assess the transition temperature. Additionally, the reversibility of this reaction upon returning to normal temperature was evaluated before proceeding with the encapsulation of BMP2. To release the encapsulated BMP2, a competitor protein is required[1], ovalbumin was employed[2] in this experiment. To evaluate the amount and bioactivity of BMP2 released from the encapsulated SPIONs, an *in vitro* model system C2C12 cells were used. These cells were cultured and treated with BMP2.

Results and Discussion: The characterisation of SPION-based nanoparticles revealed a mean particle size of approximately 13 nm. Assessment of the polymer-coated SPIONs demonstrated a transition in the polymer (assessed by precipitation) occurring at 45°C. C2C12 cells treated with BMP2 exhibited a dose-dependent increase in alkaline phosphatase activity ($P < 0.01$) compared to untreated controls over 2-6 days, allowing for the determination of the amount of active BMP2 released from the encapsulated polymer-coated SPIONs.

Conclusions: The synthesis and characterization of thermally responsive polymer coated SPIONs demonstrate their potential as versatile platforms for targeted drug delivery. The dose-dependent responsiveness of C2C12 cells to BMP2 *in vitro* provides a valuable model for assessment of the bioactivity and the amount of BMP2 released from polymer coated nanoparticles. The outcome shows the efficacy of the controlled BMP2 release from nanoparticles, suggesting their viability as a therapeutic platform. Such a targeted delivery system holds promise for enhancing bone regeneration outcomes while minimizing adverse side effects associated with systemic BMP2 administration. These nanoparticles could therefore be a promising avenue for the release of molecules to be used in the field of regenerative medicine. Further research is warranted to explore the clinical translation of these findings and their implications for therapeutics.

References:

1. Walker M et al. *ACS Applied Nano Materials* 3,6: 5008-5013, 2020
2. Sharma R et al. Nanoscale 16,5: 2466-2477, 2024.

DEVELOPMENT OF IN VITRO TRIPLE NEGATIVE BREAST CANCER MODEL FOR TME CHARACTERISATION

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Abstract theme: Biomaterials

Introduction

HA has been a widely investigated in the area of drug delivery for several fields, due to the versatility associated with its modification, as well as its biocompatibility and biodegradability. In the field of cancer treatment the affinity of the material for the CD44 receptor has been of particular interest, mainly due to its overexpression in several cancers of epithelial origin.[1] These properties make HA a prime candidate to deliver metallacarboranes, a family of boron cluster compounds that have been found to be promising therapeutics in a number of areas in biomedicine, and can be tracked in vitro using stimulated Raman scattering (SRS) microscopy.[2–4] As regards drug delivery systems for boron clusters examples are relatively sparse, especially in the case of HA materials.

Materials and Methods

Hyaluronic acid (HA) materials were characterised using SEM, EDX, ^{1}H, ^{11}B, and DOSY NMR, UV-Vis and IR. In-vitro cytotoxicity testing was carried out with colorimetric cell viability and fluorescence microscopy live/dead analysis assays. Drug internalisation experiments were carried out using SRS microscopy.

Results and Discussion

Metallacarborane-loaded hyaluronic acid materials were successfully prepared through a non-covalent interaction between the appended positively charged lysine groups on the HA and the intrinsically anionic metallacarboranes. Release was shown to be quite fast with over 50% of the loaded compound released after one hour in PBS. GPC traces showed the compounds to remain complexed to the polymer 3 hours after solvation of the material in water. Toxicity testing showed no changes compared to the toxicity of the free compound vs human breast cancer cell lines and normal human cell lines after complexation with the HA. Rapid internalisation was demonstrated with the presence of the B–H signal within human breast cancer cells using stimulated Raman microscopy imaging of live cells following treatment with a solution of the material.

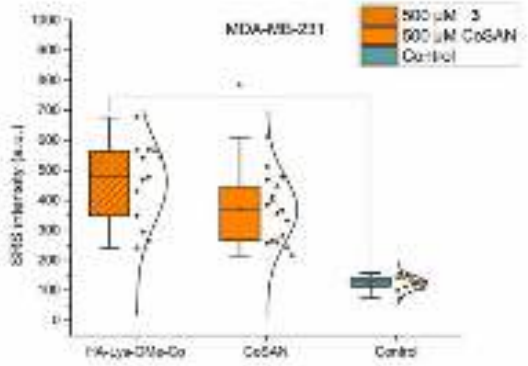

Fig.1 Measured SRS intensities at 2500 cm^{-1} (B–H stretch) in MDA-MB-231 cell line.

Conclusions

Hyaluronic acid appended with positively charged components may be loaded with metallacarboranes, a family of metal complexes showing a lot of potential in an array of areas within medicinal chemistry including new cancer therapeutics. Significantly, the complexation with the HA didn't affect the cytotoxicity of the compounds, thus providing a building block for future drug delivery strategies within the field of metallacarboranes.

References

[1] C. Buckley, E. J. Murphy, T. R. Montgomery, I. Major, Polymers (Basel). 14:1–23, 2022.
[2] N. Murphy, E. McCarthy, R. Dwyer, P. Farràs, J. Inorg. Biochem., 218:111412, 2021.
[3] P. Farràs, E. J. Juárez-Pérez, M. Lepšík, R. Luque, R. Núñez, F. Teixidor, Chem. Soc. Rev., 41:3445–3463, 2012.
[4] N. Murphy, W. J. Tipping, H. J. Braddick, L. T. Wilson, N. C. O. Tomkinson, K. Faulds, D. Graham, P. Farràs, Angew. Chemie - Int. Ed., 62:1–7, 2023.

Acknowledgements

The authors thank Dr. Wenming Tong for assistance with the release studies of the loaded biopolymers.

PERFLUOROPENTANE NANODROPLETS FOR OXYGEN DELIVERY TO OSTEOCLASTS AND OSTEOBLASTS FOR BONE REPAIR

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Abstract theme: Biomaterials

Introduction

Hypoxia is associated with common bone diseases and complications. Current clinical interventions to improve bone healing by improving tissue oxygenation are invasive or unsuccessful. Perfluorocarbon nanodroplets are capable of dissolving large amounts of oxygen and may be a method for delivering oxygen to poorly healing bone fractures. In this work we tested the hypothesis that oxygenated nanodroplets affect the growth and differentiation of osteoclasts and osteoblasts.

Materials and Methods

Nanodroplets were made by sonication of mixtures of DSPC, PEG(40)s and perfluoropentane in PBS. To determine oxygen delivery to cells, the relative concentration of HIF-1α in lysates of SAOS-2 cells grown in hypoxia, in the presence and absence of nanodroplets, was measured by western blot and compared to those grown at normoxia. Bone marrow stromal cells (BMSCs) and peripheral blood mononuclear cells (PBMCs) from human donors were seeded at 2.5×10^4 and 7.6×10^5 cells/cm^2 respectively. The cells were then treated with a dilution of air-saturated nanodroplets (0, 0.01, 0.1 and 1% v/v) and placed in either normoxia or hypoxia. Bone cell activity was quantified using alkaline phosphatase (ALP) expression, alizarin red staining and manual cell counting.

Results and Discussion

HIF-1α concentration in the presence of air-saturated nanodroplets was found to be 71.8% ± 10.3% compared to the absence of droplets in hypoxia ($n = 3$, $p = 0.0414$). This was not observed in the oxygen- (113.2% ± 18.6%, $n = 3$, $P > 0.05$) and nitrogen-saturated (95.8% ± 21.1%, $n = 2$) groups. ALP was found to decrease significantly after 14 days of treatment (87.6% ± 3.1%). However, alizarin red staining for mineralisation was found to significantly increase when cells were treated with nanodroplets and lipids only under normoxic osteogenic conditions (8-fold and 3-fold increase, respectively). Osteoclast number was found to decrease in normoxia with treatment ($p = 0.0002$). In hypoxia, average osteoclast size decreased ($p = 0.0001$).

Conclusions

The HIF-1α decrease in the presence of air-saturated nanodroplets, suggests that the nanodroplets are capable of increasing the oxygen delivered to cells in hypoxia. Early-stage osteogenic activity appears to decrease in BMSCs treated with nanodroplets. However, the mineralisation in the same cells is significantly increased with the same treatment. Osteoclast number and size is decreased with nanodroplet treatment. Together, this work indicates that nanodroplets may be suitable for aiding bone repair.

Acknowledgements

Orthopaedic Research UK

A CONDUCTIVE HYDROGEL WITH SELF-HEALING PROPERTIES

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Introduction: Hydrogels, crosslinked three-dimensional polymeric structure, serve as supportive matrices for cells in tissue regeneration applications. These biomaterials emulate the characteristics of the native extracellular matrix (ECM), including porosity, high water content, soft mechanical properties, and biocompatibility, thus providing crucial physical support for cells in engineered tissues. By integrating electroconductive elements into hydrogels, their mechanical properties can be combined with conductivity, facilitating electrical stimulation of excitable cells and tissues such as muscle, heart, or the nervous system. Conjugated polymers, electrically conductive materials, are suitable for the fabrication of electroconductive hydrogels due to their organic nature which enables their interaction with conventional polymers to produce tailored hydrogels while retaining the ECM-mimicking properties [1].

Materials and Methods: In this study, an electroconductive hydrogel, Cs-PEDOT, was developed by blending the conjugated polymer, poly(3,4-ethylenedioxythiophene)-polystyrene sulfonate (PEDOT:PSS) and the biopolymer chitosan (Cs). The morphologies of the Cs-PEDOT hydrogel were examined by scanning electron microscope (SEM). The electric properties of the hydrogel were investigated by recording I-V curves and cyclic voltammetry (CV). Rheological properties and dynamic mechanical analysis were employed to study their mechanical properties and mechanical self-healing ability. Electrical healing properties of the hydrogel were also confirmed by powering up a light-emitting diode (LED). Moreover, adhesive properties were tested by visual inspection. Biological functions of the hydrogels were evaluated for their biocompatibility for 2D cell culture of myoblast cells, using confocal microscope.

Results and Discussion: A electroconductive Cs-PEDOT hydrogel was formed by blending chitosan and PEDOT:PSS together. We aimed to develop a conductive hydrogel that displays mechanical responsiveness, including flexibility, stress relaxation, shear-thinning, self-healing and adhesive behaviour.

The morphologies of the Cs-PEDOT hydrogel were observed by SEM, showing that the hydrogel had a highly porous 3D network structure with an average pore diameter of 328 ± 75 µm. The electric properties of the hydrogel were investigated by recording I-V curves that revealed a conductivity of 0.016 ± 0.002 S/cm. The electroconductive Cs-PEDOT hydrogel exhibited viscoelastic properties associated with dynamic networks such as stress relaxation with $\tau 1/2 = 70$ s, and shear thinning with the viscosity decreasing with shear rate (from 0.1 to 12 s^{-1}). The hydrogel could be injected through a microneedle of 800 µm diameter, completely recovering its shape upon its release. According to the viscoelastic properties, the storage modulus of the Cs/PEDOT hydrogels was measured to be approximately 1.6 kPa. Furthermore, the hydrogel network could self-heal within seconds, regaining its conductivity as confirmed by powering up a light-emitting diode (LED), as well as retaining its mechanical properties. The Cs-PEDOT hydrogel also exhibited adhesive properties confirmed by visual observation. The hydrogel showed biocompatiblity, in which cell morphologies of C2C12 myoblast cells cultured on the hydrogel were recorded by DAPI (nuclei) staining images.

Conclusion: Overall, a Cs-PEDOT electroactive hydrogel was successfully formed by blending chitosan solution and PEDOT: PSS dispersion. In addition to its conductivity, the hydrogel showed multiple mechanical- responsiveness properties such as shear-thinning, stress relaxation, self-healing and self-adhesive, properties relevant for its minimally invasive placement on tissue and long-term use as a bioelectronic implant [2].

References

1. Fidanovski K et al. Adv. Healthcare Mater. 8: 1900053, 2019. et al. Adv.Sci. 6: 1802077, 2019.
2. Xu Y et al. Adv.Sci. 6: 1802077, 2019.

THE INFLUENCE OF IRRADIATION ON THE GROWTH AND SURVIVAL OF HSG CELLS ON ANTIOXIDANT SCAFFOLDS

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Introduction

It is well established that radiotherapy (RT) for cancer treatment also damages surrounding healthy tissue, leading to phenomena such as oxidative stress (1). Characterised by an increase in reactive oxygen species (ROS), oxidative stress can lead to alterations within the tumor microenvironment that cause subsequent, and in some cases debilitating, side effects post-treatment (2). Antioxidants have been employed as a means to tackle excess levels of ROS; and recently, vitamins and their derivatives have been investigated (3).

Previous work has established that healthy, undamaged human submandibular gland (HSG) cells (HeLa derivative) could successfully grow and proliferate on antioxidant scaffolds containing retinyl acetate (RA), a derivative of vitamin A (4). The aim of this study was to investigate how irradiated HSG cells interacted with antioxidant polycaprolactone (PCL) electrospun fibres, and how this compares to healthy, non-irradiated HSG cells.

Materials and Methods

Antioxidant scaffolds were fabricated by electrospinning 0.5% RA (w/v) dissolved with 10% PCL (w/v) in hexafluoroisopropanol (HFIP). Characterisation of the scaffolds was performed by scanning electron microscope (SEM) imaging and mechanical testing.

HSG cells were subjected to 10 Gy gamma radiation. Irradiated or non-irradiated cells were seeded onto the scaffolds and cultured over a period of seven days. Cell responses were measured via cell viability and DNA quantitation assays, and transcriptional changes were measured by gene expression analysis.

Results and Discussion

Electrospinning was used to create 0% and 0.5% RA fibres with smooth and randomly aligned morphologies, and fibre diameters of 1.21 ± 0.09 μM and 1.18 ± 0.10 μM respectively, as shown by SEM analysis (Figure 1). Characterisation testing including tensile testing was performed. Consistent with previous results, inclusion of the antioxidant did not have any significant effect on the characteristics of the fibre (4). Cell studies showed that the damaged cells were able to recover from irradiation exposure over a seven-day period.

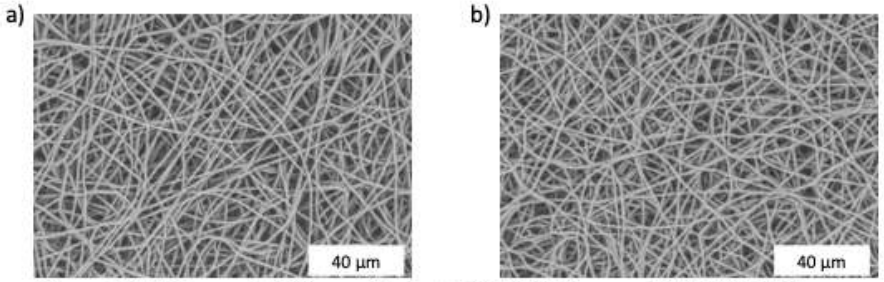

Figure 1 SEM Images (x2000 magnification) of (a) PCL fibres and (b) 0.5% RA fibres

Conclusions

This study has shown the efficacy of antioxidant containing scaffolds to support the survival and proliferation of HSG cells which had been exposed to radiation. Encouragingly, this suggests that such a conduit has the potential to support the repair of the damaged site post-radiation therapy.

References

1. Wei *et al.,* Oxid Med Cell Longev., 2019, 2019
2. Westwood *et al.,* Front. Biomater. Sci., 3, 2024
3. Patil *et al.,* IJPER, 54, 2020
4. Westwood *et al.,* Front. Bioeng. Biotechnol., 11, 2023

Acknowledgements

This work is funded by EPRSC studentship EP/T517884/1 and MRC grant MR/L012766/1.

MANUFACTURE OF PSEUDO-RETE RIDGES IN A BILAYER FOR SKIN REGENERATION

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Abstract theme: Biomaterials

Introduction

Rete ridges (RR) at the dermal-epidermal junction of human skin are putative stem cell niches for cells that replenish the epidermis and improve shear resistance of the epidermis/dermis layers. Loss of RR due to full thickness wounding may therefore impair the formation of healthy native microarchitectures during healing. Where some commercially available wound healing aids do consider the skin's intrinsic bilayer structure (Integra™, for example), RR are overlooked at the layer interface. Herein, pseudo-RR are designed into a synthetic bilayer by combining high internal phase emulsion polymerisation (polyHIPE) as a dermal matrix, and an electrospun membrane as an epidermal scaffold. Undulating topography is incorporated with the aim of providing a pseudo-niche for cells involved in tissue regeneration, potentially ameliorating wound healing.

Materials and Methods

A micro-ridged surface is designed using the cos(x)*cos(y) function and 3D printed using the FormLabs 3 printer, as previously demonstrated[1]. These templates were used to make topographical silicone moulds. 4-arm methacrylated polycaprolactone (PCL-MA) HIPE emulsion is cast into the moulds and UV cured. Stir speed and temperature of the emulsion was varied to select appropriate parameters for a pore size suitable for fibroblast integration. The bilayer was created either by: (1) 15% (w/w) PCL solution (9:1 DCM:DMF) is electrospun directly onto the cured patterned polyHIPE or; (2) a patterned mould is lined with an electrospun PCL membrane and HIPE emulsion is poured on top before curing. Scanning Electron Microscopy (SEM) imaging and ImageJ were used to assess bilayer morphology (porosity, fibre diameter, RR geometries and layer integration).

Results and Discussion

SEM images of debossed pseudo-RR on polyHIPE suggest the incorporation of topography is achievable on these scaffolds (Fig. 1A and 1B). Increasing the emulsion temperature of HIPE causes an increase in pore size, yet increasing emulsion stir speed decreases pore size (Fig.1C and D). Parameters are selected to produce quality pore morphology and a mean pore size within 20-125 µm for fibroblast infiltration as seen in literature[2]. Handleability (layer integration) is much improved by method (2). However, pouring the emulsion leads to solvent melting and a decrease in fibre quality on the electrospun layer.

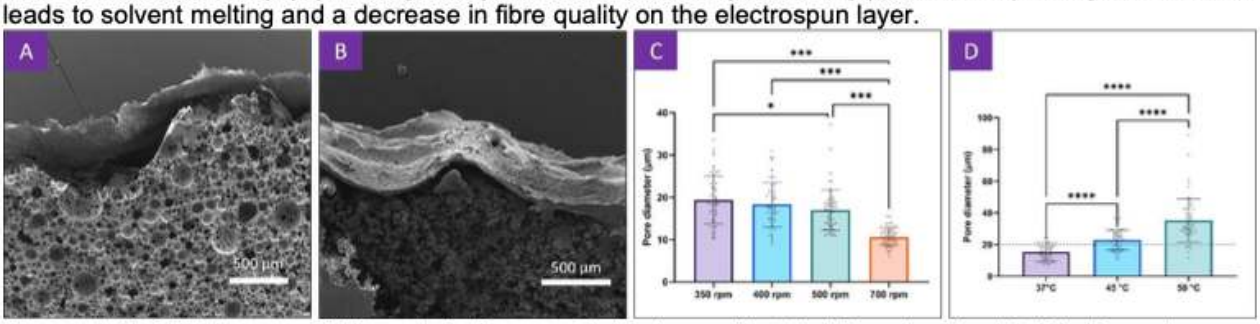

Figure 1 A) SEM images of bilayer interface created using method 1 (A) and method 2 (B). Pore size as a function of emulsion stir speed (C). Pore size as a function of emulsion temperature (D). (*) = P≤0.05; (***) = P≤0.01; (***) = P≤0.001.

Conclusions

PolyHIPE scaffold surfaces can be debossed by ridged moulds to form pseudo-RR. Mould design should be improved to achieve finer resolution ridges more alike native sizes. This bilayer scaffold may eventually contribute to generating more native-like skin upon healing.

References

1. Ramos Rodriguez D *et al*, ACS Biomater. Sci. Eng. 7: 2803-2813, 2021.
2. Dikici B *et al*, *Biomater. Sci.* 9: 7297-7310, 2021.

Acknowledgements

The presenter would like to acknowledge the EPSRC for funding this project through the Advanced Biomedical Materials CDT.

Decellularized Human Liver and Skin Tissues: Profiling with Ambient Vibrations Optical Coherence Elastography and Insights into Decellularization and 3D Hydrogel Fabrication

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Abstract theme: Optical Coherence Elastography, Stiffness, Decellularization, Liver, Skin

Introduction

By simulating the natural environment, tissue engineering offers a viable path towards understanding tissue functions and disease modelling. Monitoring the mechanical property of in-vitro studies during culture is critical for their maturation and functionality. Using decellularized extracellular matrix (dECM) in 3D-printed gels has the potential to mimic tissue microenvironments. This abstract introduces decellularization, involving dECM hydrogels with varying stiffness levels and a novel modality called MechAscan post-processing algorithm, coupled with the Optical Coherence Tomography for Engineered Tissue (OCTET). This facilitates non-invasive and sterile assessment of tissue mechanical properties and enables high-throughput screening.

Materials and Methods

Human liver and skin samples underwent decellularization using diverse methods. Human liver samples were decellularized with Trypsin, EDTA, Triton-X-100 and Sodium Deoxycholate and disinfected with Peracetic acid. OCT imaging was performed on both native and dECM tissues, and mechanical stiffness was assessed using MechAscan. Following liver matrix decellularization and mechanical property analysis with Mechascan, human skin samples were decellularized using 1% (v/v) Triton X-100- and 0.1% (v/v) ammonium hydroxide (NH4OH). In vitro experiments involved the preparation of hydrogels with different stiffness levels using dECM solutions derived from human skin. Subsequently, 3D samples were fabricated using 3D printers and imaged with OCT.

Results & Discussion

A quantitative analysis with Mechascan was conducted to assess the mechanical properties of human liver samples, as depicted in mechanical contrast maps (Figure 1). This comparative analysis showcased the mechanical characteristics of both native human liver tissue and dECM offers valuable insights into their structure and mechanical behaviors. Additionally, 3D-printed scaffolds, fabricated from hydrogels derived from human skin dECM at various concentrations, underwent thorough examination. The evaluation of these hydrogels revealed notable differences in stiffness levels across different groups of 3D-printed scaffolds. Evaluation of the hydrogels showcased distinct stiffness levels among different groups of 3D-printed scaffolds.

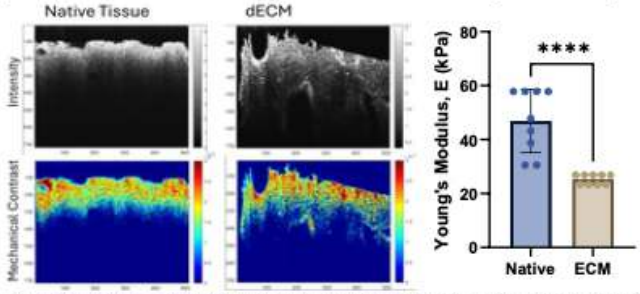

Figure 1: *Mechanical property analysis of decellularized human liver tissue, native human liver and cirrhotic tissue by using OCT imaging and Mechascan post processing algorithm. Images show the stiffness distribution in mechanical property map. The graph is representing the difference in mechanical properties of native tissue and decellularized matrix (P<0.0001).*

Conclusion

The obtained results, inclusive of intensity and mechanical contrast images derived from imaging data, along with the quantitative analysis results, highlight the efficacy of MechAscan for non-destructive spatial mechanical assessment of engineered constructs and decellularized human liver tissue. This abstract provides important insights into decellularization, hydrogels, 3D printing and tissue dynamics by explaining how cutting-edge technologies are integrated to advance tissue engineering.

Acknowledgments

Funding was provided by Republic of Turkiye Ministry of National Education, EPSRC (EP/PO31137/1 and EP/PO31250/1), MRC UKRMP Hub: Engineered Cell Environment (# 623924).

References

1. Mason, J.H., Luo, L., Reinwald, Y. *et al.* Debiased ambient vibrations optical coherence elastography to profile cell, organoid and tissue mechanical properties. *Commun Biol* **6**, 543 (2023). https://doi.org/10.1038/s42003-023-04788-0
2. Saldin, L. T., Cramer, M. C., Velankar, S. S., White, L. J., & Badylak, S. F. (2017). Extracellular matrix hydrogels from decellularized tissues: Structure and function. Acta biomaterialia, 49, 1-15.

ROLE OF EXOGENOUS ELECTRICAL STIMULATION ON *IN VITRO* CELL FUNCTIONING IN HUMAN MESENCHYMAL STEM CELLS

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Introduction: Electrical stimulation has recently gained huge attention as a promising tool for tissue engineering. Benefits of electrical stimulation can include influencing cell proliferation, migration, differentiation, improving vascularisation and tissue integration, and extracellular matrix production. The use of exogenous electrical stimulus has been widely applied in clinical interventions such as in cardiac pacemakers, transcutaneous electronic nerve stimulator devices and more recently considered in chronic wound dressing products. For advancing electrical stimulation-based therapies, it is essential to study the underlying signalling mechanisms affected by exogenous electrical stimuli, at both the cellular and tissue level. In this project, the role of externally applied electrical stimuli on cell activity, proliferation, and extent of trilineage differentiation of human mesenchymal stem cells (hMSCs) is investigated.

Materials and Methods: A simple and customised 24-well plate electrical stimulation bioreactor with platinum wire electrodes was developed for applying direct current (DC) electrical stimuli on bone marrow-derived hMSCs for *in vitro* systems. Three different DC electrical voltage regimes (50, 100 and 150 mV/mm) were applied to the cells for 60 min per day, for seven days, along with chemical induction of the cells to differentiate into adipogenic, chondrogenic and osteogenic lineages. Cell metabolic activity and relative gene expression during trilineage differentiation were investigated at various time points post electrical stimulation until day 21, to understand the effect of externally applied electrical stimuli on the proliferation and differentiation of hMSCs.

Results and Discussion: A high throughput 24-well plate electrical stimulation bioreactor was developed using a Computer-Aided Design (CAD) model. Its biocompatibility was tested to demonstrate the advantage of using platinum electrodes as it showed minimal corrosion effects and generated less harmful faradaic by-products, unlike other electrode candidates. Improved cell proliferation and metabolic activity were observed in electrically stimulated cells when compared to the control (cells with no electrical stimulation and cultured in respective differentiation media). qPCR-based relative gene expression of adipogenic, chondrogenic and osteogenic gene markers post-electrical stimulation was compared to the control, showing varying effects of different ES voltage regimes on lineage commitment.

Conclusions: This study demonstrates the promising application of the custom-designed 24-well plate electrical stimulation bioreactor for *in vitro* experiments for application in tissue engineering. The effect of three different DC voltages shows enhanced cell metabolic activity with increased DNA content per cell with the application of direct electrical voltage regimes compared to the control. The difference in relative gene expression, by applying different external ES can be correlated to the intrinsic transmembrane potentials that innately direct cells to differentiate to a particular lineage. Also, the bioreactor can be extended to incorporate additional electrical stimulation parameters such as frequency, waveforms, duty cycle, *etc.* to further investigate the effect of electrical stimulus on stem cell-based tissue engineering technologies.

Acknowledgements

We acknowledge the Henry Royce Institute for Advanced Materials (grant no. EP/R00661X/1, EP/S019367/1, EP/ P025021/1, and EP/P025498/1) for support and equipment use.

DEVELOPING AN INNOVATIVE BIOPROSTHETIC HEART VALVE UTILISING DECELLULARISED PERICARDIUM

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Introduction: Calcific aortic stenosis (AS) is the progressive narrowing of the aortic valve due to degenerative calcification over time, leading to an increasingly severe reduction of blood flow and subsequent risk of heart failure[1]. Current bioprosthetic aortic valve replacements (BAVR) also calcify, and suffer from long-term durability issues, which are often attributed to the glutaraldehyde (GA) crosslinking and residual immunogenicity of the biomaterial[2]. Alternative tissue processing methods are needed for the construction of calcification resistant BAVRs. The aim of this study was to evaluate the physicochemical properties of PEGylated decellularised pericardium, compared to GA and carbodiimide crosslinked samples.

Materials and Methods: Patches (25 cm^2) of bovine pericardium were decellularised using low-concentration sodium dodecyl sulphate, and histological analysis was performed to confirm decellularisation. Samples were subsequently treated with polyethylene glycol (PEG) N-Hydroxysuccinimide (NHS) at increasing molar ratios (PEG:Lysine residues) of 1:1, 1:10, 1:1000, glutaraldehyde concentrations (0.1 % & 0.6 %) or 1-ethyl-3-(3- dimethylaminopropyl) carbodiimide (EDC, 30 mM).

The degree of crosslinking was determined using the colorimetric TNBS (2,4,6-trinitrobenzenesulfonic acid) assay, differential scanning calorimetry (DSC), and resistance to collagenase digestion. The hydration degree and water activity of bound water was analysed. Surface topography and collagen D-banding was assessed using scanning electron microscopy (SEM) and atomic force microscopy. The biomechanical parameters (collagen phase modules [Ec] and Ultimate tensile stress [UTS]) were determined though uniaxial tensile testing to failure.

Results and Discussion: Successful decellularisation was determined through the absence of nuclear material in histologically processed samples. Resistance of decellularised samples to both thermal and enzymatic degradation increased with GA and EDC treatment, whilst PEGylated samples were comparable to untreated samples. A reduction of available amines was observed across each chemically treated group using the TNBS assay. Visual analysis of surface features using SEM revealed a complex and interwoven basement membrane, and preliminary AFM studies provided information on collagen D-band periodicity for further investigation. No significant differences in biomechanical properties were found across all groups.

Conclusions: This study explored the use of PEG-NHS as an innovative chemical treatment to produce pericardium-derived biomaterials that may resist *in vivo* calcification and prolong the life of BAVR implants. An array of biological and material property tests to investigate these novel anti-fouling molecules, and their effect on decellularised pericardium has been developed. High magnification analysis techniques such as AFM and SEM provide further data that may support the selection process for future BAVR biomaterials. Data presented here forms the basis for future investigations into the development of the next generation of bioprosthetic heart valves using decellularised tissue coupled with PEGylation.

References

1: N. Kiesendahl, *et al.* Ann Biomed Eng. 48: 282-297, 2020. 2: Salaun E, *et al*. Heart. 104: 1323-1332, 2018.

Acknowledgements

The authors would like to thank our collaborators at Loughborough University: Dr Simin Li, Dr Andy Gleadall and Dr Jingwen Zhang, and the EPSRC for funding this research.

LABEL-FREE TRACKING TO QUANTIFY NANOPARTICLE DIFFUSION ABOVE CELL MONOLAYER

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Introduction

A label-free microscopy technique is described that has the potential to quantify and characterise the influence of cellular activity and biological interaction on the dynamics of nano-entities in a solution. Fluorescence microscopy techniques are one of the most widespread tools to monitor and track biological interactions; however, the influence of fluorescent tags on diffusion and biological activity is still unclear. Further, the prolonged exposure of fluorescent labels to light can cause phototoxic effects and photobleaching, limiting the use of fluorescence for real-time monitoring in biological processes. Theoretical models of diffusion cannot encompass phenomena such as cellular interaction highlighting the need for experimental regimes. Additionally, no studies have quantified the influence of corona formation on nano-entity dynamics at the cellular level, further motivating this study. In order to identify the limitations as well as the ever-expanding potential of the technique, nanoparticles have been used to explore the lower size limits of the technology. The aim of this study is to develop a model for the diffusion of nano-entities though biological media in the presence of a cell monolayer with a view to developing a technology platform for *in-vitro* testing systems to monitor living organisms interacting with cells on the micro and nano scale.

Materials and Methods

Using a standard inverted optical microscope adjusted to produce near-coherent light to generate optical signatures of nanoparticles, or caustics, as described by Patterson and Whelan [1], 100nm diameter gold nanoparticles were tracked diffusing at different time points and at different heights above a cell monolayer. The influence of nanoparticle charge and the presence of proteins in the suspension media was also investigated. Human mesenchymal stem cells were seeded at 20,000 cells/cm^2 on glass cover slips and left for 24 hours to attach. The mean squared displacement of caustic signature trajectories were used to determine the experimental diffusion coefficient for individual nanoparticles.

Results and Discussion

The influence of corona formation and biological activity on the diffusion behaviour of nanoparticles has been quantified and will be presented. The local environment of nanoparticles has been found to have a significant effect on their dynamics. Nanoparticles closest to the cell monolayer diffuse slower 15 minutes after exposure to cells. The time dependent nature of corona formation has also been found to significantly influence nanoparticle diffusion in the presence of cells.

Conclusions

Experimental regimes to obtain quantitative information on the factors influencing nanoparticle diffusion in biologically relevant environments have been developed and optimised. In doing this, it has been found that the diffusion behaviour of nanoparticles significantly changes during short time periods after exposure to a cell monolayer. The ability of the technique to visualize and track, in real-time, nanoparticles above a cell layer demonstrates the potential of the technique to quantify more complex biological interactions involving a monolayer.

References

1. Patterson, E.A. and Whelan, M, Nanotechnology, 19(10):105502,2008

ENHANCING STEM CELL VIABILITY IN CORNEAL TISSUE ENGINEERING THROUGH HYBRID PEPTIDE AMPHIPHILE FORMULATIONS

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Introduction: Corneal transplantation using human donor tissue is the "gold standard treatment" for restoring vision in patients who developed irreversible corneal damage, leading to corneal blindness (1). However, it is reported that approximately 13 million people worldwide are on the corneal transplant waiting list due to donor shortage, which mainly impact young people (2,3). Therefore, there is a high demand for bioengineered corneal replacements. The use of peptide amphiphiles (PAs) in corneal tissue engineering has shown promising advancements in recent research. They have been utilized to design bio-prosthetic corneal tissue in vitro (4). PAs can be used to create self-assembled nanostructures that offer properties supporting cellular adhesion, proliferation and differentiation (5). PAs with the RGDS peptide motif have been evaluated to promote the corneal cell adhesion (6), which is essential for corneal tissue regeneration. Studies have also highlighted the importance of presence of the RGDS sequence in an optimal manner for cell recognition and adhesion (7). For the regenerative purposes, the development of scaffolds that can support the viability and functionality of stem cells is vital. However, the specific influence of RGDS-modified PAs on stem cell behavior within corneal tissue engineering scaffolds remains unclear. This study aims to investigate the efficacy of incorporating PAs with RGDS motif into PAs with ETTES motif at varying concentrations to enhance stem cell viability for corneal tissue engineering applications.

Materials and Methods:1.25mM 100% C16-ETTES sequenced PAs (ETTES PAs) and 1.25mM %80 C16- ETTES sequenced PAs & 20% C16 RGDS sequenced PAs (RGDS PAs) were separately dissolved in double sterile water. These two PAs formulations were diluted to the concentrations of 125 µM, 12.5 µM and 1.25 µM. 3 biological replicates of 24 well plates were coated with ETTES PAs and composite of RGDS & ETTES PAs in the concentrations of 1.25mM, 125 µM, 12.5 µM and 1.25 µM. Adipose Stem cells (ASCs) were incubated on these coatings for 3 days, and their metabolic activity were quantitatively measured as florescent intensity using Alamar Blue Assay in comparison to the control groups incubated on uncoated 24 well plates. Two-way ANOVA was used for statistical analysis. Moreover, ASCs proliferation and morphology were assessed using advanced microscopy techniques with Leica Microsystems.

Results and Discussion: Results revealed that 100% ETTES PAs did not adequately support cell viability across all concentrations tested. Conversely, the hybrid formulation of 80% ETTES + 20% RGDS PAs demonstrated superior performance in supporting stem cell viability in the concentrations of 125 µM, 12.5 µM and 1.25 µM, showing improved metabolic activity compared to the 100% ETTES in same concentrations at two time points. Although the results of concentrations at 125 µM, 12.5 µM and 1.25 µM did not make a significant difference on the impact of both formulations, cell viability was not supported at 1.25mM for ETTES PAs and mixture of RGDS and ETTES PAs. These outcomes highlight the importance of RGDS PAs involvement into the design of PAs scaffolds in promoting cellular adhesion and viability for corneal tissue engineering applications while strongly emphasising the optimal threshold concentration under 1.25mM to be effective.

Conclusion: The findings underscore the critical importance of peptide amphiphile composition in dictating cellular responses. While 100% ETTES exhibited limitations in supporting cell viability, the hybrid formulation emerged as a more promising candidate for corneal tissue engineering across various concentrations. This study provides valuable insights into optimizing peptide amphiphile formulations for corneal tissue engineering and highlights the significance of considering both composition and concentration in scaffold design.

References:

1.Moshirfar M et al. Clin Ophthalmol. 12;15:2963-2973, 2021
2.Holland, G et al. Frontiers in Medicine, vol. 8, 2021
3.Marcus A et al. British Journal of Ophthalmology, vol. 104, no. 11, 2020 4.Miotto, M et al. Journal of Functional Biomaterials, 6(3), 687-707. 2015 5.Webber M et al. Nat Mater, 15(1): 13–26, 2016
6.Gil, E et al. Biomaterials, 31(34), 8953-8963, 2010
7.Webber, M et al. Acta Biomaterialia, 2010

PRESSURE-MEDIATED TOPICAL NON-VIRAL GENE THERAPY UP TO MILLI/CENTIMETRE-SCALES

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Introduction: Topically applied gene therapies often result in limited accessibility to the deep target sites (milli/centimetre-scales). This is inhibitory for peptide and nucleic acid macromolecule drug delivery strategies, particularly with poorly perfused tissues and significant volume and geometry. Our technology, GAG-binding enhanced transduction (GET) [1], efficiently deliver a variety of cargoes intracellularly in the form of nanoparticles. We previously introduced GET gene delivery from temperature-sensitive hydrogel patches/bandages [2]. Transfection was effective with direct contact with cells, but only if the diffusion characteristics of GET-NPs in the hydrogels were improved, and if formulations were used at very high concentrations. Changing nanoparticle physicochemical properties can enhance penetration [3], however the use of a pressure differential, generating fluid-flow would enhance gene delivery over milli/centimetre scales.
Materials and Methods: Plasmid DNA (pCMV-gluc2 termed pGluc) was complexed using a mixture of GET peptides (FLR/FLH) at a charge ration (+/-) 5. A model of NIH-3t3 mouse fibroblasts cell-laden collagen hydrogels ($1x10^7$ cells/mL), with targeted/untargeted regions for transfections (IN and OUT areas) the surface of cell strainers (40µm pore-size) was employed. We then adapted clinically used pressure systems to administer negative pressure (NP) down to -150mmHg (Negative pressure wound therapy; NPWT).
Results and Discussion: Poor penetration of complexes was observed when transfecting collagen hydrogel-laden cells at atmospheric pressure. Short exposures to NP (5min-25 mmHg) focused on a defined area of hydrogel allowed us to use NPWT system to regionally transfer GET-pDNA to cells in 3D (Fig 1) without affecting metabolism or viability. A significant increase ($p<0.001$) in the transfection efficiency (RLU) was observed in the region where 25 mmHg NP was applied compared to no NP. This was equally evident using fluorescence microscopy following traces of TM-FLR-Gluc nanocomplexes after localized pressure-mediated transfection.

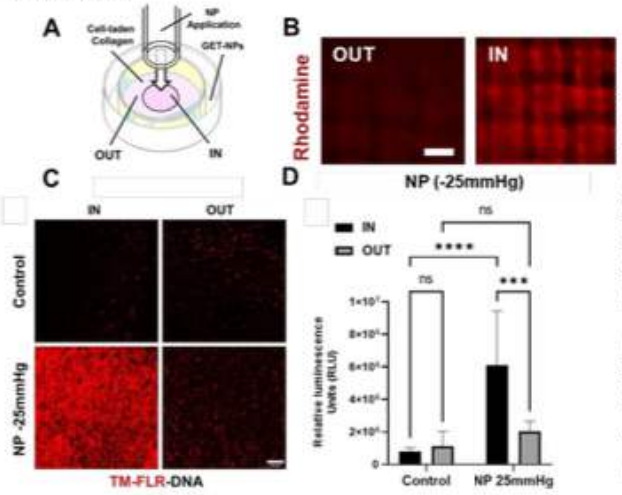

Fig. 1. Negative pressure-mediated localized delivery. A. Schematic of the 3D *in-vitro* model assembly of collagen-engineered tissue in a cell strainer to allow localized NP to be administered B. Demonstration with fluorescence microscopy of Rhodamine distribution in IN and OUT areas following NP application. C. Localisation of GET-pDNA (with labelled FLR, TM-FLR) after NP application. D. Localization of transfection efficiency. GET-pGluc nanocomplexes (10µg) + 5min NP (25mmHg) applied. The IN area was removed by biopsy punch and both IN and OUT samples transferred to fresh media at day 1 post-transfection for extra 24 h to record luminescence from defined areas (N=6, bars are S.D.).

Conclusions: Directed gene delivery to sites in tissue, such as skin as an intradermal constituent of wound healing strategies, could be transformative in traumas and other skin conditions. The ability to simply deliver genes to specific regions of tissue by applying NP to the tissue will allow site-specific delivery of novel nanotechnologies and gene therapeutics.
Acknowledgements: The authors acknowledge funds by the Defence Accelerator (DASA, DSTL) (Award reference: ACC6007330), the European Research Council under the European Community's Seventh Framework Programme (FP7/2007–2013)/ERC grant agreement 227845.

References:
1. Dixon J.E. *et al.* PNAS. 113 (3) E291-E299, 2016.
2. Eltaher H.M. *et al.* Biomaterials Advances. 143, 213177, 2022
3. Dixon J.E. *et al.* ACS Omega. 9, 4, 5014–5023, 2024

Abstract theme: Enabling Technologies / Biomaterials

QUANTUM BIOELECTRONICS FOR THE TREATMENT OF HARD-TO-TREAT CANCERS

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Abstract theme: Enabling Technologies

Introduction: Quantum biological tunnelling for electron transfer (QBET) is involved in controlling essential functions for life such as cellular respiration and homoeostasis. Understanding and controlling the quantum effects in biology has the potential to modulate biological functions.

Materials and Methods: We merged wireless nano-electrochemical tools with cancer cells for control over electron transfer to trigger cancer cell death. Gold bipolar nanoelectrodes functionalized with redox-active cytochrome *c* and a redox mediator zinc porphyrin were developed as electric-field-stimulating bio-actuators, termed bio-nanoantennae.

Results and Discussion: We show that a remote electrical input of alternating current (A.C.) at high frequency (3MHz) and low voltages (0.65V/cm) regulates electron transport between these redox molecules, which results in quantum biological tunnelling for electron transfer to trigger apoptosis in cancer cells from various hard-to-treat tumors. Transcriptomics data show that the electric-field-induced bio-nanoantenna targets the cancer cells in a unique manner, representing electrically induced control of molecular signaling. Arrhenius-kinetics confirmed the occurrence of moderate QBET in the system. *In vivo* studies in tumor bearing rats are underway to demonstrate first example of quantum medicine to treat cancers.

Conclusions: The work shows the potential of quantum-based medical diagnostics and treatments.

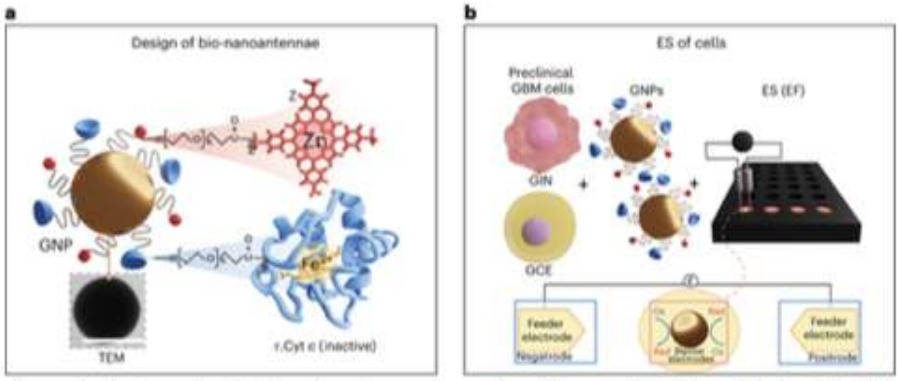

Figure 1: Illustration of wireless electrical–molecular quantum signalling mediated by AC-electric field-responsive bio-nanoantennae to induce cell death. **(A)** Bio-nanoantennae were synthesized by covalently conjugating r.Cyt and Z to carboxylic gold nanoparticles. **(B)** Patient-derived glioblastoma cells, namely, GIN (Glioma infiltrative margin) and GCE (Glioma core enhanced) cells, were incubated with bio-nanoantennae and electrically stimulated with AC EFs. ES, electrical stimulation; Ox, oxidized; Red, reduced.

References

[1] Jain, A., Gosling, J., Liu, S. *et al.* Wireless electrical–molecular quantum signalling for cancer cell apoptosis. *Nat. Nanotechnol.* (2023).
[2] Xin, H., Sim, W.J., Namgung, B. *et al.* Quantum biological tunnel junction for electron transfer imaging in live cells. *Nat Commun* **10**, 3245 (2019).

hiPSC-DERIVED ENDOTHELIAL CELL NERVE REPAIR CONSTRUCTS

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Abstract theme: Enabling Technologies

Introduction Traumatic peripheral nerve injury has a sudden, debilitating effect on millions of people every year, resulting in loss of sensation and movement. The clinical treatment for severe peripheral nerve injury, the autograft, has multiple limitations including donor tissue availability and donor site morbidity. Tissue-engineered nerve repair constructs have the potential to overcome these limitations while harnessing autograft biology with aligned cells and biomaterials [1]. Mimicking the polarised vasculature formed by endothelial cells early in the natural regenerative process offers an innovative feature of nerve repair constructs [2]. However, cell source and production time can limit the clinical translation of repair constructs [3]. Here, a novel source of endothelial cells was differentiated and incorporated into the constructs in an automated, scalable fashion, and in a manner conducive to the formation of endothelial cell tube-like structures.

Materials and Methods ReNeuron Ltd. human induced pluripotent stem cells (hiPSCs), derived by reprogramming research equivalents of a clinically approved neural stem cell line, were differentiated into endothelial cells using a monolayer culture protocol, monitored by immunofluorescence and RT-qPCR. Collagen hydrogels seeded with endothelial cells were stabilised by controlled aspiration using the Gel Aspiration-Ejection (GAE) technique [4] which was automated by integrating a robotic positioning system with a programmable syringe pump. Viability, alignment, and construct maturation were assessed.

Results and Discussion Endothelial cells were successfully differentiated from hiPSCs as determined by decreased expression of the pluripotency marker OCT4 and increased expression of endothelial markers CD31 and CD144 (Fig. 1a), RT-qPCR, and tubulogenesis assays. The GAE technique was successfully automated by combining a syringe pump with a 3D printer adapted to act as a robotic arm, resulting in the production of nerve repair constructs from hiPSC-derived endothelial cellular collagen hydrogels in ~1 minute, with the potential to generate up to 48 constructs per batch. The nerve repair constructs exhibited ~80% cell viability with significantly improved cell alignment compared with the starting hydrogels ($p=0.0038$) (Fig 1b). Endothelial cell constructs significantly improved *in vitro* neurite alignment compared with acellular constructs ($p=0.0169$) (Fig. 1c). On culturing endothelial cell constructs for 2 days, aligned tube formation and a 3D angiogenic network was observed. The network continued to mature, with lumen number significantly increasing over time (Fig. 1d), mimicking the polarised vasculature seen in native, regenerating nerve tissue [2].

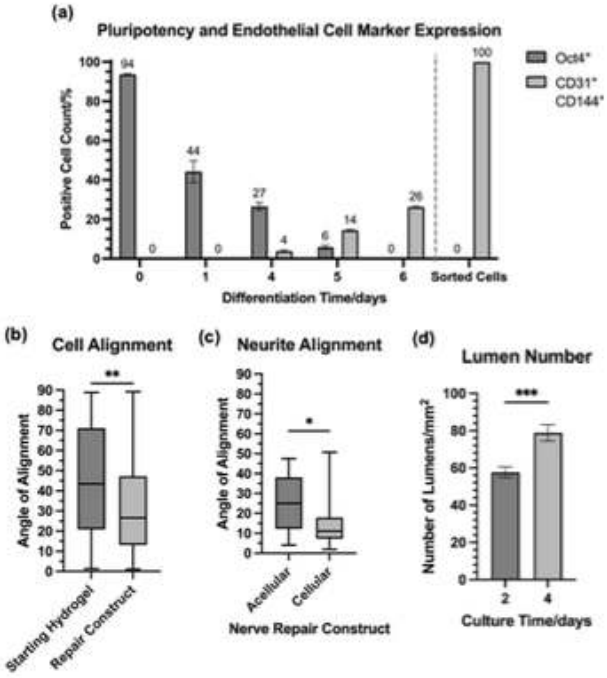

Figure 1. As differentiation progressed, cells exhibited a decrease in pluripotency markers and an increase in endothelial cell markers **(a)**. Cells in nerve constructs were aligned **(b)**, with the potential to support directed neurite growth *in vitro* **(c)** and form tube-like structures with lumen number increasing with time **(d)**.

Conclusion Human endothelial cells were successfully differentiated from hiPSCs for which GMP equivalents are available. These cells were used to produce viable, aligned nerve repair constructs from collagen hydrogels using an automated and scalable technique. These nerve repair constructs supported neurite growth and angiogenic behaviour.

References

1. Phillips JB Emerg. Top Life Sci. 5(5):699-703, 2021. 2. Cattin AL *et al.* Cell 162:1127-1139, 2015.
3. Meena P *et al.* Cell Tissue Res. 383:617-644, 2021. 4. Muangsanit P *et al. J. Neural Eng.* 2020; 17:046036

Acknowledgements

Work funded by the London Interdisciplinary Doctoral (LIDo) Programme and Canada NSERC.

VISCOELASTICITY IN THE INTEGRIN-GROWTH FACTOR CROSSTALK

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Abstract theme: Mechanobiology

Introduction: The extracellular matrix (ECM) is a dynamic environment that not only supports cells, but can also influence their behaviour, signalling and fate through mechanical and biochemical cues. Much of the earlier work on stem cell responses to ECM mechanics focused on elastic substrates and on the role of integrin receptors in mechanotransduction, a process through which physical cell-matrix interactions lead to gene expression changes via cytoskeletal alterations. However, the ECM is viscoelastic and time-dependent changes to the mechanics of the ECM can greatly impact cell responses. Increasingly complex models are therefore necessary as they offer a fuller understanding of how cell mechanotransductive responses can be tuned via matrix engineering. Growth factors (GFs) such as the osteogenic BMP-2 have also been used to promote desired stem cell fates; however, problems with efficacy and side effects have led to the need for improved delivery strategies. These approaches exploit the recently demonstrated link between GF activity and ECM mechanics: cells respond differently to matrix-bound GFs compared to soluble ones; interestingly, there is also a minimum matrix stiffness below which BMP-2 has no osteogenic effect.[1] Furthermore, mimetic peptides – short sequences derived from the functional region of a GF – may improve clinical strategies by granting easier functionalisation and fewer side effects compared to full-length counterparts. One such peptide derived from BMP-2 is DWIVA, which has shown promising albeit mixed results in recapitulating the effects of BMP-2.[2] This study therefore aims to leverage ECM dynamics to harness the osteoinductive potential of DWIVA, using an ECM model that features controlled surface viscosity and matrix-bound GF presentation.

Materials and Methods: 2D dynamic culture surfaces with controlled viscosity were generated using supported lipid bilayers (SLBs), formed from either DOPC (more mobile) or DPPC (more viscous) lipids, and employed to recapitulate the dynamic nature of the ECM. Surfaces were functionalised, via biotin-neutravidin interaction, with RGD to allow integrin binding, and with DWIVA to promote BMP-2 signalling. Functionalised glass was used as a non-mobile surface, and soluble BMP-2 was added as osteogenic control. Mechanotransductive and GF signalling responses of human mesenchymal stem cells were investigated using immunofluorescence and in-cell western techniques after up to 5 days of culture.

Results and Discussion: Surface viscosity was found to regulate cell adhesion and morphology via RGD-mediated mechanotransductive pathways, independently of the presence of DWIVA on the surface. Indeed, cells spread more at increasing viscosity, with a round phenotype on DOPC and the largest cell area on glass. This correlated with an increase in focal adhesion formation and translocation of the mechanosensitive transcription factor YAP to the nucleus (Figure 1a). There was no indication that DWIVA had major effects on early adhesion-based processes; furthermore, DWIVA alone was unable to sustain cell adhesion on the SLBs in the absence of RGD. In terms of GF signalling, DWIVA was shown to promote osteogenic differentiation on viscous DPPC compared to mobile DOPC and non-mobile glass. This was indicated by an increase in RUNX2 phosphorylation to levels similar to the soluble BMP-2 control (Figure 1b), mediated by differential activation of non-canonical BMP-2 signalling pathways including pERK1/2 and phospho-p38. Canonical BMP-2 signalling was instead not observed on any condition other than the soluble GF control.

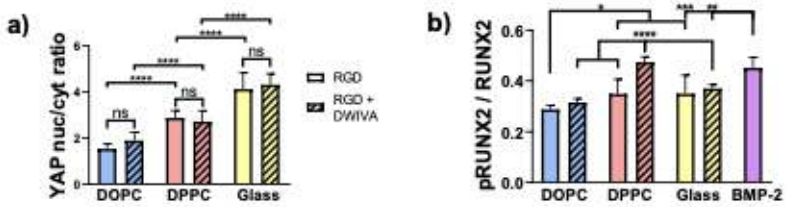

Figure 1 Mechanotransduction and GF signalling on dynamic culture sufaces, as measured by YAP nuclear translocation (a) and RUNX2 phosphorylation (b).

Conclusions: Our results demonstrate that ECM dynamics can be leveraged to harness the osteoinductive potential of the DWIVA GF-mimetic peptide through non-canonical MAPK BMP-2 pathways. Indeed, while adhesion-based mechanotransductive pathways remain unaffected by matrix-bound DWIVA presentation, osteogenic differentiation is promoted on viscous DPPC, with no GF activity instead observed on less viscous DOPC and on non-mobile glass. This suggests that a dynamic environment and a minimal threshold of cell-generated mechanical forces are both necessary to harness DWIVA's osteogenic potential. These findings will inform future work using DWIVA in more complex, viscoelastic hydrogel-based ECM models.

Acknowledgements: The authors acknowledge funding from EPSRC and from The Royal Society.

References: 1. Crouzier T *et al.* Adv. Mater. 23(12): H111-1118, 2011.
2. Oliver-Cervello L *et al.* Adv. Healthcare Mater. 10(7): 2001757, 2020.

DESIGNING TOPOGRAPHICALLY-TEXTURED MICROPARTICLES AS CELL-INSTRUCTIVE BONE MATRIX MIMETICS VIA MODULATION OF HEDGEHOG SIGNALLING

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Abstract theme: Mechanobiology

Introduction: Hedgehog (HH) signalling is a key pathway in early stages of bone development. Efforts in bone research have mainly relied on engineering 2D substrates to study the role of HH signalling in mesenchymal stem cell (MSCs) differentiation and bone remodelling. However, there is a substantial need for engineered 3D substrates that provide more physiologically relevant characteristics in regenerative therapies. Therefore, we have engineered topographically-textured polymeric microparticles to investigate the effect of tailored 3D architectural features on MSCs behaviour *in vitro*. Poly(D,L-lactic acid) microparticles are ideal for bone tissue engineering because their stiffness (1.22- 1.33 GPa)[1] is comparable to that reported for trabecular bone[2]. We have demonstrated that these topographically-patterned microparticles, which mimic biomechanical features of bone matrix[1], induce osteogenesis in bone marrow-derived human MSCs (hMSCs)[1] and murine C3H10T1/2 cells[3], without the need for additional osteo-inductive supplements. In this study, we investigate the precise molecular mechanisms driving this osteo-inductive effect, focusing on the HH signalling pathway.

Materials and Methods: Polylactic acid microparticles were fabricated by a solvent evaporation oil-in-water emulsion technique, using phase separation to create 3D topographical features on their surfaces[1]. Primary hMSCs (5 donors, aged 19-34 years) were cultured on smooth and dimpled microparticles, with tissue culture plastic as 2D controls. Cell viability, morphology and various markers of osteogenesis and HH signalling were assessed. Two-photon polymerisation is being optimised as a high-precision microfabrication strategy for better control over this osteo-induction mechanism.

Results and Discussion: hMSCs showed excellent viability and proliferation up to day 14. Cells exhibited varying morphologies on different designs- elongated morphologies on dimpled and spreading out on smooth microparticles (Fig. 1). Culture of hMSCs on dimpled microparticles significantly increased the expression levels of early and late markers of osteogenesis, including *RUNX2* (2.52-fold, $p \leq 0.01$) at day 3, and *BGLAP* (2.25-fold, $p \leq 0.01$) at day 10, relative to smooth microparticles. The osteo-inductive effect of dimpled microparticles coincided with a significant increase in the expression levels of HH signalling pathway components at day 3 relative to smooth microparticles, including *GLI1* (3.14-fold, $p \leq 0.05$), *SMO* (1.92-fold, $p \leq 0.05$), and *SHH* (41.84-fold, $p \leq 0.01$). Remarkably, the increase in *GLI1* expression induced by dimpled microparticles was comparable to the effect seen in 2D cultures treated with purmorphamine, a widely used SMO receptor agonist, relative to 2D vehicle-treated control (3.47-fold). The involvement of canonical HH activation was confirmed by the significant reduction in the expression of *GLI1* ($p \leq 0.05$) and *BGLAP* ($p \leq 0.01$) following treatment with KAAD-cyclopamine (SMO receptor inhibitor) in cells cultured on dimpled microparticles relative to vehicle-treated counterparts at day 3 and 10, respectively. Optimisation of two-photon lithography to print topographically-textured microparticles has also been carried out (Fig. 2).

Conclusions: Our data demonstrates that topographically textured microparticles induce osteogenesis in hMSCs via activation of the canonical Smo-dependent HH pathway without adding biochemical differentiation supplements. This will offer opportunities to explore alternative methods for modeling bone diseases, particularly those linked to HH signalling, and for crafting innovative regenerative therapies.

References:1. Amer M *et al.* J. Biomater. 266: 120450, 2021. 2. Wu, D *et al.* J Act Biomater. 78: 1-12, 2018. 3. Ghuloum, F *et al.* J. Biomater Adv. 154: 213652, 2023.

Acknowledgements: FG is supported by a scholarship from Kuwait University, Kuwait.

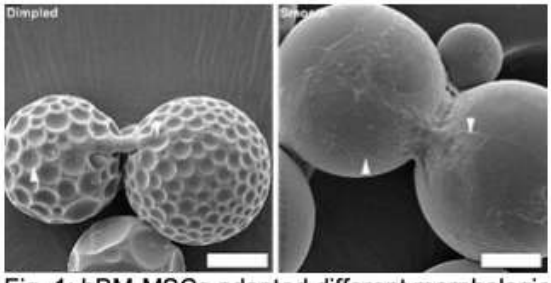

Fig. 1: hBM-MSCs adopted different morphologies on different 3D microtopographies (Scale bar: 20µm).

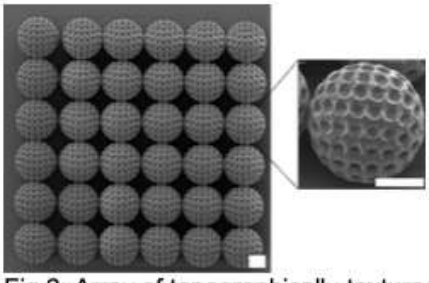

Fig 2: Array of topographically-textured microparticles printed using two-photon lithography (Scale bar: 40µm).

HIGH THROUGHPUT MECHANICAL PHENOTYPING OF NANO-VIBRATED MESENCHYMAL STEM CELLS USING REAL-TIME DEFORMABILITY CYTOMETRY

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Introduction: Osteoporosis is a natural progression of the condition osteopenia and is characterised by low bone mineral density (*1*). Globally, an estimated 500 million people are affected, predominantly coming from older demographics (*2*). The root cause of this condition lies in an imbalance of osteoblastic bone formation, and osteoclastic bone resorption. Mechanical stimulation, via nano-amplitude vibration (1 kHz, 30 nm), may present an option to treat this condition in a chemical free approach. This technique targets the response of the patient's own mesenchymal stem cells, located in the bone marrow, to differentiate into osteoblasts and populate the porous bone, reconstituting it. Furthermore, osteoblast-osteoclast co-culture has shown that osteoblasts reduce bone resorption conducted by osteoclasts (*3*). As shown by Yen, et al; 2020, the cell stiffness is positively correlated with osteogenesis (*4*). In this study, we used Real-Time Deformability Cytometry (RT- DC), a high throughput technique, to assess cell stiffness.

Materials and Methods: Bone marrow mesenchymal stem cells (MSCs) from three donors (male 28, male 80, and female 72) were cultured in supplemented high glucose DMEM in an incubator at 37 °C in 5% CO2. Cells were seeded 24 hours before mechanical treatment with 1 kHz, 3 nm nano vibration. Cellular mechanical properties were tested at 0 h and 72 h via RT-DC. Detachment of cells from culture-ware was performed using 0.05% (w/v) trypsin preceded by two washing steps of EDTA. Cells were resuspended in Cell Carrier B. The cell's deformability measurements were then conducted in the Polydimethylsiloxane (PDMS) 30 µm channel chip at flow rate 0.32 µl/s on both channel and reservoir. All measurements were performed at the same time from the flask's trypsination. Raw data was exported from ShapeOut2 (version: 2.11.9).

Results and Discussion: Results showed that cellular stiffness changed in culture from time 0 h to 72 h, and, between control and nano- vibrated groups. The male donor (28 year old), showed a stiffening of the cells from time 0 h to 72 h. Furthermore, the nano vibrated group, in turn, presented itself stiffer than the control group ($p<0.0001$). Cells also increased in volume compared to time 0 h. However, nano vibrated cells remained smaller. The male donor (80 year old) responded differently to the 28 year old, presenting decreased cell stiffness ($p<0.0001$) in the vibrated sample, compared to control and 0 h. Area was reduced in both control and nano vibrated groups. The female donor (72 year old), presented with decreased stiffness by 72 h. However, the nano vibrated group presented higher stiffness than control ($p<0.0001$). Area of the cells followed the same trend as the young male with the nano vibrated cells being of smaller volume than controls. Data suggests that the cytoskeleton of the cells is reacting to the mechanical stimulation and therefore actin staining will be necessary to evaluate the changes. This is similar to the behaviour to the osteosarcoma cells, MG63s, tested previously utilising the same methodology.

Conclusions: In conclusion, MSCs are sensitive to the mechanical cues, and they are responding to it by adjusting their cytoskeleton and potentially the expression of osteogenic markers. The high throughput technique being employed was sensitive enough to detect natural cell drift in stiffness and the cell's response to stimulation.

References

1. J. Lamb, in *Advancing Medicine with Food and Nutrients: Second Edition* (StatPearls Publishing, 2012; https://www.ncbi.nlm.nih.gov/books/NBK441901/), pp. 657–675.
2. International Osteoporosis Foundation, Epidemiology of osteoporosis and fragility fractures | International Osteoporosis Foundation. *Int. Osteoporos. Found.* (2020), p. 1.
3. P. A. Hulley, I. Papadimitriou-Olivgeri, H. J. Knowles, *JBMR Plus.* **4** (2020), doi:10.1002/jbm4.10370.
4. M.-H. Yen, Y.-H. Chen, Y.-S. Liu, O. K.-S. Lee, *Biochem. Biophys. Res. Commun.* **526**, 827–832 (2020).

Acknowledgements

EPSRC: EP/N013905/01, EP/W524670/1
BBSRC: BB/S018808/1
Melanie Jimenez is supported by the Royal Academy of Engineering under the Research Fellowship scheme (RF/201718/1741)

Computational Design Of Cultured Tissue Structures With Biophysics And Machine Intelligence

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Introduction: Tools to assist mould and scaffold designs for cultured tissue growth with bespoke cell organisations are needed for applications such as regenerative medicine, drug screening and cultured meat. Computational and machine-learning models of the cell-matrix interactions vital to tissue organisation can help with this unmet need. In polarised tissues, self-organisation is driven by local interactions between cells and the extra-cellular matrix (ECM), and the onset of macroscopic forces in tethered tissues. The goal of this work is to understand and predict this self-organisation using biophysical models and machine learning. This has led to a rational design process for moulds with a high level of tissue organisation.

Materials and Methods: Several thousand moulds suitable for growing cellular hydrogels were automatically generated, including a proportion that were constrained to have high symmetries. These were used for high-throughput calculations of self-organisation in cellular hydrogels using the contractile-network dipole-orientation (CONDOR) model [1], which simulates cell-matrix interactions and tissue scale forces. We solved the biophysical model using simulated annealing. The resulting high-throughput data were used to identify promising moulds for highly aligned tissue [2] and to train an implementation of the pix2pix deep learning model [3].

Results and Discussion: We carry out validation work on CONDOR, finding close agreement with experiments on artificial neural and other tissue types. We introduce a fitness function to automatically identify moulds from the high-throughput computation with favourable properties for growing polarised tissues, and use this to find tethered mould designs, suitable for growing cultured tissue with very highly-aligned cells. We find that the machine learning technique makes excellent predictions, commensurate with the biophysical model and experiment, with a speedup of several orders of magnitude over the biophysical model. We will discuss recent approaches to using this faster approach for further improvements and new applications of mould design.

Conclusions: The CONDOR model matches well with experimental data for a wide range of polarised tissues, showing that microscopic cell-ECM dipole interactions and tissue-scale forces cooperatively drive macroscopic self- organization. We find that a CONDOR approach is suitable for high-throughput rational design of artificial tissues, predicting that two specific tethering strategies within elongated moulds lead to highly-aligned cells: (1) placing tethers within bilateral protrusions guides alignment (2) placing tethers within a single vertex reduces opportunities for misalignment [2]. Moreover, a machine-learning approach trained on this data [3] is both significantly faster than the biophysical method, opening the possibility of very high throughput rational design of moulds, scaffolds and 3D printing strategies for a range of cultured tissue applications.

References

1. Hague JP, Mieczkowski PW, O'Rourke C, Loughlin AJ, Phillips JB. Microscopic biophysical model of self-organization in tissue due to feedback between cell- and macroscopic-scale forces. Physical Review Research 2: 043217, 2020.
2. Hague, JP, Andrews, AE and Dickinson, H. High-throughput design of cultured tissue moulds using a biophysical model: optimising cell alignment. Physical Biology, 20: 066006, 2023.
3. Andrews, AE, Dickinson, H and Hague, JP. Rapid prediction of lab-grown tissue properties using deep learning. Physical Biology 20: 066005, 2023.

Acknowledgements

We acknowledge funding from the STFC Impact Accelerator Account 2022-2023 and the Open Societal Challenges programme of the Open University.

EXTRACELLULAR VESICLE BIOACTIVITY AND POTENTIAL FOR CLINICAL UTILITY IS DETERMINED BY MESENCHYMAL STROMAL CELL CLONAL SUBTYPE.

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Introduction: Mesenchymal stromal cells (MSCs) have been used in numerous clinical trials but very few reach phase 3 or market authorisation. Progress is hampered by the use of non-clonal, heterogeneous and uncharacterised MSC cultures and lack of mechanistic understanding. There is limited evidence of MSC engraftment in vivo and disease resolution may be the result of the paracrine effects of the MSC secretome, rather than the cells *per se*. Extracellular vesicles (EVs) are key components of the MSC secretome and there is growing interest in the use of EVs as cell-free therapies. EVs may be able to offer a more accessible, cost-effective route to clinic compared to their parent cells, with improved safety profiles and amenable transport, storage and administration options. However, like MSCs, heterogeneity within any therapeutic EV pool will hamper clinical development. Here we used immortalised clonal MSC lines, termed Y201 and Y202, to examine how MSC phenotype influences EV character and function. Y201 and Y202 were isolated from the same donor and identify as “MSCs” by surface protein expression and independent transcriptomic profiling. Y201 are model mesenchymal *stem* cells with strong trilineage differentiation and immune-suppressive properties, whereas Y202 MSCs have weak differentiation capacity and appear to function primarily as immune-regulatory cells.

Materials and Methods: EVs were isolated by ultracentrifugation and characterised by nano-sizing, ultrastructural morphometric analysis (diameter, perimeter, area, roundness), western blotting, mass spectrometry (proteome and lipidome) and miRNA screening. Bioactivity was determined by phosphorylation of ERK1/2, proliferation (live imaging and single cell tracking; cell counts, confluency, dry mass and doubling times), suppression of activated T cells, T cell polarisation and in vivo models of peritoneal inflammation and adjuvant-induced arthritis (AIA).

Results and Discussion: EVs isolated from Y201 and Y202 MSCs had similar peak average sizes, morphological characteristics and lipidomic profiles, however, Y201 EVs were more abundant in ALIX, flotillin-1, CD63 and CD81 versus Y202 EVs, with an enhanced miRNA content (10 vs 2 significantly elevated) and EVome (68 vs 2 significantly elevated). Computational analysis of the Y201 EVome identified significant enrichment in matrix-associated proteins that were predicted to contribute to an elaborate EV corona particularly abundant in RGD-containing proteins fibronectin and MFG-E8, which was confirmed by western blotting.

Both Y201 and Y202 EV subsets significantly reduced proliferative index scores of activated T cells and Y202 EVs also reduced number of T cell proliferative cycles. Y201 EVs, but not Y202 EVs, caused a significant increase in the development of anti-inflammatory Th2 cells from naïve T cells as indicated by intracellular IL4 staining. We demonstrated that Y201 EVs, but not Y202 EVs, significantly increased the proliferation of articular chondrocytes in a dose-dependent manner, and that the proliferative effect of Y201 EVs was mediated at least in part via an RGD (integrin)-FAK-ERK1/2 axis. In models of inflammatory peritonitis, Y201 EVs, but not Y202 EVs, suppressed the accumulation of peritoneal exudate cells including a reduction in macrophage and neutrophil recruitment to the peritoneal cavity. Finally, in a disease-relevant AIA model we demonstrated that intra-articular injection of EVs derived from Y201 MSCs induced a significant decrease in all measures of disease activity compared to vehicle controls, including reduced joint swelling, synovial infiltrate, joint exudate, synovial hyperplasia and overall arthritis index. Y202 EVs significantly reduced joint swelling compared to controls but otherwise did not affect any other disease score measures.

Conclusions

EVs released by closely related MSC subtypes within the same heterogeneous population differ significantly in terms of cargo abundance, bioactivity, and pre-clinical in vivo efficacy. Analysis of defined EV subsets will aid mechanistic understanding and prioritisation for EV therapeutics. Y201 EVs have potentially high therapeutic value in the treatment of inflammatory disorders associated with tissue loss.

COMBINING CELL-INDUCED POLYMERISATION AND ELECTRIC FIELD STIMULATION FOR CANCER TREATMENT

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Introduction: Glioblastoma (GBM) is an aggressive brain tumour, accounting for 49% of all primary malignant brain tumours with just 6.8% of patients surviving five years post-diagnosis.[1] There is a growing interest in utilising electrical stimulation, including low-intensity, intermediate-frequency alternating current (AC) electric fields, known as tumour treating fields (TTF), in GBM treatment. Despite TTFs showing promise in the treatment of GBM when combined with the alkylating agent temozolomide, overall survival in patients remains low. Consequently, there is a distinct need to enhance TTFs. As TTFs can affect molecules intracellularly by electrophoresis, we hypothesise that conductive and semi-conductive nanoparticles can be used to enhance electric field effects.[2] In addition, advances in harnessing the redox capabilities of cells to drive polymerisation reactions *in situ* have also opened the door to novel approaches for instructed cell death.[3]

Materials and Methods: Patient-derived cells from both the core and invasive edge of GBM are used to reflect the heterogeneous nature of glioblastomas.

To investigate the optimum setup for TTF, cells were seeded in a 96-well plate and treated with 300 kHz, 3 V/ cm AC electric fields. This was coupled with treatment with zinc oxide nanoparticles to enhance the TTF based on previous findings in the group.

To better understand the *in situ* redox polymerisation of pyrrole to polypyrrole, cells were incubated with the monomer and the inactive initiator potassium ferrocyanide (Fe^{2+}). After 48 h the solution was freeze- dried and analysed using solid-state nuclear magnetic resonance. Controls containing either the initiator or the monomer were also analysed.

Results and Discussion: We propose the combination of electrical stimulation and cell-mediated polymerisation as a novel treatment for GBM. Using this approach, we have shown that the toxic redox-active monomer, pyrrole, induces cell death through a combination of apoptosis and necrosis and is polymerised to conductive, biocompatible polypyrrole when exposed to cells. It is hypothesised that the main driver of the redox polymerisation is the presence of oxidising ions in cellular exudate. In this reaction, the toxic monomer is inactivated in a '*reverse prodrug*' approach.

Work is currently being carried out to optimise a tumour-treating field setup where the application of low- intensity, AC electric fields can be used to wirelessly induce further cell death, enhanced by the presence of polypyrrole. This could pave the way for the development of improved therapies to overcome hard-to-treat cancers including GBM.

References

1. Ostrom, Q *et al.* J. Neurooncol, 21:1-100, 2019. 2. Jain, A. *et al.* Bioelectron. Med, 8 (17), 2022. 3. Shen, Q *et al.* Acc. Mater. Res, 4:57-70, 2022.

Acknowledgments

Cell stocks provided by Drs S Smith & R Rahman and TTF 96-well plate designed by Dr K Nguyen (School of Medicine, University of Nottingham).

TOWARDS THE GENERATION OF A GLIOSIS IN VITRO MODEL

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Introduction: Reactive gliosis and the formation of a glial scar are dominant pathologies after traumatic brain injuries (TBI), an important public health problem affecting over 2.5 million people worldwide.[1] Gliosis also called astrocytic gliosis or astrocytosis, is a common term that refers to the reactive astrocytic response to a brain injury. Particularly, in response to TBI, astrocytes change their morphology and extracellular protein expression and glycans generating a cascade of immunological events.[1] These responses suggest that altered astrocytic number and protein profile impact in neuronal function leading to long-term defects with phenotypic sequelae.[1] It is becoming clear, as the field evolves that new molecular targets together with materials approaches will be necessary to modulate gliosis after TBI. However, a **system to study gliosis in vitro** is a **critical and unmet need** for real time analysis of neural mechanobiology and glial cell interactions.

3D organoids are generated through spontaneous self-organization and differentiation of human pluripotent stem cells or human tissue-derived progenitor cells.[2] These cultures offer a viable platform for recapturing the main histological features of both healthy and diseased human organs, and have great potential in the fields of organ development and disease modelling. Particularly, cerebral organoids resembling different parts of the brain provide a basis for *the organoid fusion approach, also called assembloid*. With this type of approach, neuronal migration, brain circuits, and oligodendrogenesis have been studied.[3,4] In turn these studies, bring a new prospective to the cerebral organoid research where “fusion” or “merging” of organoids could provide a lot more information than a single organoid configuration towards modelling diseases with complex cell interactions.

In this study, a 3D culture in vitro platform of glial scar formation is developed using the organoid merging approach to promote growth and maturation of astrocytes primarily. This platform will enable real-time evaluation of local cell forces, their relationship to cytoskeletal - cell shape changes, and visualization of glycan expression after mechanical induction of TBI. This, with the aim to exploit this knowledge to design new materials tailored for mitigation of gliosis.

Materials and Methods: Single and merged cerebral organoid groups were obtained using a modified protocol by Eigenhuis *et al.*[5] The single and merged cerebral organoids grown in culture for 35, 40, 60 and 100 days, respectively. Immunostaining of relevant neural biomarkers and neural outgrowth was performed.

Results and Discussion: A significant increase in astrocyte numbers was observed in merged organoids when compared to single organoid configurations. As early as day 40 in in vitro culture, astrocytes were identified in merged organoids only, and increasing their numbers over the 100 days in culture. These observations highlight the importance of physical merging in the maturation of astrocytes, a key player of gliosis.

Conclusion: Designing complex 3D *in vitro* culture models requires multicomponent approaches. The molecular taxonomy and migratory dynamics of merging organoids, showed to potentiate the maturation of astrocytes over time when compared to single organoid configuration. These findings are key towards next steps with the integration of microglial cultures into our 3D *in vitro* system, recapitulating complex biological processes.

Acknowledge: Thanks to Dr. Lomora´s group at University of Galway - CURAM for their training in organoid formation.

References

1. R. G. Mira, *et al.* J Front Physiol 12. 740939, 2021.
2. M. A. Lancaster, *et al.* Sci. 345, 2014.
3. A. Chen *et al.* Front. cell. neurosci. 14, 2020.
4. J. A. Bagley *et al.* Nat. Methods 14, 743, 2017.
5. K. N. Eigenhuis *et al.* Front. cell. neurosci. 17, 2023.

THE BORON TRANSPORTER NaBC1 MEDIATES MECHANOTRANSDUCTION VIA FIBRONECTIN- BINDING INTEGRINS

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Abstract theme: Mechanobiology

Introduction

This study is based on the functional interaction of integrins, the boron (B) transporter NaBC1, and growth factor receptors (GFRs) upon their activation. In our previous research, we demonstrated that active NaBC1 co-localizes with integrins (specifically Fibronectin (FN)-binding integrins) and GFRs, forming a functional cluster that enhances biochemical signals and facilitates crosstalk mechanisms [1]. This synergy accelerates muscle repair following an injury and restores dystrophic phenotypes in vivo, even in muscular dystrophies with different underlying causes. We propose that NaBC1, in addition to its natural role in controlling boron homeostasis, can also function as a mechanotransducer protein, compensating for abnormal cell-extracellular matrix interactions observed in mechanotransduction disorders.

Materials and Methods

The objective of this study was to demonstrate a novel role for NaBC1 in mechanotransduction mechanisms, particularly in collaboration with FN-binding integrins. To achieve this, we engineered three sets of PAAm hydrogels with varying stiffness ranging from 0.5 to 35 kPa that were functionalized with FN. We examined whether the rigidity of the hydrogel surface influenced the activation of NaBC1-integrin clusters. C2C12 myoblast cells were seeded onto these hydrogels, and we assessed cell behaviour, including adhesion, spreading area, myogenic differentiation, and the activation or repression of gene/signalling pathways, after the simultaneous activation of NaBC1 by soluble B and FN-binding integrins by FN.

Results and Discussion

Our results demonstrated that soluble B induced a concentration-dependent primed state for cell adhesion and spreading area in cells grown on stiffer substrates. Furthermore, measurements of retrograde actin flow and traction forces exerted by cells on the different substrates significantly increased in response to B concentration when cultured on hydrogels with varying mechanical properties. Additionally, the simultaneous stimulation of NaBC1 and FN-binding integrins promoted myotube formation, which was dependent on substrate stiffness. To validate our findings, we replicated all experimental conditions using laminin-111-functionalized PAAm hydrogels, since laminin-111 cannot activate mechanotransduction mechanisms [2]. The observed effects resulting from NaBC1 activation were abrogated in the absence of FN onto 3D hydrogels and after NaBC1 silencing, demonstrating the causality role of NaBC1 and its cooperation with FN-binding integrins.

Conclusions

In conclusion, this study unveils a novel role for the NaBC1 transporter beyond its involvement in controlling B homeostasis, functioning as a mechanoresponsive protein through specific cooperation with FN-binding integrins.

References

1. Ciriza J et al. Mater. Sci. Eng. C. 123: 112003, 2021. 2. Kechagia Z et al. Nat. Mater. 22(11):1409-1420, 2023.

MSC DONORS SHOW VARIED RESPONSE TO NANOVIBRATIONAL STIMULATION

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Introduction: Mesenchymal stem cells (MSC) hold promising potential to treat a range of diseases currently lacking effective therapies such as osteoporosis and spinal cord injury. However, directing stem cell differentiation toward specific lineages for clinical use is challenging in the absence of chemical induction. Previous studies have applied mechanical stimulation to cells, and observed osteogenic, chondrogenic, myogenic, adipogenic and neurogenic responses [1,2]. The Universities of Glasgow and Strathclyde have previously developed a vertical nanovibration device capable of delivering stimulation of 1 kHz, 30 nm to cultured cells *in vitro* [3]. The application of such vibration conditions has been shown to induce an osteogenic response in MSCs [4]. However, recent studies have suggested higher amplitudes and horizontal vibration may induce a higher osteogenic response in cells [5,6]. Here, we investigated two sets of vibration conditions applied to cells, in an attempt to optimise cell response. Three MSC donors were used to explore donor variability and identify whether a similar response may be observed between donors.

Materials and Methods: Three MSC donors of different ages and genders were used (Donor 1: male, 80 years old; Donor 2: male, 28 years old; Donor 3: female, 80 years old). Cells were seeded and left to adhere for 24 hours before being stimulated at one of two vibration conditions (either 1 kHz, 30 nm, vertical vibration or 1 kHz, 90 nm, horizontal vibration) for 28 days. Non-vibrated controls were incubated in a separate incubator. Gene expression analysis was conducted following 7, 14, 21 and 28 days of stimulation, investigating osteogenic genes as well as a neurogenic, chondrogenic and adipogenic response. Immunofluorescent staining was performed on Day 28. Atomic force microscopy (AFM) was used to investigate the mechanical properties of the nucleus and cytoplasm during nanovibrational stimulation at multiple timepoints within the first 3 days of stimulation. Mineralisation was also measured on Day 28 using Alizarin Red staining.

Results and Discussion: Morphologically, nuclear area was found to increase in all donors vibrated at 1 kHz, 90 nm, horizontally, indicating increased nuclear tension. Beyond this, all donors responded to vibration differently. Donor 3 showed a strong morphological response, with an increase in cell area and actin intensity in response to vibration at 1 kHz, 90 nm, horizontally. Mechanically, older donors showed an increase in stiffness (not significantly) within the first few hours of stimulation, particularly when vibrated vertically at 1 kHz, 30 nm. Donor 1 showed the highest osteogenic response to vibration, with an increased expression of Runx2 on Day 7, and OPN on Day 14 and Day 28. Meanwhile Donor 2 did not show a significantly high osteogenic response to vibration, but did show increased expression of adipogenic and neurogenic genes.

Conclusions: This study demonstrates the variability in donor response to nanovibrational stimulation. As such, identifying key vibration conditions to induce a desired response is difficult, and may require systematic optimisation, leaning toward more personalised medicine approaches. This may be required to translate such technology toward a clinical setting.

References

1. Meier E *et al.* Biotech. Bioeng. 3(3): 1069, 2016.
2. Choi Y *et al.* Life Sci. 91(9-10): 329-337, 2012.
3. Campsie P *et al.* Sci. Reports. 9: 12944, 2019.
4. Tsimbouri M *et al.* Nat. BiomedEng. 1(9): 758-770, 2017.
5. Orapiriyakul W *et al.* ACS. Nano. 14(8): 10027-10044, 2020.
6. Halonen H *et al.* J. Mech. Behav. Biomed. Mater. 101: 103419, 2020.

THE ROLE OF VISCOELASTICITY IN SOFT 3D MATRICES.

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Abstract theme: Bioengineered Models / Mechanobigology / Enabling Technologies / Biomaterials / CDT Session

The extracellular matrix (ECM), a dynamic network composed of proteins, glycosaminoglycans and growth factors, regulates various cellular processes (1). This microenvironment is not purely elastic but exhibits a time-dependent response to mechanical loading, a property known as viscoelasticity (1). However, efforts to understand the role of ECM viscoelasticity in regulating cell behaviour have only recently been made using 3D mimicking hydrogels (2). These have shown that viscoelasticity can affect cell behaviour independently from the hydrogel's stiffness (3,4), but little is known about its effects on cell mechanics. Here, we propose a novel approach to develop isoelastic hydrogel pairs with varying stress relaxation rates. These hydrogels will then be used to investigate the effect of viscoelasticity on mesenchymal stem cell (MSC) morphology and mechanics at different stiffnesses.

Non-degradable polyethylene glycol-Maleimide (PEG-MAL) hydrogels were prepared using Michael-type addition, and functionalized using full-length Fibronectin (FN), a protein abundantly found in the ECM. Stiffness and viscoelasticity were varied by altering the percentage or molecular weight (MW) of the hydrogel components. Bulk mechanical properties including storage modulus (G') and stress relaxation were measured using a stress-controlled rotational rheometer. Furthermore, spheroids of human BM-derived mesenchymal stem cells (hMSCs) were encapsulated within hydrogels for a period of 5 days, after which spheroid morphology was investigated using confocal microscopy. hMSC spheroid mechanics were explored after 3 days using Brillouin spectroscopy.

We engineered three pairs of FN-functionalised PEG-based hydrogels, with elastic moduli *E'* of 1, 5 and 12kPa. Each pair comprises of a slow-relaxing, more elastic hydrogel and a fast-relaxing, more viscoelastic counterpart. When hMSC spheroids were encapsulated in the 5kPa hydrogel pair, we observed that spheroids in slow-relaxing matrices impaired spheroid outgrowth, with no significant changes in spheroid morphology by day 5. On the contrary, spheroids in fast-relaxing hydrogels demonstrated increased size, accompanied by the formation of finger-like projections that invaded the matrix. This morphological difference correlated with a change in spheroid mechanics, whereby spheroids in fast-relaxing hydrogels presented a significantly decreased Brillouin frequency shift compared to spheroids in slow-relaxing hydrogels. At higher stiffnesses (12kPa hydrogels) viscoelasticity did not affect spheroid morphology or mechanics, as spheroids in both slow- and fast-relaxing gels remained spherical and showed similar Brillouin frequency shifts. However, matrix stiffness significantly affected spheroid mechanics, with spheroids in rigid gels showcasing significantly higher Brillouin frequency shifts than those in the 5kPa hydrogel pair.

We engineered FN-functionalised synthetic hydrogels with tuneable stress relaxation independently of their elastic modulus. Upon encapsulation of hMSC spheroids, fast-relaxing hydrogels of medium stiffness (5kPa) promoted cell invasion compared to slow-relaxing ones which instead restricted cell movement. Crucially, this change in cell behaviour could be detected via Brillouin spectroscopy as spheroid mechanics were affected by the stress relaxation of the surrounding matrix. Finally, increased hydrogel stiffness (12kPa) significantly impacted spheroid mechanics, while viscoelasticity did not affect spheroid morphology or mechanics.

References

1. Kular J. et al, Journal of Tissue Engineering. 5: 1-17, 2014.
2. Chaudhuri O. et al, Nature. 584: 535-546, 2020.
3. Chaudhuri O. Biomaterials Science. 5: 1480-1490, 2017.
4. Elosegui-Artola A. et al, Nature Materials. 22: 117-127, 2022.