QRW Regenerative Medicine and Tissue Engineering 2019 Abstracts

R1

Innovative New Strategies in Skeletal Regenerative Medicine

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The impact of chronic diseases and trauma as well as the aging population and soaring healthcare costs have prompted an acceleration of regenerative medicine to develop biological substitutes to restore tissue function. Tissue engineered scaffolds are an integral part of future regenerative efforts to support the repairing tissue and can act as carriers for growth factors and stem cells. Millions of dollars are spent on the development of biomaterial scaffolds, with many put into animal studies and clinical use without proper evaluation, often leading to unfavourable outcomes and associated patient morbidity.
We have developed a package of *in vitro* assays to evaluate the biomaterials prior to evaluation in a number of pre-clinical animal models for bone regeneration and tendon repair.

R2

Creating and Evaluating a Novel Sheep Abductor Tendon Tear Model

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Purpose:
No animal model currently exists for hip abductor tendon tears. We aimed to 1. Develop a large animal model of delayed abductor tendon repair and 2. To compare the results of acute and delayed tendon repair using this model.

Method:
Fourteen adult Romney ewes underwent detachment of gluteus medius tendon using diathermy. The detached tendons were protected using silicone tubing. Relook was performed at six and 16 weeks following detachment, histological analysis of the muscle and tendon were performed.
We then attempted repair of the tendon in six animals in the six weeks group and compared the results to four acute repairs (tendon detachment and repair performed at the same time). At 12 weeks, all animals were culled and the tendon–bone block taken for histological and mechanical analysis.

Results:
Histology grading using the modified Movin score confirmed similar tendon degenerative changes at both six and 16 weeks following detachment. Biomechanical testing demonstrated inferior mechanical properties in both the 6 and 16 weeks groups compared to healthy controls.
At 12 weeks post repair, the acute repair group had a lower Movin's score (6.9 vs 9.4, p=0.064), and better muscle coverage (79.4% of normal vs 59.8%). On mechanical testing, the acute group had a significantly improved Young's Modulus compared to the delayed repair model (57.5MPa vs 39.4MPa, p=0.032)

Conclusion:
A six week delay between detachment and repair is sufficient to produce significant degenerative changes in the gluteus medius tendon. There are significant histological and mechanical differences in the acute and delayed repair groups at 12 weeks post op, suggesting that a delayed repair model should be used to study the clinical problem.

R3

Gene editing tools for functional genomics and gene editing

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Modern advances in gene editing technology associated with CRISPR/Cas9 are increasingly used as laboratory-based tools and currently being developed for diagnostic and clinical uses. To date, there has been limited application of these tools in the musculoskeletal space and our team has focused on developing practical and efficient uses of gene editing in bone. The two key projects focus on functional genomics and on vectors for in vivo gene editing.

Increases in efficiency and decreases in costs of exome and whole genome sequencing have enhanced the rate of detection of gene variants. For individuals with genetic bone disease such as osteogenesis imperfecta, validation of variants of uncertain significance (VUSs) assists in understanding the long-term prognosis, assists in genetic counselling and pre-genetic diagnostics, and is the first step towards curative gene therapies. Our team is establishing high throughput *in vitro* and xenograft systems for screening VUSs, however understanding the reliability of functional tests is critical for implementation in a hospital setting.

While multiple groups have had varying success at rescuing bone mutations in cultured cells, we identified systemic delivery as a major hurdle to implementing gene therapy for bone. Adeno-associated viruses (AAVs) have great potential as vectors for systemic transgene delivery and may be adapted for emerging gene editing technologies. We screened 18 natural and engineered recombinant AAV variants in murine and human cell models, and identified several variants with a high capacity to transduce bone. Second-generation vectors were found to produce high levels of gene editing *in vivo* with a single intravenous injection of virus, and using an Sp7 promoter sequence were able to be restricted to bone. These and third-generation vectors currently being developed will have a broad utility in preclinical modelling of genetic bone disease, and ultimately may be adapted for gene therapy applications.

R4

Efficient Engineering of IPS Cells using Genome Editing

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IPS cells continue to have tremendous promise as a source of cells for regenerative medicine. Genetically engineering human IPS cells has the potential to make IPS cell derived therapies both safer and more potent. We have developed a genome editing system using the CRISPR/Cas9 system combined with non-integrating AAV6 viral delivery that allows highly efficient genome editing of human IPS cells. I will discuss the development of this system and our use of this system to engineer IPS cells to develop safe and potent regenerative medicine therapies.

R5

Challenges in characterizing tissues in 4D

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It is clear that there is and will continue to be increasing demand for reproducible and predictable 3D in vitro models that effectively replicate the tissue of interest. These systems need to

- replicate specific physical and biochemical aspects of the biological system.
- be readily manipulated to address specific research questions or target specific biological pathways
- be reproducible, scalable and critically, validated against the gold standards.

With these developments of organoids, spheroids and tissue engineered 3D systems, there is a challenge to integrate from the earliest inception both *in situ* (ie embedded biosensors, reporter constructs, imaging) and *ex situ* tools (chemical spectroscopy, in silico models). These sensing systems enable real-time monitoring of the behaviour both during preparation and use, creating a 4^{th} dimension to the cell culture systems. Creating approaches that minimise the perdubation of the biological system while providing insight into both local and systemic responses is hugely challenging. It is only with the development and incorporation of these tools that we can expect to develop systems that are validated, reproducible and scalable and thus implementable across the Medical Technology and Pharmaceutical sectors.
This talk will discuss the tools and systems we are using to characterise and monitored tissues in 4D.

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R6

Dissecting cellular heterogeneity of the human adipose tissue-derived stromal vascular fraction using single cell technologies

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Human subcutaneous fat is an abundant and accessible source of stromal vascular fraction (SVF) cells that contains pre-cursor cells capable of differentiating into adipocyte lineages, and possibly also progenitor cells capable of differentiating into other mesenchymal lineages, such as bone and cartilage. For this reason, they have been the subject of intense investigation, especially in the context of regenerative medicine. However, the precise subpopulation within the SVF with the greatest therapeutic potential remains unclear and is one of the most controversial areas in stem cell biology. The key cell population of interest within the SVF is often termed adipose tissue-derived “stem” cells (ASC) or mesenchymal stem cells (MSC), inferring their suitability for a wide range of regenerative applications. However, the cellular diversity of this population, referred to hereafter as adipose-derived stromal cells (AD-MSC), and their true therapeutic potential remains undefined. Single-cell transcriptomic technologies have emerged as powerful tools to explore cellular heterogeneity of complex systems, however the correlation between gene and protein expression is only beginning to be explored. Therefore we sought to use multicolour flow cytometry and single cell transcriptomics (genomic cytometry) to identify cell populations of interest while also cross-validating these techniques, ie comparing cell surface proteins to gene expression. Spectral flow cytometry was used to characterise and define mesenchymal stromal cell populations using a 16 colour panel. Cell populations of interest were enriched by FACS cell sorting, and single cell RNA sequencing (scRNAseq) was performed using the Chromium 10x Genomics system. We reveal that the mesenchymal population in adipose tissue is indeed heterogeneous, and report discrepancies between protein and gene expression in a few common cell surface markers. Using knowledge from these pilot studies, we sought to further validate tissue heterogeneity by utilising CITE-seq (Cellular Indexing of Transcriptomes and Epitopes by sequencing), to simultaneously characterise surface proteins and transcriptomes at the single cell level (scRNAseq).

R7

Non-destructive imaging of collagen architecture in biological membranes

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Bioprosthetic heart valves constructed from bovine pericardial tissue are used for the replacement of diseased heart valves. These valves are limited in their durability, showing complications in 50% of patients after 10 years[1]. Failure rates may be improved by better characterisation of tissue mechanical properties. Collagen is the main load bearing component in pericardium and is thought to give rise to its complex mechanical properties. Bovine pericardium has been shown to have a high intra- and inter-specimen variability of its collagen fibre architecture. A non-destructive imaging technique that is sensitive to collagen may allow a tissue selection protocol for artificial valve leaflets that leads to reduced failure rates.

Mueller matrix imaging polarimetry is an optical technique that can non-destructively estimate the orientation and anisotropy of the intrinsically birefringent collagen in turbid, highly scattering tissues, such as pericardium[2]. An instrument utilizing this technique, in a transillumination arrangement, has been developed and integrated with a mechanical testing rig for performing simultaneous optical and mechanical experiments on biological membranes. Measurements of bovine pericardia, harvested post mortem from Angus-Hereford steers aged 18 to 24 months and fixed with glutaraldehyde, demonstrate anisotropic and heterogeneous structural arrangement of collagen across the membrane.

In addition, Micro-CT imaging of pericardium was carried out to compare the 3D architecture of collagen with the bulk tissue optical properties acquired from Mueller matrix imaging. Pericardia were stained with phosphotungstic acid and imaged in a micro-CT scanner (Bruker, SkyScan 1172) at a pixel resolution of 1.08 µm. Results show a highly resolved fibrous structure on the epipericardial surface of the pericardium, with layers of densely packed bundles of collagen fibres through the thickness of the membrane. Within layers there are distinct regions with a predominant orientation of fibres. Techniques are being developed to correlate the collagen architecture between the two imaging modalities.

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R8

Modulation of chondroitinase sulphate proteoglycans combined with exercise rehabilitation leads to axonal regeneration and functional improvement after spinal cord injury.

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Reactive astrocytes are key contributors to the deposition of chondroitin sulphate proteoglycans (CSPGs) after injury, and potent inhibitors to axon regeneration and plasticity. Modulation of CSPG activity after spinal cord injury has been demonstrated to be protective and lead to functional improvements. A disintegrin and metalloproteinase with thrombospondin motifs-4 (ADAMTS4) is a human enzyme that catalyses the proteolysis of CSPGs. Infusion of ADAMTS4 into the damaged spinal cord was previously shown to improve functional recovery after SCI; however, this therapy is limited in its enzyme form. Gene therapy is a method that allows for long-term expression of therapeutic molecules. Since astrocytes play an important role in SCI pathology, we created an AAV vector with a GFAP promotor to drive astrocyte specific expression and determine if this was an effective method to modulate astrocyte function after spinal cord injury. This vector elicits selective, robust and widespread gene expression in spinal cord astrocytes. A spinal cord contusion rodent model was transduced with a AAV-ADAMTS4 vector and showed sustained expression of ADAMTS4 and widespread degradation of CSPGs. Transduction with AAV-ADAMTS4 resulted in significantly decreased lesion size, increased levels of neuroplasticity markers 5-HT and PKCg, regeneration of axonal fibres from the corticospinal tract and improved hindlimb function. Hindlimb-specific exercise rehabilitation was then used as a method to drive and strengthen the formation of beneficial connections whilst pruning aberrant ones. Indeed, the combination of hindlimb rehabilitation with AAV-ADAMTS enhanced the therapeutic effect, leading to an even greater improvement in hindlimb function compared to AAV-ADAMTS4 or contusion alone. Widespread and long-term degradation of CSPGs using gene therapy in combination with other approaches such as exercise rehabilitation and/or cell transplantation therapy represents a promising candidate to improve axonal regeneration after spinal cord injury and as a potential treatment.

R9

Use of biomaterials to enhance neural tissue regeneration.

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With the number of deaths due to stroke decreasing, more individuals are forced to live with crippling disabilities resulting from the stroke. To date, no drug therapeutics exist after the first 4.5 h following stroke onset, aside from rest and physical therapy. The translation of therapeutic compounds into the clinic has been hampered due to the fact that most pharmacological compounds developed fail to cross the blood-brain-barrier. Post-stroke tissue remodelling results in a compartmentalised cavity that can directly accept a therapeutic material injection, such as biomaterials. Biomaterials can help to increase the regenerative potential of cells and biomolecules by controlling transplanted cell fate and provide a local, sustained release of biomolecules, such as trophic factors like brain-derived neurotrophic factor. Herein, the therapeutic potential of biomaterial to deliver trophic factors and other small molecules will be discussed.

R10

Photo-click Gelatin-Norbornene Hydrogels for Biofabrication of Vascularized Tissue

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Biofabrication approaches allow recapitulation of the complex and organised architecture of native tissue. However, the lack of functional vasculature within large biofabricated tissue engineered constructs remains an issue for sustained long-term viability. Therefore, the aim of this study is to evaluate the vasculogenesis and biofabrication potential of a photo-polymerisable thiol-ene gelatin based hydrogel. Gelatin (10wt%) was reacted with carbic anhydride (20wt%), at 50˚C for 24h with pH kept in the range of 7.5 – 8 to produce gelatin-norbornene (GelNor). 5wt% GelNor hydrogels were photo-polymerised (400-450nm, 30mW/cm^2, 3min, Ruthenium (Ru)/Sodium Persulphate (SPS) as photoinitiators) with thiolated molecules as crosslinking agents. The physico-mechanical properties were characterised with varying crosslinking parameters (Nor/thiol ratio, Ru/SPS concentration and crosslinking agent). Human umbilical vein endothelial cells (HUVEC) were co-encapsulated within GelNor hydrogels with human mesenchymal stromal cells (MSCs). Co-cultures in casted hydrogel disks (Ø5x1mm) were maintained in endothelial growth media for 7 and 14 days, following fixation and immunohistochemical evaluation (CD31/F-actin). Scaffolds with interconnected channels were fabricated by casting GelNOR macromer over 3D plotted Pluronic127© which served as a sacrificial template. GelNOR was successfully synthesised with a 45% degree of modification. Varying Nor:SH (DTT) ratios, photoinitiator concentrations and crosslinking agents resulted in tailorable sol fractions, mass swelling ratios and compression modulus. HUVECs were co-encapsulated with MSCs in gelNOR hydrogels, showing high viability (>90%) and a retained HUVEC phenotype over the cell-culture period. These conditions were able to facilitate the formation and stabilisation of interconnected vessel-like structures. Interconnected channels were successfully generated. In conclusion, we have shown that GelNOR hydrogels, with tailorable physico-chemical properties, can be used to promote *in vitro* vasculogenesis for large biofabricated tissue engineered constructs.

R11

Lens protein biomaterials for use in ocular surgery

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Crystallins are the dominant structural protein in the eye lens, where they are responsible for maintaining optical transparency. The structure and packing of crystallin proteins enables this function, and *in vivo*, some classes of crystallin proteins also have therapeutic properties. Our research focuses on the creation of biomaterials from crystallins for use in ocular health applications. Although crystallin proteins may be produced recombinantly, there are multiple classes of crystallins, and gaining significant yield is an issue. As such, our source material is extracted from Hoki fish (*Macruronus novaezelandiae*) eyes[1], a low value by-product of the New Zealand fisheries industry.

We have recently used crystallin proteins to formulate transparent biomaterials – namely, thin films, gels and adhesives[2]. Crystallin thin films are preparing via casting, and have a range of mechanical properties and degradation rates dependant on the concentration of crosslinker and plasticizer. One application that is currently being explored for these films is as stem cell carriers for the treatment of limbal stem cell deficiency, an ocular disease that results in significant pain and loss of visual acuity. We have shown crystallin thin films to be cytocompatible and support *ex vivo* expansion from limbal explants. Proof-of-concept experiments demonstrate that cell transfer takes place from films to a corneal surface, validating transfer capacity in an *in vitro* model of clinical treatment.

In ophthalmology there is also a need for new specialised adhesives for use in ocular surgery. Current adhesives are limited by a lack of transparency, scarring issues, and toxicity. This has led to the formulation of crystallin proteins into a bioadhesive that is biocompatible due to its protein-based formulation, and transparent due to the source material. Current work is focused on the tuning of degradation rate to match host cell infiltration, whilst maintaining adequate adhesive strength and curing time for surgical use.

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R12

Isolation and Identification of a Trophic Factor for Mesenchymal Stem Cells; a Functional Decorin Protein Fragment Cleaved from Ovine Forestomach Matrix by Macrophage Cells

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In living tissue, the relationship between cells and the extracellular matrix (ECM) is multifaceted and intricate. ECM proteins impart structural properties, such as strength and elasticity to tissues; however the ECM also interacts with cells to alter biological processes required for the maintenance, development, growth and repair of tissues. ECM and ECM-derived proteins known as matrikines can directly interact with cells in order to illicit a biological response [1].

Biomaterials derived from the ECM of allogeneic or xenogeneic tissue sources have been used in soft tissue repair for decades, and are a potential source of molecules that affect biological processes. One such material is OFM (ovine forestomach matrix), a biomaterial for soft tissue repair that mimics the structure and composition of native ECM, acting as both a structural scaffold and a supply of bioactive proteins for cells during soft tissue repair.

In this work, OFM has been modified using cell-mediated processing to release bioactive extracts that were tested in chemotactic assays with relevant wound healing cells. This led to the discovery of a bioactive fragment of ECM that was identified using mass spectrometry. This putative OFM-derived bioactive fragment was recombinantly expressed and tested using in vitro chemotaxis assays and in an animal model of mesenchymal stem cell recruitment to demonstrate efficacy.

This approach has the potential to uncovered ECM components that affect many different biological processes, such as angiogenesis, fibro genesis and immune responses. The results can be used to explain biological processes within ECM-cell dynamic reciprocity and also uncover potential therapeutics to alter biological processes such as soft tissue repair, angiogenesis, immune responses and cancer progression.

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R13

Marine-derived collagen and gelatin: Expanding the ingredients pantry for biomaterial fabrication

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Collagen and gelatin are two of the most commonly used ingredients for biomaterials because of their excellent biocompatibility and biodegradability. Collagen can be extracted from all animals; however, the most common format and source available is gelatin (hydrolysed form of collagen) extracted from pigs. The absence of structural, functional and compositional options potentially available from other raw material sources restricts the use of collagen-based materials for some research applications.

Marine collagen (collagen extracted from fish and marine invertebrates) is fundamentally different to porcine collagen. It offers a range of physical and molecular properties that are impossible to achieve with porcine gelatin, while still being biocompatible and biodegradable. Marine collagens are waiting to be exploited in the biomaterial space.

This presentation will showcase a range of marine collagens, including different types (type I, II, V, IX, XI)[1] and formats (such as fibrillar, native triple helical, whole-chain denatured or gelatin). It will also cover how, with subtle differences in the extraction process and a deep understanding of the collagen molecule, we can finely tune the physical and molecular properties of marine collagen for specific fabrication techniques.

We have found marine collagen to be an ideal ingredient for 3D printing and electrospinning. Our work with electrospinning has led to commercial fish collagen nanofibres[2], novel intrafibrillar crosslinking techniques[3] and the use of Synchrotron small-angle X-ray scattering analyses to understand whether collagen electrospun fibres form native structures.

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R14

Regeneration for dental implant therapy: clinical requirements and preclinical models.

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In dentistry, regeneration originated with the use of Teflon membranes for selected repopulation of the periodontal ligament in chronically-diseased periodontal wounds around teeth, a process called Guided Tissue Regeneration (GTR). GTR involved regeneration of multiple tissue compartments (collagenous ligament, cementum, bone). Subsequently this technology expanded to focus on the regeneration of oral and maxillofacial bone, with the objective of creating sufficient support for titanium dental implant screws that replace teeth or support dentures. This technique was termed Guided Bone Regeneration (GBR). Both GTR and GBR changed over time, from non-resorbable Teflon to resorbable collagen membrane sheets, supported by particulate grafting materials (alloplasts, allografts or xenografts) that act as a scaffold for ingrowth of regenerating tissue. More recent developments include the application of biologically-active cytokines, the use of stem cells, and solutions to the problem of microbial colonization, both of titanium implant surfaces and the grafting materials. The primary preclinical models for new therapeutic approaches (dog and monkey) are ethically problematic and unavailable in New Zealand. We have developed a number of models using sheep, that have allowed us to test different approaches to regeneration in New Zealand. This presentation will review the history of oral regeneration and the development of our preclinical models, and highlight how our regeneration research uses these as a preclinical pathway to acceptance by FDA, prior to human clinical trials.

R15

Targeting Bacterial Biofilms in Translational Research

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Biofilms underpin the disease etiology of nearly all opportunistic bacterial infections especially when biomaterials remain implanted. An understanding of how biomaterials potentiate infection was formalized by Costerton and colleagues from observations made in the 1970s.[1] Opportunistic bacterial pathogens preferentially dwell in sessile communities by excreting sticky extracellular polymeric substances (EPS) to form cohesive aggregates and adhesive attachments to implant biomaterials or devitalized tissues; this strategy deranges phagocytic clearance by host leukocytes.[2] Quiescent phenotypic variants in the diffusion-limited biofilm core are tolerant of antibiotic concentrations many orders of magnitude greater than will otherwise kill planktonic phenotypes, concentrations greatly exceeding toxic thresholds bounding safe systemic antibiotic use.[3] Yet despite an understanding of how biofilm phenotypes potentiate infection, the use of actively dividing planktonic cultures predominates in the clinical and regulatory tests for determining the efficacy of antibiotics, antimicrobial coatings, and medical devices: the minimum inhibitory concentration (MIC), the E-test, Kirby-Bauer, the minimum bactericidal concentration (MBC), and MicroScan. Lab-grown biofilms were used to test clinical and experimental antibiotic compounds. These biofilms withstood antibiotic concentrations greatly exceeding respective MIC values. One example, vancomycin was ultimately pushed to its solubility limit (64 mg/ml); at this inordinate concentration for 24 h produced less than a 0.5 $\log_{10}$ reduction against *Staphylococcus aureus* biofilms (>9 billion CFUs). Importantly, the MICs failed to predict the order of efficacy for antibiotics when tested against biofilms. Two experimental antibiotic compounds were thereby identified with promising antibiofilm activity unanticipated by the MICs. Translation of these *in vitro* technologies to appropriate *in vivo* animal models is essential. We show that the use of biofilms as initial inocula quickly produces reliable infections with the hallmark antibiotic recalcitrance observed in the most difficult to treat clinical scenarios. These models are indispensable for testing our experimental antibiofilm technologies.[4]

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R16

The Horse as a Model and Translational Stepping Stone in Orthopaedic Research

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Osteoarthritis and muskuloskeletal injury is one of the most significant causes of morbidity in humans and veterinary patients alike. In particular, equine orthopaedic medicine and surgery represents a unique intersection between the human and veterinary fields. Due to comparable cartilage thickness and joint size, the horse has become the accepted preclinical model for cartilage research. The equine stifle (anatomically equivalent to the knee) offers an ideal location for testing of cartilage repair modalities and has been extensively utilised to assess the efficacy of numerous techniques. In addition, the clinical equine patient offers an opportunity for translational medicine with naturally occurring joint diseases, tendon and ligament injuries that parallel many human disease processes. Both developmental orthopaedic disease and degenerative joint disease (osteoarthritis) may result in large full thickness cartilage defects that limit athletic performance in the horse and necessitate surgical intervention. Equine athletes are also frequently presented for acute an chronic tendon and liagment injuries, which approximate those of their human counterparts. The use of autologous bone marrow and adipose derived mesenchymal stromal cells (MSCs) has become common in equine clinical practice due to the high economic value of these animals and the relative lack of regulation in implementation of novel therapies, compared to the human field. Numerous studies have evaluated the efficacy of intra-articular and intratendinous injection of MSCs. The use of allogeneic MSCs and alternative sources such as umbilical cord blood derived MSCs have become increasingly prevalent in the literature in an effort to identify an MSC source with optimal regenerative properties that is readily available without the requirement for culture expansion. Over the last decade a broad body of literature has emerged from the equine sphere encompassing both *in vitro* and clinical data, which may be applicable to the management of osteoarthitis and muskuloskeletal injury in people.

R17

Melt electrowriting: An emerging additive biomanufacturing technique for soft tissue engineering.

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Abstract

Melt electrowriting (MEW) is a fascinating additive biomanufacturing technique that merges the physical principles of fused deposition modelling and electrospinning, bringing together the best of both worlds. Scaffolds manufactured by MEW are tailorable in terms of fibre architecture, porosity and thickness. Moreover, MEW is a solvent-free process, compatible with the use of medical grade thermoplastic polymers. These exclusive advantages make MEW an ideal manufacturing technique for the production of polymeric scaffolds for a wide range of biomedical applications. However, despite the great advantages of MEW, this technique is still at its infancy in laboratory setups and a lot of work needs to be done to facilitate a widespread use of it. In this talk, I will give an overview of the physical principles and unique capabilities of MEW, followed by some examples on how to capitalize its potential to produce scaffolds with controlled mechanical properties for soft tissue engineering.

R18

Biofabrication of the Musculoskeletal Tissue Niche: Tissue Spheroid and Bioink Platforms

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INTRODUCTION: Biofabrication technologies, including 3D bio-printing and bio-assembly, enable the generation of engineered constructs that replicate the complex 3D organization of native tissues via the automated placement of cell-laden bioinks, tissue modules, growth factors and/or bioactive agents. A major bottleneck lies in designing hydrogel bioinks that are both cell-instructive and compatible with 3D-biofabrication techniques. We investigated the development of versatile, cell-instructive bioinks for biofabrication of the musculoskeletal niche targeting chondrogenic, osteogenic and vascular tissue formation.
METHODS: We describe a platform visible-light photoinitiator system (Vis + Ru/SPS) [1-4] exhibiting increased cell viability, shape fidelity and penetration depth compared to traditional ultraviolet (UV) crosslinking. Furthermore, we developed photo-clickable hydrogels based on allylated gelatin (GelAGE) as platform bio-inks and bio-resins [3]. We investigated bioink physico-chemical properties, shape fidelity, mechanical stiffness, and incorporation of growth factor binding molecules (e.g. heparin; HepSH) to promote cell differentiation in co-cultured human articular chondrocyte (hAC), bone marrow-derived mesenchymal stromal cell (bmMSC), and endothelial cell (HUVEC) spheroids.

RESULTS: We show for the first time that GelAGE hydrogels can be successfully applied as platform bioinks used across multiple biofabrication technologies inclusding extrusion Bioprinting, 3D Bio-assembly and high resolution lithography-based (DLP) Bioprinting [3]. Furthermore we modulated the cell-instructive musculoskeletal tissue niche for multiple cell type via covalent incorporation of thiolated heparin (HepSH), mechanical stiffness and shape fidelity, yielding enhanced tissue formation *in vitro* [2-5]. We demonstrate a 3D spheroid Bioassembly platform for biofabrication of complex hybrid and mechanically functional constructs [6, 7], promoting high density cell-cell interactions, tissue fusion and investigation of co-cultured hAC/bmMSC/HUVEC spheroid microenvironments resulting in enhanced chondrogenic, osteogenic and vascular tissue differentiation capacity.
DISCUSSION & CONCLUSIONS: This study demonstrated novel photo-click GelAGE hydrogels as multifunctional, cell-instructive bioinks promoting musculoskeletal differentiation of bmMSCs via functionalised growth factor incorporation and/or mechanical stiffness, and are compatible with multiple 3D-biofabrication approaches for clinical relevance and practicality.
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R19

Investigating the Immunology of Type 1 Diabetes using Pluripotent Stem Cells.

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Problem: Type diabetes is an autoimmune condition in which T-cells attack and kill insulin producing cells of the pancreas. Much of our understanding of Type 1 diabetes has been derived from animal models in which the course of disease is quite different from that seen in humans. As such, new approaches to studying the pathogenesis of type 1 diabetes in humans are needed. In this study, we used the in vitro differentiation of pluripotent stem cells to to create key cell types involved in type 1 diabetes.

Methods: We generated induced pluripotent stem cells (iPSCs) from a tissue donor who had type 1 diabetes and then differentiated these iPSCs in vitro to generate macrophages. Differentiated cells were characterised using flow cytometry, confocal microscopy and phagocytosis assays. The ability of the macrophages to present islet antigens to a HLA matched T cell line was studied using flow cytometric analysis of T cell activation.

Results: Flow cytometry analysis of iPSC derived macrophages indicated these cells expressed typical macrophage markers, CD14, CD11b, CD86 and HLA-DR, had a macrophage morphology in cytospin preparations and displayed a capacity for phagocytosis. Activated macrophages efficiently presented C peptide to a T-cell line expressing a T-cell receptor derived from islet-infiltrating $CD4^+$ T-cells in an HLA-restricted manner. Non-HLA matched iPSC derived macrophages failed to activate T-cells, confirming the specificity of the HLA/TCR interaction.

Conclusion: We generated macrophages from induced pluripotent stem cells derived from an individual who had Type 1 diabetes. iPSC Macrophages expressed typical lineage markers, responded to inflammatory stimuli, and presented antigen to HLA match islet infiltrating CD4+ T-cells. This system has potential as a model for type 1 diabetes and as a platform to search for islet derived antigens that drive autoimmunity.

R20

Identification and tracking of periosteal progenitors during fracture healing

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The periosteum is a major source of cells involved in fracture healing, but the identity of osteoprogenitors in the periosteum is undefined. A number of different markers and approaches have been utilized to identify osteoprogenitors in different settings. My research has focused on progenitors that express alpha smooth muscle actin (αSMA), and characterising the fate of these cells using lineage tracing.

To identify and trace αSMA+ cells we crossed αSMACreERT2 with Ai9 tdTomato reporter mice. In many instances this was combined with the Col2.3GFP reporter to identify mature osteoblasts. We have shown that αSMA-labelled cells contribute to a large proportion of osteoblasts, chondrocytes and fibrous tissue within a fracture callus. Global gene expression analysis identified numerous changes occurring in αSMA-labelled cells following fracture, including downregulation of Notch signalling.

In order to evaluate if the αSMA-labeled population in the periosteum contains long-term progenitors, tibia fractures were generated in αSMACre/Ai9/2.3GFP mice treated with tamoxifen 13, 6 or 2 weeks prior to fracture, or on the day of fracture, and contribution to osteoblasts and chondrocytes quantified in a 7 day fracture callus. The results indicate that the majority of osteoprogenitors involved in fracture healing express αSMA. A subset of these cells remain capable of contributing to osteoblasts after 3 months indicating long-term progenitor potential. Functionally, ablation of αSMA-expressing cells around the time of fracture using a DTA model impaired formation of a mineralised fracture callus. Efforts are ongoing to identify subpopulations of cells that represent long term progenitors.

R21

Stem cells for regenerative medicine in dentistry

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Tooth regeneration using stem cell therapy targets repair of pulp, dentine, enamel and periodontal ligament. Regeneration of alveolar bone is also important for dental implant placement and in association with trauma, osteonecrosis or cancer. In addition, dental pulp stem cells (DPSC) provide a readily available source of neural crest-derived stem cells with broad potential therapeutic utility. The aims of this research are to investigate stem cells in association with bone regeneration and dental implants; the location, differentiation capacity, and regulation of DPSC and palatal periosteum; and to investigate the control of neural crest-derived stem cells using deer antler as a mammalian model of organ regeneration.

Adipose-derived stem cells were obtained from human and sheep sources and grown in serum- and xeno-free medium prior *in vitro* assays, qRT^2-PCR and placement within circular bone defects. Human palatal periosteum and DPSC have been examined using: qRT^2-PCR, FACS, differentiation assays, inhibition and proliferation assays. Proteomic analysis of antler neural crest stem cells used 2D-DIGE and label-free proteomics (LC-MS/MS).

Stem cells have been isolated from adipose, periosteum and tooth pulp in serum-free medium. Adipose-derived stem cells were tri-lineage differentiated, labelled, and transplanted into critical sized bone defects in sheep. Human DPSC were differentiated into ectodermal, endodermal and mesodermal lineages. Palatal periosteum stem cells were identified *in vivo* and *in vitro*, and the role of HGF and MET in osteogenesis investigated. Proteomics on antler stem cell niches has identified a number of pathways/proteins critical to maintaining and controlling stem cells during endochondrial bone formation.

This research has developed xeno- and serum-free stem cell culture systems which allow the development of DPSC, palatal periosteum and adipose-derived stem cells for clinical translation. Identification of the proteins/pathways associated with stem cell niches which can control tissue growth and differentiation is of critical importance for successful tissue repair and regeneration.

R22

Radical concepts underscore paradigm shifts – from enigmatic infantile haemangioma to cancer

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Abstract:

Infantile haemangioma (IH), affects 4-10% of infants and has a predilection for female, Caucasian and premature infants. It is characterised by a rapid post-natal growth, followed by spontaneous slow involution often leaving a fibro-fatty residuum. IH had previously been regarded as a primary tumour of the microvasculature with excessive angiogenesis. In this presentation, the author outlines his team's quest to understand the origin of this enigmatic condition. The observation of the association of segmental cervicofacial IH with midline structural anomalies that constitute PHACES syndrome led to the proposed involvement of neural crest cells. The investigation led to the discovery of the stem cell origin of IH characterised by a haemogenic endothelium with mesenchymal and haematopoietic plasticity, with these embryonic stem cell (ESC)-like cells being the putative source of the fibro-fatty tissue that naturally occurs during involution. The observation of increased incidence of IH following pregnancies complicated by placental events such as pre-eclampsia, and that the endothelium of IH sharing expression of a unique combination of markers led to the finding of a placental origin of IH. The expression of angiotensin converting enzyme (ACE) by the haemogenic endothelium of IH, coupled with the observation of (1) increased renin levels in female, Caucasian and premature infants (compared with their counterparts) all of whom have an increased incidence of IH; (2) a 10-fold greater serum levels of renin in the first 3 months of life than that of adults, and decreasing to 3 folds at 3-12 months, and gradually decline to normal adult levels from 8 years of age - reflecting the programmed biologic behaviour of IH, led to the discovery of the involvement the RAS in IH. The novel findings of the stem cell origin of IH and the involvement the RAS, underscore the accelerated involution of this tumour induced by β-blockers and ACE inhibitors. This work has led to the demonstration of the presence of ESC-like cells in fibrotic conditions such as keloid lesions and Duputren's contracture, and the identification and characterisation of cancer stem cells in many cancer types. The therapeutic implications of the expression of the RAS and enzymes that constitute bypass loops of the RAS by these primitive populations will be discussed.

R23

Intelligently Engineered Skin

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Burn wounds that cover a large area of a patient's body are traumatic, life threatening events. The gold standard for treatment of large burns is autologous split thickness skin grafting. In patients with burn wounds covering more than 30% total body surface area, there is often not enough unburnt skin available to cover all of the burn wound area with skin graft primarily. In these instances the un-grafted wound sites are dressed and the patient is kept in hospital until the donor graft sites heal and can be harvested again. This is a painful process that can result in patients spending long periods in hospital. Autologous engineered skin grafts could provide an alternative to cover patient wounds, reducing the time to complete wound closure and decreasing hospital stay. A significant limitation of growing autologous skin grafts in the laboratory is the time required. We have developed innovations that reduce the time required for autologous skin graft production, while producing a more robust skin graft. These innovations include a method to increase cell yield from a patient's healthy skin sample and increased speed at which the skin cells grow. We have increased the strength and ease of handling of the skin grafts by incorporating a bioresorbable synthetic mesh, as well as developing a novel minimal manipulation method for skin graft growth. These innovations combine to produce a robust skin graft 100 times larger than the original patient sample in as little as 16 days. Translation of this research will significantly reduce the time to complete wound closure, reducing pain and recovery time for burn patients.

R24

The Role of *in vitro* Immune Response Assessment for Biomaterials

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This abstract pertains to a suggested oral presentation based on a literature review recently accepted for publication by the *Journal of Functional Biomaterials*[1].

Grafts are required to restore tissue integrity and function. However, current gold standard autografting techniques yield limited harvest, with high rates of complication. In the search for viable substitutes, the number of biomaterials being developed and studied has increased rapidly. To date, low clinical uptake has accompanied inherently high failure rates, with immune rejection a specific and common end result. Our review article recently accepted for publication by the *Journal of Functional Biomaterials* examined published immune assays evaluating biomaterials, to stress the value that incorporating immune assessment carries. Immunogenicity assays have had three areas of focus; cell viability, maturation and activation, with the latter being the focus in the majority of the literature due to its relevance to functional outcomes. With recent studies suggesting poor correlation between current *in vitro* and *in vivo* testing of biomaterials, *in vitro* immune response assays may be more relevant and enhance ability in predicting acceptance prior to *in vivo* application. Uptake of *in vitro* immune response assessment will allow for substantial reductions in experimental time and resources, including unnecessary and unethical animal use, with a simultaneous decrease in inappropriate biomaterials reaching clinic. This improvement in bench to bedside safety is paramount to reduce patient harm.

1. Lock, A. M., Cornish, J., Musson, D. S. (2019) *The Role of in vitro Immune Response Assessment for Biomaterials* (accepted for publication by the *Journal of Functional Biomaterials* July 2019)

R25

Preptin knockout mice have increased bone volume without overt metabolic changes

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Many hormones that regulate whole-body glucose homeostasis also influence bone homeostasis. Preptin, a 34 amino acid peptide derived from the *Igf2* gene, is co-secreted with insulin from pancreatic β-cells and can increase glucose-stimulated insulin secretion[1]. Preptin also has direct effects on bone cells, promoting proliferation and differentiation of isolated osteoblasts[2]. However, preptin's function *in vivo* is unknown. The goal of this study was to determine the effect of preptin deficiency on bone by evaluating the phenotype of a preptin knockout (KO) mouse.

Experimental KO and wild type (WT) mice were generated by heterozygous breeders. RT-qPCR on livers from 12-15-week old mice (n=4-9) confirmed *Igf2* expression was similar between genotypes, whereas preptin expression was undetectable in KO mice. Bone phenotypes were evaluated by whole-body DEXA and femoral microCT at 14-weeks (n=10-12/sex/genotype), and 1-year of age (n=7/genotype, males only). To characterise the metabolic phenotype, a separate cohort underwent weekly fasting blood glucose measurements between 6-28-weeks of age, and intraperitoneal insulin tolerance tests (ipITT) at 9-weeks of age (n=12-14/sex/genotype). Analyses were performed using two-way ANOVA, with sex and genotype as factors.

Bodyweight, body fat, and total body bone mineral density were not different between genotypes at 14-weeks of age, nor was fasting blood glucose at 6-28-weeks of age. Glucose AUC during ipITT were not different between genotypes. At both 14-weeks and 1-year of age, trabecular bone of femurs had significantly increased bone volume fraction (BV/TV; 21% and 47% increase, respectively), and trabecular number (Tb.N; 17% and 45% increase, respectively) in male KO mice compared to WT mice. These effects were absent in female mice. Cortical bone parameters were unchanged.

Our data indicate that preptin deficiency promotes increased trabecular bone mass in male mice, even in the absence of an overt metabolic phenotype.

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R26

Adipose Stem Cells Versus Cardiac Progenitor Cells: The Better Candidate for Cardiovascular Disease

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Cardiovascular disease (CVD) continues to be a leading cause of death globally. Ischaemic heart disease (IHD) is characterised by insufficient cardiac blood supply. Current pharmacological treatments for IHD delay disease progression but cannot reverse damage to the heart[1]. Stem cell therapy may halt IHD progression and reverse damage caused. One challenge for stem cell therapy is identifying the best cell to use. Two potential candidates are cardiac progenitor cells (CPCs) and adipose stem cells (ASCs)[2,3]. Both types have shown to secrete paracrine factors promoting cardiac repair[4,5]. Hence, we carried out a series of experiments comparing ASCs and CPCs collected from the same individuals.

ASCs and CPCs were isolated using explant culture. An important characteristic of stem cells is the ability to migrate to the ischaemic area, proliferate and secrete therapeutic paracrine factors. To compare migration, a scratch assay was performed with images taken every six-hours for 24 hours. Next, cells were exposed to ischaemic conditions by culturing in serum-free normoxia and hypoxia for 72 hours. A CyQuant proliferation assay was used to compare proliferation between cell types in normoxia and hypoxia. Following the culture period, conditioned media was collected and IGF-1 and VEGF-A concentrations measured using commercial ELISA kits. RNA lysates were also collected and will be analysed using reverse transcription PCR for genes HIF1A, AKT1, FGF2 and PDGFA.

Preliminary results showed better migration potential with CPCs ($P<0.05$ vs. ASCs) while ASCs showed higher proliferation in hypoxia (3.351±0.841 mean fold increase in cell number in ASCs vs. 1.848±0.2779 in CPCs, n=5). No significant difference was found between cell types with VEGF-A and IGF-1 secretion. In conclusion, we established a culture for CPCs and ASCs and found CPCs migrated better than ASCs while ASCs showed higher proliferation in hypoxia. However, more experiments are required to identify further differences between cell types.

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R27

Gene and protein expression of endogenous serine proteases and protease inhibitors in alveolar epithelial cells

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The amiloride-sensitive epithelial sodium channel (ENaC) contributes to controlling extracellular fluid ion composition, lung alveolar clearance and airway mucociliary clearance. The proteolytic effect of endogenous serine proteases on the activation of ENaC in many epithelia by cleavage of ENaC's subunits, specifically α and γ, has been indicated [1, 2].
We have previously established that the human cell line NCI-H441 is an appropriate model for the study of ion transport in the alveolar epithelium [3] .

In this study, the gene and protein expression of endogenous serine proteases Channel Activating Proteases 1,2 and 3 (CAP1, CAP2, CAP3) and serine protease inhibitors Alpha 1 Anti Trypsin (A1AT), Serine Proteinase Inhibitor clade B (SERPINB12) and Short Palate, Lung, Nasal Epithelium clone 1 (SPLUNC1) were studied in the NCI-H441 cell line under control and simulated lung injury conditions using oleic acid treatment. Cells were cultured at air-liquid interface condition for 9 days, and barrier function and active ion transport were monitored by measuring the trans-epithelial electrical resistance and potential difference. For simulating lung injury, cells were treated with oleic acid (350uM, 600uM) in the basolateral chamber for 24 hours. Electrical resistance and potential difference were measured before and after treatment and then RNA and protein were extracted for analyzing gene and protein expression by qPCR and western blotting.
A significant decrease in TEER and TEPD values of monolayer under treatment conditions by oleic acid have been indicated. QPCR technique showed that all genes except SERPINB12 were detected under normal and treatment condition. CAP1 among proteases and A1AT among protease inhibitors were more abundant. In addition, in protein level, measured by western blotting technique, there was not a significate alteration under control and oleic acid treatment for each protease and protease inhibitors.

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R28

Non-invasive imaging of pericardium using micro-CT

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Valve replacement surgery would be revolutionised by a tissue-engineered heart valve that can grow, repair, and remodel, once implanted. Decellularisation technology may help to achieve this goal by making the donor tissue immunocompatibile. However, decellularisation protocols can have detrimental effects on the extracellular matrix microarchitecture. Thus, architectural stability must be confirmed experimentally during the design process. Unfortunately, the laboratory techniques required to perform histological and biochemical measurements are time-consuming and they result in the irreversible destruction of the tissues undergoing examination.

Micro-computed tomography (micro-CT) presents a non-invasive method of imaging datasets in three-dimension. We present a protocol for imaging pericardium using the heavy metal stain phosphotungstic acid. Bovine pericardium was obtained from a local abattoir and then dehydrated with graded ethanol solutions. The tissue samples were fixed in 3% paraformaldehyde for 48 hours and then immersed in either 0.3% phosphotungstic acid or 5% potassium iodide for 48 hours. Micro-CT images were obtained at 1 μm resolution and then reconstructed using software developed by Bruker.

Preliminary results suggest that micro-CT may be a useful method of non-invasively imaging soft tissues. 0.3% phosphotungstic acid produces good contrast for imaging pericardium and permits quantification of parameters such as fibre angle orientation. By comparing our data with those obtained using other imaging tools, some of the benefits and drawbacks of micro-CT-use are discussed.

R29

Efficacy and safety of alpha lipoic acid-capped silver nanoparticles for oral application

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Oral biomaterials can fail as a consequence of microbial infection. Silver nanoparticles (AgNPs) are a potential antimicrobial agent that can be used in conjunction with such biomaterials to reduce microbial ingress. In this study, we evaluated the safety and the efficacy of alpha lipoic AgNPs as an antimicrobial agent for oral application. AgNPs were synthesised and characterised by inductively coupled plasma mass spectroscopy, transmission electron microscopy and dynamic light scattering analysis. Antibacterial activity, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC), was determined against six key oral pathogens. Cell viability and apoptosis assays were conducted on primary human gingival fibroblasts (HGF) at 4, 24, 48, 72 and 96 hr and the effect of AgNPs was compared to the effect of commercial products. The expression profile of genes associated with cellular stress and cell cycle were investigated using qRT2-PCR. AgNPs were spherical and ranged in size between 1-12 nm. The MICs for AgNPs ranged between 2.5 (*Escherichia coli*) to 12.5 □g/ml (*Staphylococcus aureus*); while MBC values were between 5 (*E. coli*) and 100 □g/ml (*Streptococcus mutans*). HGF viability was significantly reduced at doses >22.5 □g/ml whilst lower doses significantly increased the cell viability at the 24 and 72 hrs time points. Caspase 3/7 was not induced by AgNPs in this study. HGF treatment with AgNPs (0.225 □g/ml) produced an upregulation of TP53 (p=0.0058), PARP1 (p=0.026), SOD2 (p=0.051), and CDK1 (p=0.093) genes at 4 hrs only. In conclusion alpha lipoic acid capped AgNPs were found to be stable and have antimicrobial activity on key oral pathogens with a cytotoxicity profile similar or better than current products. Gene regulation showed initial oxidative stress on gingival fibroblasts which was corrected over time. This study indicated that AgNPs could be safely incorporated into oral biomaterials to improve their antibacterial properties without inducing cytotoxic effects.

R30

Investigating the structural assembly of keratins in biological materials using electron microscopy

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Biomaterials research includes the development of scaffold systems that mimic the structure and function of native tissues or organs. Proteins such as collagen have been heavily investigated as scaffold substrates, but keratin has received less attention. Keratins are insoluble proteins that form ordered arrays of intermediate filaments. These arrays are interconnected by various inter-filament mechanisms to form the bulk of cytoplasmic epithelia and epidermal appendages such as hair and wool. Currently available keratin-based materials are intrinsically biocompatible, biodegradable and mechanically durable, but lack mechanical strength and are mixed with other natural or synthetic materials to enhance mechanical strengthening (1,2). Understanding assembly of hair is a good place to start for creating future materials. A hair follicle is a complex miniature organ where multiple cell lines undergo differentiation to produce a hair shaft comprising 95% keratin. Despite being a linear developmental system, how these keratins self-assemble to form a keratinised hair shaft is poorly understood. The key to developing revolutionary keratin biomaterials is to biomimetically harness the self-assembly of the different structural formats in hair. A current barrier is an inability to observe keratin assembling inside cells without disrupting structure. This could be overcome using high-resolution transmission electron microscopy and tomography. Existing studies of follicles all rely on chemically processed samples. Chemically-processing procedures may distort the delicate keratin structures and surrounding cells. We found that a different approach, high-pressure freezing and freeze-substitution, preserves the subcellular structures close to the native state and therefore forms an essential tool in efforts to investigate the ultrastructural aspects and three-dimensional architecture of the keratin structure self-assembly.

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R31

Limitations of *in vivo* bioluminescent imaging of S. aureus Xen36 biofilm-based Infection

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Bioluminescent bacteria can play a powerful role in animal models of infection, allowing realtime, semiquantitative tracking of infection in vivo in order to pinpoint the appropriate timing for CFU data enumeration. While contemporary versions such as *S. aureus* Xen36 hold their plasmids reliably and can be used as proportionate measurements of metabolic activity, it is critical to understand the limitations of in-vivo bioluminescence imaging in the biofilm phenotype. In a recently validated model of rat knee prosthetic joint infection, we have demonstrated that bioluminescence does not reliably penetrate cortical bone or periprosthetic joint infection, and can be confounded by extraneous sinus or peripheral tissue infection.

R32

Two-step ruthenium-catalysed bioprinting of low-viscous cell-instructive bioinks as a platform for tissue-specific bioink development

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Extrusion-based 3D bioprinting (3DP) has become a pivotal tool in tissue engineering for patterning of cell-containing hydrogel materials (bioinks) into physiologically relevant architecture\s. However, designing cell-supportive bioinks remain a major challenge in 3DP, as printable formulations typically consists of dense polymer networks (i.e. stiff constructs) - restricting functional tissue formation. Thus, the aim of this study is to print soft, cell-instructive bioinks, utilising a sequential dual-step crosslinking approach.
We have developed a cutting-edge polymerisation strategy to print low-viscous/soft bioinks by controlling the degree of polymer crosslinking through progressive steps: 1) partial pre-printing crosslinking for increased bioink viscosity, and 2) subsequent post-printing crosslinking to provide construct shape stability. We implemented a ruthenium (Ru) and sodium persulfate (SPS)-based initiating system [1] to initiate a click reaction between thiolated crosslinkers, and allyl moieties grafted onto gelatin (Gel-AGE) [2], thus driving crosslinking. Time-dependent sol-gel transition was observed in Gel-AGE bioinks in the absence of light, resulting in partial crosslinking (2-20%) and a viscosity increase. Partial crosslinking was mediated through a thiol-persulfate redox reaction, of which the kinetics could be controlled through increasing Ru, SPS and thiol concentrations, as well as crosslinker size. Partial crosslinking successfully enhanced viscosity of 3wt% Gel-AGE bioink (from 0.004 to 3.4 Pa·s), allowing shear thinning behaviour followed by mechanical recovery of extruded filaments - achieving high construct shape fidelity. Furthermore, the method still allowed complete, post-printing, photo-initiated crosslinking, achieving soft mechanical properties (10.6±0.6 – 27.8±6.9 kPa) that were tailorable through modifying the thiol concentration. Finally, printed constructs with encapsulated human articular chondrocytes (5 million cells/mL) displayed high cell viability of over 7 days of culture (day 1: 81.4±7.6%, day 7: 85.5±4.3%). In conclusion, Ru-catalysed dual-step crosslinking allowed high shape fidelity, cytocompatible bioprinting of low-viscous Gel-AGE bioinks – providing a facile method for bioprinting complex architectures whilst allowing designing of cell-instructive soft bioinks.

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R33

High fidelity bioprinting of nanocomposite bioinks supporting osteogenic and angiogenic tissue formation

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3D bioprinting is an attractive approach to engineer tissue equivalents for musculoskeletal regeneration. However, insufficient functionality of available cell-laden hydrogels (bioinks) has impeded the clinical translation of this technology. Advanced bioinks for skeletal application should: i) Display high print fidelity, ii) promote cell viability and bone-specific differentiation and iii) encourage vascularisation and tissue integration upon implantation [1].

To address these complex requirements we developed a multifunctional nanocomposite bioink (LPN-GelMA) comprising Laponite nanoclay (LPN), gelatin methacryloyl (GelMA) and the visible-light Ru/SPS crosslinking system [2,3]. Multiple blends were characterised to identify the ideal bioink composition based on rheological and mechanical properties, crosslinking kinetics and print fidelity. Human mesenchymal stromal cells (hMSCs) were encapsulated within LPN-GelMA constructs to assess cell viability and osteogenic differentiation over 21 days of *in vitro* culture. LPN-GelMA constructs were loaded with vascular-endothelial growth factor (VEGF) and implanted into a chick chorioallantoic membrane (CAM) model to investigate the capacity to stimulate blood vessel ingrowth and construct integration.

A bioink composition of 1 wt% LPN and 7.5 wt% GelMA was found to significantly enhance the biofabrication window for successful extrusion-printing of constructs with high shape fidelity. LPN addition did not significantly affect the hydrogel stiffness, which promoted high cell viability and osteogenic differentiation, evidenced by bone matrix formation after 21 days. Furthermore, CAM studies demonstrated that VEGF infused LPN-GelMA composite could stimulate angiogenesis with excellent integration across the CAM membrane.

This study demonstrates the unique potential of multi-functional LPN-GelMA nanocomposite bioink to enhance print fidelity, support cell viability and differentiation, as well as facilitate growth factor localisation. LPN-GelMA bioinks offer new possibilities for musculoskeletal regeneration.

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R34

The effects of different buffers on the physico-mechanical properties of gelatin-methacryloyl (GelMA) hydrogels

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Bioprinting is an emerging field that employs hydrogels (bioinks) containing cells to fabricate functional biological structures. The design of bioinks is a complicated process, as they need to be customised for specific cells of interest. Cells require particular physiological factors to remain viable, such as temperature, pH, osmolarity, the types of ions required by the cell, and the resulting mechanical properties of the construct. For example, cardiac myocytes isolated from rat hearts require a physiological buffer solution in order to function properly, the most commonly used being Tyrode's solution. This buffer is considerably different from both water and phosphate-buffered solution (PBS), which are typically used to reconstitute dry macromere hydrogel precursor, and photoinitiators used in the cross-linking process. Furthermore, the resulting construct must also have the right physico-mechanical properties, as myocytes are mechano-biologically responsive. To optimise myocyte function we aim to produce hydrogels with a Young's modulus of 10 kPa to 20 kPa, the same stiffness as native non-contracting heart tissue[1]. In this work, we explored the effect of three buffer solution formulations on the properties of GelMA based hydrogels. In particular, we examined the effects of a time delay between mixing the printing solution and photo-initiated cross-linking of the solution on gel properties. When using a visible light cross-linking system (Ru/SPS)[2], an increase in time delay caused a reduction in the cross-linking efficiency. When using Tyrode's solution, the amount of polymer in the network was lower compared with gels made with water and PBS, and when immediately cross-linked the Young's modulus was (13.0 ± 2.5) kPa, which is within the range required. However, this value decreased with increasing time delay. When using a Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) photo-initiating system the same time-dependent reduction in cross-linking density was not present, and a time delay did not appear to impact the resulting stiffness of the gels for each buffer. This suggests that the time-dependency seen with Ru/SPS is due to the photo-initiating system itself. An increase in the time delay reduced the amount of polymer cross-linked when using the Tyrode's solution for both initiators, suggesting that Tyrode's solution has an effect on the ability to cross-link GelMA. The Ru/SPS system may be more appropriate to use with myocytes as the stiffness for gels made with LAP was much higher (30.5 kPa to 44.5 kPa) than the required 10 kPa to 20 kPa. The pathway for creating and adapting bioinks is complex, with cells requiring specific conditions. In the case presented here, immediate curing of the gels resulted in hydrogels with desirable mechanical properties, while using a cell-friendly buffer.

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R35

Development and *in vitro* biocompatibility of 3D printed scaffolds for intervertebral disc tissue engineering

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Intervertebral disc (IVD) degeneration is a major cause of lower back pain occurring in 97 % of the population over 50 years old.[1] Although some surgical techniques are available to treat IVD degeneration including discectomy and spinal fusion, these often cause adjacent IVD degeneration, implant failure and inflammation.[2] An alternative treatment strategy is transplantation, however, it is extremely difficult to obtain healthy donors. To address this limitation, we aim to develop 3D printed scaffolds combining suitable biomaterials, and to investigate their biocompatibility with human, bone-marrow-derived mesenchymal stem cells (hBMSCs).

Five combinations of biomaterial blends consisting of poly-caprolactone (PCL), chitosan and tri-calcium phosphate (TCP) were placed in a heatable cartridge of the GeSiM Bioscaffolder 3.1 with numerous temperatures tested in order to improve their pneumatic, melt extrusion behaviour. Then, printing parameters as well as processing parameters were optimised for each blend until they formed layer-by layer 3D scaffolds with regular pores, and straight continuous uniform strands. Once scaffolds were fabricated with optimised parameters, they were visually investigated for inter-scaffold variation, and the *in vitro* biocompatibility investigated with immortalised hBMSCs using a cell proliferation assay on day 7.

Whilst all five blends generated scaffolds with minimal printing difficulties, as the ratio of TCP increased, the nozzle became more clogged. A one way ANOVA showed no statistically significant difference in biocompatibility between the cells only control and all the scaffold blends seeded with hBMSCs. From these results blend containing 20% chitosan was chosen as optimal.

In conclusion, various biomaterial blends 3D printed with precise architecture have been developed and shown to be biocompatible with hBMSCs. These biocompatible 3D scaffolds may potentially be useful not only for IVD tissue engineering, but also for articular cartilage and bone tissue engineering with the added benefit of custom design by the seeding of autologous BMSCs.

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R36

Developing CRISPR/Cas9 gene-editing methods to generate patient-specific, gene-edited skin substitutes for patients with Epidermolysis bullosa

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Epidermolysis bullosa (EB) is a rare but severe genetic skin disorder. EB causes fragile skin characterised by blistering through touch or friction and can be fatal. To date, there is no treatment available for patients with EB other than palliative and wound care. Our aim is to correct the EB causing mutations using CRISPR/Cas9 gene-editing in patient-derived skin cells. These will then be used to grow corrected 3D skin substitutes. In brief, we will take a small sample of patient skin, expand the isolated skin cells *in vitro* and use CRISPR/Cas9 genome-editing to repair the disease-causing mutation. The corrected cells will be used to grow sheets of skin *in vitro*, which could be used to permanently cover/treat the chronic wounds and blisters of EB patients.

We are currently developing CRISPR/Cas9 gene-editing methods to efficiently correct primary keratinocytes, the main type of skin cells affected in EB. Through optimisation of CRISPR/Cas9 methods in healthy, primary keratinocytes, we have achieved up to 90% non-homologous end joining (NHEJ)-mediated repair efficiencies. Following this we are now developing methods for CRISPR/Cas9-mediated homology directed repair (HDR), which will be required to make specific edits in the gene of interest, in healthy, primary keratinocytes as well as in EB-patient-derived keratinocytes.

If successful our methods could provide an *ex vivo*, non-viral gene therapy as a new, permanent treatment option for patients suffering from EB.

R37

Identification of stem cell biomarkers using deer antler as a model of mammalian organ regeneration

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As the only known mammalian organ that can fully and annually regenerate, deer antler has significant advantages over lower-order animal models. Antler regeneration is known to be maintained and controlled by stem cells in different states of activation, however, the underlying regenerative mechanisms are not fully understood. Pleiotrophin (PTN) was previously found to be highly up-regulated using suppression-subtraction hybridisation technology. The aims of this research were to identify the stem cells within the antler, investigate the pleiotrophin family of proteins and their significance during regeneration, and identify new proteins involved in stem cell regulation using proteomics**.** The mesenchymal stem cell (MSC) markers CD73, CD90 and CD105, along with PTN and its receptors, were examined within the antler using immunohistochemistry. Label-free mass spectrometry was used to detect the protein expression profiles of antler stem cell tissues. The tissues included: dormant pedicle periosteum (DPP), growth centre (GC), post-active stem cells from mid-beam periosteum (MAP), and deer facial periosteum (FP) as a control (N=3, biological replication). PEAKS and Ingenuity Pathway Analysis software were used to analyse the proteomics data. Our research confirmed the central role of stem cells in the development of this mammalian organ by localising the MSC markers within the antler. The high expression levels of PTN and its key receptors within the growth centre indicated its importance during rapid antler regeneration. Label-free proteomics identified unique markers of dormant (6), active (87) and post-active (3) antler stem cells. Activation of antler stem cells was associated with up-regulation of a number of canonical pathways and molecular/cellular functions such as PI3K/AKT signalling. This work identifies biomarkers as well as activation and control proteins associated with stem cell regulation during bone formation, and provides further research directions in the field of mammal organ regeneration.

R38

Wound healing biomaterials as cell and drug delivery vehicles

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Cells live in an intricate and dynamic extracellular matrix (ECM) where they intermingle with a host of biophysical and biochemical signals that guide their function, regulation, and wound repair. Numerous efforts have been made to engineer biomaterials, based on both natural and synthetic polymers, in order to simulate the ECM microenvironment, in order to facilitate the wound healing process. Herein, we present an overview of developed and ongoing biomaterials and 3D biofabrication projects with the primary goal of building functional, clinically relevant, wound-healing regenerative tissues of the future. These include the development of a commercialized, chitosan/dextran (CD)-based post-surgical, wound healing, ear, nose and throat (ENT) hydrogel (Chitogel™) (Cabral, Roxburgh et al. 2014, Aziz, Cabral et al. 2015, Cabral, McConnell et al. 2015), a peptide functionalized CD hydrogel as a stem cell delivery vehicle (Nelson 2017) and bio-ink for 3D bioprinting using novel core/shell technology (Turner 2019), and a dual cytokine (Wise, Bodaan et al. 2018) release cubosome/alginate gel dressing. Our studies indicate that in addition to serving as a successful, FDA-approved, ENT post-surgical aid, the CD hydrogel was found to serve as an appropriate adult stem cell delivery vehicle; and when functionalized with bioactive peptides, optimized as a core bioink to co-axially 3D bioprint regenerative, vascularized scaffolds. In addition, an *in vitro* dual release growth factor cubosome/alginate hydrogel dressing was developed and shown in an *in vivo* small animal pilot study to be adhesive, absorb wound exudate, and promote healing. In conclusion, a variety of biomaterial constructs have been developed and found to be appropriate cell and drug delivery vehicles for potential wound healing applications.

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