# QRW Programme

## Stem Cells & Regenerative Medicine Satellite Day 1

## RM1: Translating skeletal stem cells and environmental niches for bone regeneration: from bench to clinic

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Medical advances have led to a welcome increase in world population demographics. However, increased aging populations pose new challenges and emphasize the need for innovative approaches to augment and repair tissue lost through trauma or disease.

We have developed protocols for the isolation, expansion and translational application of skeletal stem cell populations with cues from developmental biology informed by in vitro and ex vivo models as well as, nanotopography and nanoscale architecture and biomimetic niche development informing our skeletal tissue engineering approaches. Furthermore, we have developed ex vivo approaches to skeletal tissue formation evaluation and analysis and, central to clinical application, large animal in vivo translational studies to examine the efficacy of skeletal stem and cell populations in innovative scaffold compositions for orthopaedics. The talk will also highlight current clinical translational studies to examine the efficacy of skeletal populations for orthopaedic application.

Advances in our understanding of skeletal stem cells and their role in bone development and repair, offer the potential to open new frontiers across the hard tissue interface and offer exciting opportunities to improve the quality of life of many.

Acknowledgements: Funding from the BBSRC, MRC and EU FP7 (Skelgen and Biodesign) is gratefully acknowledged.

## RM2: Mimicking developmental drivers of osteogenic commitment in perivascular stem cells for bone regeneration

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The effective use of adult mesenchymal or perivascular stem cells (PSCs) in regenerative medicine and drug screening depends on our ability to effectively direct their differentiation into desired tissue cell types. Exquisite control over PSC fate is needed to efficiently produce sufficient, defined cell populations for such applications, yet this is substantially hindered in many cases by undefined culture components, signal crosstalk between multiple exogenous and endogenous (secreted) factors, and spatiotemporal variations in microenvironmental composition inherent to conventional culture formats. With these challenges in mind, we have sort to develop a range of biomaterial substrates, scaffolds and microdevices aimed at providing defined culture environments for PSC expansion and differentiation. We have developed a number of tailored substrates and scaffolds that enable one to probe the impacts of substrate viscoelasticity, ligand type, ligand lateral spacing and inorganic nanoparticle encapsulation on PSC fate choice, exemplifying that deterministic control over PSC behaviours is possible. These surfaces and scaffolds have more recently been tuned to screen combinations of peptides from a range of ECM molecules and CAMs known to exist during mesenchymal condensation through to osteo-chondral tissue specification. Soluble factors (whether provided exogenously or secreted by neighbouring cells) are also vital contributors to PSC fate choice. Our scalable, valveless, perfused microbioreactor arrays (MBAs) can provide to cells a full-factorial sets of exogenous factor compositions and in addition, allow for controlled accumulation of paracrine factors. These MBAs have been used to survey up to 8100 individual perfused cellular microenvironments in parallel. Through screens of stem cell potency maintenance and differentiation, we demonstrate the unique ability of this platform to separate, visualise, identify and modulate paracrine effects that are not otherwise readily accessible to standard in vitro culture. We have applied this MBA platform, that has been adapted to include 3D scaffolds and ECM coatings, to the optimisation of both osteogenic and myogenic differentiation outcomes from PSC starting points. Our multiplexed MBAs have enabled us to decipher factor interplay and signalling hierarchies that control PSC fate, and are applicable as a universal microenvironmental screening platform for bioprocess optimisation, media formulation design, quality control for cellular therapeutics, cell-based drug toxicity and stratification, and biomaterials discovery.

## RM3: Translational Regenerative Medicine in Articular Cartilage Repair

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Arthroscopic surgery revolutionized equine orthopaedics as it did human orthopaedics. However, limitations were recognized including acute articular cartilage loss and chronic disease leading to osteoarthritis. A desire to validate the technique of microfracture to promote articular cartilage repair in humans led to the development of our first model of articular cartilage in horses simulating femorotibial lesions in humans.[1] Various equine models have been developed from our group and the group at Cornell and reviewed.2 Initially the model was used in a 12 month study to show significant increase in the amount of repair tissue with microfracture as well as a significant increase in type II collagen content. Other studies showed that there was significant upregulation of type II collagen expression at 8 weeks and that complete removal of the calcified cartilage was critical for optimal repair. Studies on further manipulation of endogenous healing of microfractured defects showed usefulness with intraarticular adenoviral based gene therapy with interleukin-1 receptor antagonist (IL-1ra) and insulin like growth factor-1 (IGF-1) bone marrow-derived stem cells (BMSCs) administered intraarticularly.[1] Further studies in the horse have shown implantation of minced cartilage without cell culture as an effective intraoperative treatment to enhance cartilage repair.[3] This study has shown translational success in humans and led to demonstration of efficacy in human clinical cases. Studies with direct implantation of BMSCs in fibrin as well as a fibrin platelet rich plasma (PRP) have shown no significant improvement compared to fibrin alone and inferior improvement compared to fibrin/PRP respectively. Evaluation of articular cartilage progenitor cells for the repair of equine articular defects has shown significant improvement with autologous derived cells but allogeneic derived cells were inferior to autologous and similar to fibrin.[4] A clinical study in horses has shown significant improvement in meniscal repair and full-thickness articular cartilage erosion in horses.[5]

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2. McIlwraith, C.W., Fortier, L.A., Frisbie, D.D., and Nixon, A.J. (2011). *Equine models of articular cartilage repair*. Cartilage 2:317-326.

3. Frisbie, D.D., Lu, Y., Kawcak, C.E., DiCarlo, E.F., Binette, F., and McIlwraith, C.W. (2009). *In vivo evaluation of autogous cartilage fragment-loaded scaffolds implanted into equine articular defects and compared with autologous chondrocyte implantation.* Am J Sports Med 37:71S-80S.

4. Frisbie, D.D., McCarthy, H.E., Archer, C.W., Barrett, M.F., and McIlwraith, C.W. (2015). *Evaluation of articular cartilage progenitor cells for the repair of articular defects in an equine model.* Bone Joint Surg Am 97:484-493.

5. Ferris, D.J., Frisbie, D.D., Kisiday, J.D., McIlwraith, C.W., Hague, B.A., Major, M.D., Schneider, R.K., Zubrod, C.J. Kawcak, C.E., and Goodrich, L.R. (2014). *Clinical outcome after intraarticular administration of bone marrow derived mesenchymal stem cells in 33 horses with stifle injury*. Vet Surg 43:255-2

# RM4: 3D BioPrinting and BioAssembly of Tissue Organoids for Regeneration of Cartilage and Bone

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Additive Manufacturing (AM) has opened new frontiers in medicine and patient-specific design of medical devices [1]. AM allows the direct layer-by-layer fabrication of implants with porous tissue-ingrowth surfaces, and has made particular impact in development of custom titanium implants for complex revision surgery requiring large bone void filling. However, the 'holy grail' in future orthopaedic surgery is the successful implementation of strategies that regenerate rather than replace damaged or diseased joint tissues such as bone and cartilage. Advances in biofabrication technologies (e.g. 3D Bio-printing, Bio-assembly) enable the generation of engineered constructs that replicate the complex organization of native tissues via automated placement of cell-laden bio-inks, tissue modules, growth factors and/or bioactive agents [2, 3]. The greatest challenge in successful bio-printing for translational regenerative medicine is not hardware development but in improved biomaterials and bio-ink development for stem cell deliver within an appropriate niche and high spatial resolution. Hydrogels are commonly investigated as bio-inks as they provide a hydrated 3D environment for stem cell encapsulation that mimic features of native extracellular matrix including growth factor binding, adhesion motifs and degradability [3].

We describe development of novel bioinks (gelatin methacryloyl, gelMA)) using visible light crosslinking for bioprinting of human mesenchymal stromal cells (MSCs) with enhanced viability, metabolic activity and chondrogenic differentiation capacity. We also report on a synthetic 'bio-resin' (methacrylated poly(vinyl alcohol)) for encapsulation of chondroprogenitor cells using light projection stereolithography as alternative approaches to achieve high print fidelity for biofabrication of complex 3D tissues. Furthermore, alternative bottom-up or hybrid approaches combining automated bio-assembly of cellular microtissues or tissue organoids with 3D Bioprinting for regenerative medicine or 3D *in vitro* models for high throughput screening are introduced [4,5]. These approaches promote high density cell-cell interaction of co-cultured stem cells that mimic developmental stages of tissue growth while allowing biofabrication of complex constructs containing pre-differentiated microtissues [6].

Acknowledgements: Funding from the Royal Society of New Zealand Rutherford Discovery Fellowship, MBIE and EU/FP7 'skelGEN' consortium under grant agreement n° [318553] is gratefully acknowledged.

1. Murr LE, *et al.* Next Generation Orthopaedic Implants by Additive Manufacturing Using Electron Beam Melting. *International Journal of Biomaterials* 2012; 2012: 14.

2. Woodfield, T.B.F., *et al.* Rapid prototyping of anatomically shaped, tissue-engineered implants for restoring congruent articulating surfaces in small joints. *Cell Proliferation,* 2009. 42(4): 485-497.

3. Malda, J., *et al.* 25th Anniversary Article: Engineering Hydrogels for Biofabrication. *Advanced Materials,* 2013. 25(36).

4. Schon BS, Schrobback K, van der Ven M, Stroebel S, Hooper GJ, Woodfield TBF. Validation of a high-throughput microtissue fabrication process for 3D assembly of tissue engineered cartilage constructs. *Cell Tissue Res* 2012; 347(3).

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## RM5: Biological strategies for promoting bone healing for translational regenerative medicine

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Biological assessment of scaffolds/implants to augment healing can guide the success of a medical device in early stages of development. Despite the growing biomaterial/scaffold market, and the readiness of clinicians to take on new technologies, to date nothing has proven to be as effective as autologous tissue grafting. Thus, more work is needed to create scaffolds that enhance tissue repair.

Our group has developed a comprehensive *in vitro* evaluation system in cell and molecular skeletal biology for biomaterial scaffolds, covering immunogenicity and host cell growth, differentiation and matrix production. The suitability of implants for bone regeneration depends on the device being tolerated immunologically and has no foreign body response in the host tissue. The bioactive material must enhance the body's innate regenerative capacity which depends on the scaffold being able to maintain the host cell phenotype. Bioactive growth factors can be incorporated into the device to enhance tissue repair and a controlled release of these factors that can be evaluated *in vitro*. If the bioactive implant appears suitable *in vitro*, then pre-clinical *in vivo* evaluations are carried out, overseen and carried out by orthopaedic surgeons.

The scaffolds are provided to us by academic and commercial researchers and are designed to enhance the regenerative capacity of large bony defects and difficult to heal tendon-bone injuries. We have evaluated both natural and synthetic materials designed for use as stand-alone scaffolds, or as delivery systems for growth factors targeted to the tissue of interest.

## RM6: The Effect of a Novel HDACi on Osteogenic and Adipogenic Differentiation of Human Adipose Derived Stem Cells

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Controlling lineage specific differentiation of stem cells is crucial for functional tissue engineering. Histone deacetylase (HDAC) proteins play a key role in defining the epigenetic program during stem cell differentiation. A better understanding of the epigenetic mechanism governing osteogenic differentiation of human adipose-derived stromal cells (hADSCs) would provide new insights into the potential modulation of hADSC-based therapy and so improve bone tissue engineering. Our studies show that MI192, a novel HDAC3-selective inhibitor, caused hADSC death at higher concentrations (>30μM), and inhibits the proliferation at lower concentrations. After 2 days of pre-treatment with MI192, hADSC's HDAC activity were significantly reduced and the osteogenic differentiation of hADSC was markedly enhanced compared with cells without pre-treatment. The effect of MI192 on hADSC osteogenic differentiation was dose dependent, confirmed by alkaline phosphatase (ALP) staining. The MI192 optimal concentration and pre-treatment time for hADSC were 30 μM and 2 days respectively as confirmed by quantitative ALP specific activity assay. Real-time PCR analysis revealed that MI192 pre-treatment up-regulated hADSC Runx2, Col1 and OCN expression under osteogenic induction. In comparison, MI192 reduced adipogenic differentiation, leading to less lipid droplet formation compared to the control group. Flow cytometry data indicated that pre-treatment with MI192 (30 μM) for 2 days resulted hADSCs in G2/M arrest. Our results demonstrated that MI192 pre-treatment enhances osteogenic differentiation but inhibits adipogenic differentiation of hADSC, which is probably due to the inhibition of HDAC 3 and their subsequent influence on cell cycle, and could be useful for *in vivo* bone tissue engineering.

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## RM7: Biomimetic approaches toward tissue vascularisation

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Regeneration of healthy endothelium and the microvascular bed has proven a major obstacle in translating developments in biomaterials research to the clinic. Lack of endothelial regeneration is particularly detrimental in blood contacting devices, such vascular grafts and stents where an intact endothelial layer is essential for device function. Lack of timely and sufficient vascular ingrowth is also the rate-limiting step in replacing large tissue defects with engineered biomaterials and tissues. Analogues of a range of tissues have been developed *in vitro,* but the size of these constructs is generally limited to several hundred micrometers due to restrictions in nutrient, gas and waste exchange in larger constructs. To address this, we utilise silk biomaterials with tuneable physical properties as a platform to develop a range of biomaterial vascularisation strategies, including: 1) engineering of vascular-like hollow channels in 3D porous silk scaffolds, 2) *in vitro* pre-vascularisation of silk scaffolds with endothelial cells and 3) scaffold functionalisation with basement membrane components to mimic the vascular niche.

Vascular-like hollow channels played an essential role in enhancing cell infiltration and delivering oxygen and nutrients to the scaffold bulk, and promoted enhanced host tissue integration (cell infiltration & matrix deposition) and vascularisation *in vivo*. Combinatorial approaches involving hollow channels and *in vitro* pre-vascularisation further enhanced biomaterial vascularisation compared to any single strategy. Perlecan, an essential vascular proteoglycan, supported endothelial cell interactions and was explored as a biological cue for endothelial cell recruitment and silk scaffold vascularisation. The choice of immobilisation technique (adsorption vs covalent binding) and presence of glycosaminoglycan chains on perlecan affected its presentation on the biomaterial surface and modulated endothelial cell and platelet interactions. These studies demonstrate the utility of a number of physical and biological cues in fabricating biomimetic, cell-instructive environments for vascular bioengineering.

## RM8: Hybrid additive manufacturing systems for the production of 3D structures for osteochondral tissue regeneration

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Cartilage related diseases, like Osteoarthritis (OA), are rapidly increasing with tremendous individual, societal and economic impacts worldwide. Despite the research being conducted on OA, there are no effective prevention and treatment methods for this disease. Treatment strategies based on Tissue Engineering (TE) are currently under investigation, and with promising results. The initial approach comprises the development of implantable bioabsorbable systems, such as scaffolds or nanoparticles, loaded with pharmaceutical drugs capable of regenerating cartilage. Despite significant advances in materials science, tissue biology and production techniques, the application of these alternative therapeutic strategies proved to be insufficient.

Additive manufacturing processes (AM) represent a new group of non-conventional fabrication techniques recently introduced in the biomedical field. In TE, AM processes are used to produce scaffolds with customized external shape and predefined internal morphology, allowing good control over pore size and distribution. Accordingly, an innovative biomanufacturing system integrating three main modules was developed: 1) an advanced bioextrusion module combining extrusion with deposition of biomaterials, with or without hydrogels containing drugs; 2) electrospinning module for producing nano fiber layers (when necessary); 3) monitoring module, so that the scaffold manufacturing process is monitored in real time.

This new biomanufacturing system is being optimized to possess high reproducibility which allows better definition of the pores in the scaffold, in order to control both the mechanical strength and molecular diffusion. Due to its innovative internal architecture (100% interconnected), to the external geometry and flexible materials, the structures intended to be produced in this equipment will have the ability to promote a homogeneous cell colonization (by adjacent tissue cell recruitment), neovascularization, controlled drug release in predefined areas and tissue growth.

## RM9: Engineering cell-adhesive hydrogels for Tissue Engineering purposes

da Silva, L.P.[1,2], Marques, A.P.[1,2], Reis, R.L.[1,2], Correlo V.M.[1,2]

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The resemblances between soft tissues extracellular matrix (ECM), characterized by a viscoelastic polymeric network with high water content, and hydrogels has been sustaining its advance for Tissue Engineering and Regenerative Medicine purposes. However, hydrogels limitations, such as, mechanical instability and plasticity, as well as, lack of cell-adhesive properties [1] have been hampering major successes. The most followed but laborious and expensive strategy to overcome the absence of cell adhesion sites within hydrogels relies on binding cell adhesive sequences or blending ECM proteins with other polymers. Considering these constrains, we propose a simple method of processing Gellan Gum (GG) hydrogels with improved microstructure and mechanical performance, and more importantly, cell adhesive character.

Spongy-like hydrogels were obtained, following a patented methodology [2], from GG hydrogels and upon re-hydration of a freeze dried polymeric network by dropwise addition of PBS or a cellular suspension prepared in adequate culture medium. Freezing thermodynamics under different freezing temperature and time, as well as, after varying the solute amount and type, was analysed.

The microarchitecture of spongy-like hydrogels was dissimilar from hydrogels. The average pore size of spongy-like hydrogels was significantly higher than hydrogels, while its water retention capability was lower, nevertheless, presented equivalent compressive modulus. Moreover, spongy-like hydrogels, in contrast to hydrogels presented a great capacity of shape adaptability, a high degree of flexibility and a fast and complete recover after application of a compressive force. hASCs entrapped within the spongy-like hydrogels adhered to the pore walls and organized their cytoskeleton, exhibiting their typical adherent morphology, not observed in hydrogels. Furthermore, spongy-like hydrogels were able to support hASCs proliferation presenting a significantly low amount of non-viable cells after 7 days of culture.

1. Oliveira JT,at al., 2010, J Biomed Mater Res A, 93, 852.

2. da Silva LP et al WO2014167513 (2013).

## RM10: An Alternative Visible Light Initiating System for 3D BioPrinting in Tissue Engineering and Regenerative Medicine

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Photo-initiated radical polymerisation which is combining light and photo-initiators to generate radicals for crosslinking photo-polymerisable macromers, has been widely employed in 3D bioprinting of cell-laden hydrogel constructs. This technique is advantageous due to the spatial and temporal control over the polymerisation process, with fast curing rates as well as minimal heat generation for *in situ* crosslinking. However, most commonly used photo-initiators such as Irgacure®2959 (I2959) absorb in the UV region (300-400nm), which can potentially damage cellular DNA during the photo-encapsulation process. UV also has a limited penetration depth and light attenuation issues during *in vivo* photo-crosslinking of hydrogel constructs. In this study, we examine the feasibility of using a novel set of photo-initiators (VI) developed in our group, which absorb in the visible light region (400-450nm), for engineering cell-laden constructs using gelatine-methacryloyl (Gel-MA) as the base material.

We showed that hydrogels crosslinked using this developed VI has similar physico-mechanical properties as gels photo-polymerised using the conventional UV+I2959 system. However, cell encapsulation studies showed that human articular chondrocytes (HAC) encapsulated in the Vis+VI hydrogels had significantly higher cell viability as compared to the UV+I2959 samples after 21d in culture. The visible light photo-encapsulated HAC also yielded statistically greater metabolic activity throughout the 3 weeks culture period. A similar trend was observed for human mesenchymal stromal cells (hMSC) encapsulation, where the Vis+VI samples had considerably higher metabolic activity over a similar 21d culture period. As the physico-mechanical properties of gels fabricated using both systems are identical, it is hypothesised that the lower cell viability and metabolic activity observed in the UV+I2959 system might be due to the UV photo-toxicity.

We showed that the developed VI can be used to fabricate cell-laden Gel-MA hydrogel constructs with comparable physico-mechanical properties to the conventionally used UV+I2959 system, but with improved cell survival and metabolic activity.

## RM11: Understanding and exploiting structure-function relationships in naturally-derived biomaterials

Cameron Brown

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Nature abounds with interesting materials that can inspire the scientific community. An excellent example of this is spider silk. It is one of the toughest materials known, and is widely regarded as a 'super-fibre', yet it is produced at ambient temperatures using green chemistry and relatively weak bonding. We can find more examples in the musculoskeletal system. Collagen, while not necessarily exciting by itself, can use finely balanced hierarchical structuring to form tissues that perform far better than their constitutive parts would suggest. In this talk we will explore some of the mechanisms underpinning the impressive properties of these materials, with a focus on how we can use them to understand disease processes, or to develop new treatments and materials.

## RM12: Osteoimmunology in the development of bone biomaterials

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Numerous bone substitute biomaterials have been developed based on the tests of in vitro osteoblasts in terms of osteogenic differentiation and cytocompatibility. However, very few of the developed bone substitutes can be translated to clinical application due to the poor predictable outcome for bone regeneration, indicating that the current assessment methods for osteogenic property of biomaterials are not sufficient and not be able to reflect the real in vivo tissue response upon material transplantation. Clearly, one of the most important parameters missing from the current in vitro osteogenic assessment is immune response to biomaterials. As the development of osteoimmunology, immune cells have been found to play an indispensable role in bone dynamics. Accordingly, the paradigm of biomaterials for bone regeneration has been shifted from inert to immunomodulatory materials, emphasizing the importance of immune cells in the material-mediated osteogenesis. In this study, we investigated the interactions between bone cells and immune cells and elucidated the effects of immune cells in the regulation of osteogenesis in response to biomaterials. A novel bio-mimic system has been established for biomaterials assessment in terms of osteogenic properties to monitor the release of inflammatory cytokines, macrophage response and their interactions with mesenchymal stem cells. The possible strategies to endow the development of bone substitute materials with favourable osteoimmunomodulation have been proposed.

## RM13: A novel collagen scaffold for improved tendon-bone healing

Mei Lin Tay[1], Karen Callon[1], Ryan Gao[1], Donna Tuari[1], Jie Zhang[1], Dipika Patel[1], Jillian Cornish[1], David Musson[1]
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Tears of the tendon-bone interface are common, particularly in the rotator cuff, which affects 22% of the general population, and over 50% of those over 60 years old. These injuries show poor healing even after surgical repair. Augmentation with tissue-engineered grafts has been suggested for improved outcomes. Here, we evaluate a novel collagen scaffold, with organised lamellar structure and desirable mechanical properties, identified to be a potentially clinically viable tendon tissue augment.

*In vitro*, immune response was assessed by measuring expression of pro-inflammatory cytokines in human monocyte (THP-1) cells cultured with collagen scaffolds for 24 or 48 hours. alamarBlue® and fluorescent staining were used to determine if the scaffolds could sustain primary tenocyte cell growth over a 7-day period. *In vivo*, the supraspinatus was excised from the humerus of 23 sexually mature Sprague-Dawley rats. The tendon was either repaired using sutures alone, or sutures augmented with scaffolds. Biomechanical properties including elasticity and load to failure, were assessed using an Instron device at 12 weeks post-repair. H&E stained tendon sections were graded for collagen fibre density and orientation, healing at bone-tendon interface, vascularity, and presence of inflammatory cells.

*In vitro*, scaffolds did not increase the expression of pro-inflammatory cytokines (IL-1β, TNF-α, IL-8) in the THP-1 cells compared with either untreated controls or cells exposed to surgical sutures. alamarBlue® and fluorescent staining confirmed adherence and growth of tenocytes on the scaffolds. *In viv*o, scaffold augmentation increased elasticity of the repaired tendon-bone interface, but slightly lowered ultimate load to failure. There were no visible structural differences between groups.

Further work is underway to better characterise cell response to the scaffold, and to fully determine its potential for improved healing outcomes *in vivo*. However, results here suggest that the scaffold is cytocompatible, with potential to augment tendon-bone healing without inducing adverse immune responses.

## RM14: Development and characterization of chitosan/dextran-based hydrogels for adult stem cell delivery

Jaydee D. Cabral[1], Vicky Nelson[2], James Faed[2], Lyall Hanton[1], and Stephen Moratti[1]

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Delivery of stems cells in biomaterials is an encouraging method to repair damaged tissue in a marginally intrusive manner. By housing stem cells within a hydrogel matrix, viability and protection in cultivation as well as direct delivery to the damaged site in the host tissue can be achieved. The investigation of a chitosan/dextran-based (CD) surgical hydrogel as a novel approach for the delivery of human bone marrow derived mesenchymal stem cells (hMSC) was conducted. The main objectives of this study included: development of the CD hydrogel formulation, characterization of the material's composition and mechanical properties, and capacity to support hMSC growth. Our in vitro experiments using hMSCs demonstrate the CD hydrogel's ability to maintain cell viability as well as allow the cells to retain their "stemness" during encapsulation. The results of this study show that the CD hydrogel may be used as a stem cell delivery vehicle for regenerative medicine applications.

# Summary of Abstracts for the Poster Session Aug 28th

| No. | Title | Presenter | Institutions |
|---|---|---|---|
| RM15 | Finding the Links between Knee Injuries and Osteoarthritis | Leung, S.[1], Musson, D.[2], McGlashan, S.[3], Cornish, J.[2], Anderson, I.[1], Shim, V.[1] | [1]Auckland Bioengineering Institute, University of Auckland, NZ, [2]Department of Medicine, Faculty of Medical and Health Sciences, University of Auckland, NZ, [3]Department of Anatomy and Medical Imaging, Faculty of Medical and Health Sciences, University of Auckland, NZ |
| RM16 | Bioprinting of complex cell-encapsulated hydrogels with high spatial resolution via visible light photo-crosslinking and digital light processing | K.S. Lim[1], R. Levato[2], P.F. Costa[2,3], M.D. Castilho[2], K. van Dorenmalen[2,3], F.P.W. Melchels[4], D. Gawlitta[5], J. Malda[2,6], T.B.F. Woodfield[1] | [1]Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, Dept. of Orthopaedics Surgery and Musculoskeletal Medicine, University of Otago Christchurch, New Zealand. [2]Dept. of Orthopaedics, University Medical Center Utrecht, The Netherlands. [3]Utrecht Biofabrication Facility, University Medical Center Utrecht, The Netherlands. |

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| | | | [4]Institute of Biological Chemistry, Biophysics and Bioengineering, Heriot-Watt University, Edinburgh, United Kingdom<br>[5]Dept. of Oral and Maxillofacial Surgery and Special Dental Care, University Medical Center Utrecht, The Netherlands.<br>[6]Dept. of Equine Sciences, Utrecht University, Utrecht, The Netherlands. |
| RM17 | Bioassembly of complex multicellular tissues: an automated 3D microtissue assembly system for tissue-engineering and high throughput screening. | Mekhileri, N.V.[1], Schon, B.[1], Lim, K.S.[1], Mutreja, I.[1], Hooper, G.[1], Woodfield, T.B.F[1] | [1]Department of Orthopaedic Surgery, Centre for Bioengineering & Nanomedicine, University of Otago, Christchurch, New Zealand |
| RM18 | Covalent incorporation of heparin improves chondrogenic tissue formation in gelatin based hydrogels designed for cartilage tissue engineering. | Gabriella Brown[1], Bram Soliman[1], Sarah Bertlein[2], Khoon Lim[1], Gary Hooper[1], Jürgen Groll[2], Tim Woodfield[1]. | [1] Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) Group, Department of Orthopaedic Surgery & MSM, University of Otago, Christchurch, New Zealand;<br>[2]Department for Functional Materials in Medicine and Dentistry, University of Würzburg, Pleicherwall 2, 97070 Würzburg, Germany |

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| RM19 | Photo-curable Thiol-ene Gelatin Based Hydrogels as Bioinks for Bioprinting | Soliman, B.G.[1], Brown, G.C.J.[1], Lim, K.S.[1], Woodfield, T.[1] | [1] Christchurch Regenerative Medicine and Tissue Engineering (CReaTE) group, Department of Orthopaedic Surgery, University of Otago, Christchurch, New Zealand. |
| RM20 | Gelatin - Magnesium Carbonate nanocomposite hydrogels for improved osteogenic differentiation of mesenchymal stem cells | Mutreja, I.[1], Lim, K.S.[1], Maradze, D. [2], Liu, Y.[2], Hooper, G.[1], Woodfield, T.B.F[1] | [1]Department of Orthopaedic Surgery, Centre for Bioengineering & Nanomedicine, University of Otago, Christchurch, New Zealand. [2]Loughborough University, Centre of Biological Engineering, Wolfson School of Mechanical and Manufacturing Engineering, United Kingdom |
| RM21 | Self-assembling peptide supports osteoblast growth | Park, Y-E.[1], Rodriguez, L.[2], Brimble, M.[2], Naot, D.[1], Musson, D.[1], Cornish, J.[1] | [1]Bone and Joint Research Group, Department of Medicine, University of Auckland, NZ; [2]Department of Chemical Sciences, School of Biological Science, University of Auckland, NZ |

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| RM22 | Kinematic model of the hindlimb of the rat for functional assessment after peripheral nerve injury | Amado, S.[1], Ferreira, N.M.[1], Alves, N.[1], Morouço, P.[1] | [1]Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Portugal |
| RM23 | Mathematical concepts to be used in biofabrication | Martins-Ferreira, N. [1], Amado, S.[1], Alves, N.[1], Morouço, P.[1] | [1]Centre for Rapid and Sustainable Product Development, Polytechnic Institute of Leiria, Portugal |

## RM15: Finding the Links between Knee Injuries and Osteoarthritis

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The knee joint is one of the most complex organs in our bodies, and yet is one the most susceptible to injury. Traumatic injuries to the knee joint can cause pain, instability, and misalignment, altering joint loading patterns, which in turn can cause a cascade of events that leads to the development of osteoarthritis. Therefore, much research has been dedicated to understanding the onset and development of this disease by simulating *in vivo* joint loading using mechanical devices to apply various loads on 3D chondrocyte (cartilage cells) seeded in hydrogel culture models. However, these in vitro models have often not been validated, and the mechanical devices used to apply mechanical loads do not simulate physiological joint loading. Therefore we have developed the first precise multiaxial-loading device that can mimic physiological joint loading in an *in vitro* hydrogel model.

The hydrogel model was validated by determining the strain distribution of dynamic loads through different regions or zones of our hydrogel construct. Then, these construct strains were correlated with changes in cellular-shape, and angle of rotation of the cells subjected to dynamic compression, tension and shear loads in the different zones of the constructs to improve our understanding of how mechanical loads affect chondrocytes. Finally, gene expression techniques were used to determine the effects of applying different loading modes (compression, tension, shear, and a combination of the three) on chondrocyte mechanobiology, using our device. Two loading regimes, physiological and injurious loading were used. We found that more physiological loading regimes promoted cartilage homeostasis, imitating the behaviour of in vivo chondrocytes.

The system developed in this research is the closest device capable of fully mimicking in vivo conditions in health and disease. Work here has significantly enhanced our knowledge of chondrocyte mechanobiology.

# RM16: Bioprinting of complex cell-encapsulated hydrogels with high spatial resolution via visible light photo-crosslinking and digital light processing

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Digital light processing (DLP) is a photo-polymerisation based technology which involves projection of light into a resin in a spatially controlled and layer-by-layer manner [1]. However, most commercially available resins are non-cytocompatible, thus inappropriate for fabrication of cell-laden constructs. The aim of this study is to develop a bio-resin to build cell-laden constructs with high resolution and complex architectures via visible-light DLP.

Methacrylated-poly(vinyl alcohol) (PVA-MA) was used as the base platform for DLP-3D printing with the addition of gelatine-methacryloyl (gelMA) to impart bio-functionality in the resultant biosynthetic hydrogels. Constructs were fabricated using the Perfactory®4 Standard (EnvisionTec). The bio-resin used was 10wt% PVA-MA + 1wt% photo-absorber (Ponceau 4R) + photo-initiators, with or without 1wt% gelMA. Constructs were printed by exposing each layer (50µm) to 0.1mJ/cm$^2$ of light. Sol-gel analysis was conducted to evaluate the physico-chemical properties of the printed constructs (Ø5mm x 1mm cylinders). Mesenchymal stromal cells (MSCs) or multipotent cartilage progenitor cells (CPCs) were encapsulated at a density of 5x10$^6$cells/ml. DLP-printed cell-laden constructs were cultured for 21d to evaluate the potential of the gel support bone and cartilage.

3D constructs were produced either solid or with interconnected and self-supporting pores, with defined shape, high resolution, and complex architecture. Similar sol fraction (~25%) and mass swelling ratio (~9) were found for both PVA-MA and PVA-MA/gelMA constructs. Addition of gelMA significantly enhanced the viability of encapsulated MSCs (89±5%) vs. pure PVA-MA gels (78±2%). Printed MSCs and CPCs expressed markers of osteogenic and chondrogenic differentiation after 21d in culture respectively. We have shown that PVA-MA is compatible with the DLP technology as a bio-resin and addition of gelMA promotes bioactivity, cell survival and differentiation. This approach can allow to build a new generation of geometrically complex constructs with resolution higher than existing 3D-printing techniques.

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# RM17: Bioassembly of complex multicellular tissues: an automated 3D microtissue assembly system for tissue-engineering and high throughput screening.

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**Aims:** Long term repair of damaged musculoskeletal tissues is a challenge[1]. Combining microtissue fabrication techniques with 3D printed scaffolds as bottom-up approaches for tissue engineering and 3D *in vitro* models for high-throughput screening are emerging strategies. These strategies promote cell-cell/stem cell niche interactions and cell differentiation capacity[2,3]. Few technologies have been developed to automate the precise assembly of microtissues into 3D Printed scaffolds[4]. We aimed to develop a 3D microtissue assembly system for fabricating large, complex tissue-engineered constructs without adversely affecting cell viability.

**Methods:** An automated microtissue assembly system consisting a fluidic-based singularisation and injection module was developed and incorporated into a commercial 3D bioprinter (SYS-ENG). The singularisation module delivers individual microtissues to an injection module (LabView based), for insertion into specific locations in a 3D plotted scaffold. Human nasal chondrocytes or mesenchymal stromal cells (MSCs) were isolated and Ø1mm microtissues formed using a high-throughput 96-well plate format using chondrogenic media[2]. Singularisation efficiency was determined by the number of microtissues successfully singularized and injected (n=100). Bright-field microscopy measured physical dimensions of microtissues, and live/dead and trypan blue exclusion assays were used to quantify cell viability (n=4).

**Results:** Microtissues were successfully singularised with an efficiency of 97%±6.6. There was no significant difference in size and shape ($p>0.05$) or viability of microtissues before and after automated singularisation and injection. 3D plotted PEGT/PBT polymer scaffolds (1mm fiber spacing) were fabricated[1,2] and bilayered constructs containing pre-differentiated chondrogenic microtissues were successfully assembled. Culture of 3D assembled microtissues has demonstrated rapid fusion and chondrogenic differentiation in chondrocytes and MSCs.

**Conclusion:** We demonstrated an efficient system for the automated assembly of microtissues in 3D plotted scaffolds without deforming or significantly affecting microtissue viability. This technology paves a pathway for Bioassembly of complex 3D, multicellular tissues with clinically relevant size and shape.

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# RM18: Covalent incorporation of heparin improves chondrogenic tissue formation in gelatin based hydrogels designed for cartilage tissue engineering.

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Gelatin based hydrogels have been extensively researched as potential biomaterials for tissue engineering applications[1]. However, recent studies have outlined their limited capability to promote chondrogenesis for cartilage engineering while also demonstrating the potential of covalently incorporating biomimetic compounds for enhanced tissue quality in cell-laden gelatin hydrogels[2,3,4]. Such approaches typically requires chemically conjugating functional groups onto the targeted biological molecule. However, the impact of these modifications on the native bioactivity of resultant hydrogels is often overlooked. Previous studies conducted in our lab demonstrated that thiolation of heparin (HepSH), an original glycosaminoglycan (GAG) known to improve chondrocyte differentiation capacity[5], is a superior protocol for preserving biofunctionality compared to methacryloylation, a universally adapted protocol[2]. Therefore, the aim of this study was to compare the efficacy of incorporating HepSH into two different gelatin based hydrogels, gelatin-allyl (Gel-AGE) and gelatin-methacryloyl (GelMA) to achieve effective cartilage regeneration. We investigated heparin retention in the gelatin hydrogels along with their ability to promote chondrogenesis.

All hydrogels were photo-polymerised (±DTT, photo-initiators, 400-450nm, 5.4J/cm$^2$) and heparin retention (DMMB) was recorded as a function of allyl:thiol ratios. Human articular chondrocytes were encapsulated in hydrogels ($15 \cdot 10^6$ cells/ml) and cultured under chondrogenic differentiation media (5w) to assess cell viability, GAG (DMMB) and DNA (CyQuant) content, matrix deposition (SafO, Coll I/II) and morphology (CD44/F-actin).

This study shows that GelAGE can achieve significantly higher heparin retention (~75%) compared to GelMA (~25%) hydrogels. These results reflect that in GelMA-HepSH crosslinking, the step-growth thiol-ene conjugation is in rivalry with the chain-growth polymerisation of alkenes. Conversely, the pure step-growth protocol (GelAGE-HepSH) allows more homogeneous crosslinking. All constructs were shown to support long term cell survival with GelAGE-HepSH yielding significantly greater differentiation compared to both GelMA and GelAGE alone. In conclusion, gelatin allylation allows efficient conjugation of thiolated bioactives that enhances chondrogenesis in cell laden hydrogels.

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# RM19: Photo-curable Thiol-ene Gelatin Based Hydrogels as Bioinks for Bioprinting

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3D Bioprinting is a rapidly advancing technique often employed within tissue engineering. This technique requires development of specialised biomaterials (bioinks) with specific rheological properties to allow fabrication of constructs of high shape fidelity. Gelatin has been commonly used as bioink for Bioprinting as it possess unique thermosensitive rheological behaviour. Moreover, gelatin is often modified with photo-polymerisable functional groups such as methacryloyl to allow direct spatial control over the shape, size and composition of fabricated constructs. However, a major limitation using gelatin-methacryloyl (Gel-MA) in Bioprinting is oxygen inhibition during photo-polymerisation[1], whereby oxygen scavenges radicals resulting in incomplete cross-linking, negatively influencing construct shape fidelity. In the present research, we aim to utilise thiol-ene photo-click chemistry, speculated to resist oxygen-mediated radical quenching, as an alternative to methacryloyl chemistry.[2]

We applied two gelatin-based systems, gelatin-allyl (GelAGE) and gelatin-norbornene (GelNOR). Hydrogels were fabricated (Ig2959 photoinitiator, 365nm, 5.4 J/cm$^2$) using thiolated crosslinker molecules (dithiothreitol and thiolated 8-arm poly ethylene glycol). Hydrogel physico-chemical properties were characterized as a function of cross-linker concentrations, using gel-MA as control samples.

The physico-chemical properties of gelAGE and gelNOR hydrogels were highly tailorable (GelAGE: 5-30% sol fraction, 8-15% swelling ratio, gelNOR: 7-20% sol fraction, 15-25% swelling ratio). Furthermore, we demonstrated that gelAGE and gelNOR bioinks could be successfully Bioprinted, yielding porous constructs with high shape fidelity. After swelling, the shape fidelity of plotted fibres was noticeably improved in gelAGE and gelNOR-based constructs as compared to more commonly used Gel-MA, which may be attributed to the absence of oxygen inhibition.

In conclusion, we have developed a gelatin-based hydrogel system with highly tailorable physico-chemical properties. We have shown that these formulations can easily be adopted to biofabrication where high fidelity may be achieved as compared to currently used gelatin-based bioinks.

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# RM20: Gelatin - Magnesium Carbonate nanocomposite hydrogels for improved osteogenic differentiation of mesenchymal stem cells

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Nanocomposite hydrogels have gained a lot of interest for stem cell based tissue engineering applications. Different nanoparticle systems have been used for stem cell differentiation ranging from calcium phosphate (CaP) to nanosilicates and bioglass. One important class of inorganic materials that has shown promise for bone tissue engineering is biodegradable magnesium. Magnesium and its corrosion products, in particular magnesium carbonate ($MgCO_3$) have been known to promote osteogenic differentiation of human mesenchymal stem cells[2]. Therefore in this study, we report the development of extracellular matrix mimicking nanocomposite hydrogel system which combines magnesium carbonate nanostructures within gelatin-methacryloyl (GelMA) hydrogels.

$MgCO_3$ nanostructures were prepared by precipitation reaction, followed by centrifugation, washing and drying steps before being characterized and used for cell encapsulation within GelMA hydrogels. Nanoparticles were characterized using scanning electron microscopy (SEM) and X-ray diffraction (XRD) to determine their shape and crystal structure. Human bone marrow derived mesenchymal stem cells (MSCs) were encapsulated in 5% GelMA at a density of $5x10^6$ cells/ml with or without the incorporation of $MgCO_3$ nanostructures at concentrations of 0.5 and 1.5 mg/ml. MSC-laden hydrogels ± MgCO3 nanostructures were cultured in expansion and osteogenic media over a period of 28 days and cell viability and functionality was determined.

$MgCO_3$ nano-plates were formed with a size range of 50-200 nm and thickness of ~15 nm. XRD analysis confirmed that nanostructures formed had a crystal structure corresponding to hydromagnesite. The incorporation of $MgCO_3$ within the hydrogel did not alter cell viability or metabolic activity, with live/dead analysis showing >95% viability with no significant differences across all hydrogel systems. Greater levels of mineralization was observed in cells encapsulated in GelMA-$MgCO_3$ 0.5 mg/ml nanostructures cultured in osteogenic media compared to cells in GelMA alone or GelMA-$MgCO_3$ 1.5 mg/ml.

We demonstrated that we could engineer $MgCO_3$ based nanocomposite hydrogels which not only support cell survival and cell proliferation but also promotes osteogenic differentiation of MSCs.

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## RM21: Self-assembling peptide supports osteoblast growth

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The use of peptide hydrogels is a growing field in bone regeneration. Self-assembling peptides form hydrogels and are of interest as injectable drug delivery matrices. They are injected into the defect site, gel *in situ*, and release factors that aid bone growth. We have developed a hydrogel that is capable of delivering lactoferrin, a bone anabolic factor. The aim of this study was to evaluate the cytocompatibility of this novel self-assembling peptide, to determine whether it has potential for bone regeneration.

The self-assembling peptide was synthesised to be assembled into β-sheets and form hydrogels (base peptide). Also, a base peptide linked with RGD motif was synthesised to reinforce cell attachment to the gel. The mixtures of peptides examined were: 100% RGD-linked peptide, 50% RGD-linked peptide with 50% base peptide, and 20% RGD-linked peptide with 80% base peptide. The hydrogels were formed in 24-well plates. Primary rat osteoblasts were cultured for 7 days on the gels and viability was assessed on days 1, 2, 3 and 7 using alamarBlue assay, accompanied by Live/Dead fluorescent assay on day 7 of culture.

Over the culture period, there was a significant increase in osteoblast viability ($p<0.05$) for the 100% and 20% RGD-linked hydrogels, which both had the highest viability. Live/Dead staining on day 7 confirmed the presence of viable osteoblasts on the surface of the hydrogels, however, the cells on the 100% RGD gel were morphologically unusual. Preliminary experiment suggests that lactoferrin is slowly released over 12 days from the 20% RGD-linked gel.

This study demonstrated that the novel self-assembling peptides are cytocompatible to primary osteoblasts. Future experiments will focus on the 20% RGD-linked gel. In the future, osteoblast differentiation and migration into the gel will be assessed, with and without lactoferrin, to further evaluate the therapeutic potential of the peptide in bone regeneration.

# RM22: Kinematic model of the hindlimb of the rat for functional assessment after peripheral nerve injury

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Gait analysis is a promising method to assess functional recovery after hindlimb nerve injury. However, in order to provide accurate measures of functional recovery, gait analysis after hindlimb peripheral nerve injury should evolve from a simple ankle kinematics analysis to a full 3D biomechanical description of complete hindlimb motion, meaning analysis of hip, knee and ankle joints. Further refinements of gait analysis in the field of peripheral nerve research using the rat model should include the combined use of joint kinematics, ground reaction forces and electromyography data. Regenerated sciatic nerve began to regain function between 3 [1,2] to 7 [3] weeks after injury. The borderline between a sensory-motor response involving both sensory ascending and descending motor pathways and a pain-based withdrawal reflex-response is not always possible. It cannot always be sure that all responses are based on proper temperature sensing. Reflex activity should be integrated in a dynamic model, assessed during movement, since it might comprise an important aspect on motor control with functional meaning for the position of the limb and movement. Mathematical modelling is a possible solution to simplify and understand the potential environmental effect with similar neural control and exclude neural feedback. Efforts will be made to improve the biomechanical modelling with musculoskeletal model and methodologies to understand the reflex mechanisms involved in the sensory and motor recovery of function. Knowledge of the more complex, but a realistic pattern of 3D movement is important for understanding its neuromuscular control, as well as the principles of musculoskeletal design [3] that are crucial to the production of movement. During dynamic conditions, combining kinematic data with kinetics and electromyography would be desirable to make a comprehensive understanding of movement patterns and its changes as well as the study of inefficient movement after injury. Results from the last study awake us for the relevance of mechanical load, fatigue and the influence of joint rotational velocity, which should be explored to understand performance and make available results translation for motor rehabilitation.

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## RM23: Mathematical concepts to be used in biofabrication

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The aim of this work is to explain how the mathematical structure of multi-link can be applied in modelling the many steps that are involved in the whole process of direct digital manufacturing, with particular applications to tissue engineering and regenerative medicine. The main tool is the notion of a multi-link, which is a new mathematical structure that has been developed by the Laboratory of Topology and Geometry from the Centre for Rapid and Sustainable Product Development at the Polytechnic Institute of Leiria [1,2,3,4,5]. In particular, this new notion and its associated methods have been applied in solving many important problems and it is expected that they will give new insights to the current challenges and main difficulties in the field of biofabrication for tissue engineering and regenerative medicine.

A multi-link consists of: (i) a finite set (called the set of indexes), (ii) a family of endomaps (usually permutations, that encodes the topological information of the system), (iii) a family of projection maps (that is used to encode the functional information associated to each index, giving it a physical meaning) and (iv) a realization map from the set of indexes into a geometrical algebra (usually the real numbers, the complex numbers, the quaternions or the octonions, but it can also be an arbitrary vector space). The realization map gives a geometrical interpretation to each one of the indexes in the index set. Depending on the topology, which is determined by the family of endomaps and its inner properties, an index in the index set can have different geometrical interpretations. The main examples that are of interest for the purpose of direct digital manufacturing are edges, squares, cubes, and polygons in general. From our results, was obtained a structure that is rich enough to encode all the desired geometries, with many of the functional characteristics that are usually considered in the process of direct digital manufacturing, such as the type of the material, its stiffness, density, color, etc. The main procedures such as slicing, scanning, inducing porous structures, etc, are all well adapted to be easily and efficiently implemented into this formal language. Its conceptual consistency and robustness allow us to reason on a high level and hence to have a clear idea of the role played by each one of the elementary parts that are involved in the whole process of manufacturing in a wider sense.

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# Stem Cells & Regenerative Medicine Satellite Abstracts

## S1: Seeking the stemmiest stem cell; how to get more from your fat

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Human adipose derived stem cells (ASC) are of interest to the field of regenerative medicine. They are multipotent (can differentiate into fat, bone, muscle, cartilage) and they can be accessed relatively easily from lipoaspirate, the by-product of liposuction. Lipoaspirate is processed to yield a heterogenous cell pellet known as the stromal vascular fraction (SVF). Typically SVF is cultured for up to 4 weeks using standard tissue culture conditions to yield a purified population of ASC. We have been using multicolour flow cytometry to analyse and sort stem cell and progenitor populations in complex tissues such as adipose tissue. We have observed significantly higher differentiation potential in ASC isolated from the SVF using flow cytometry when compared to ASC purified using the standard tissue culture method. This data indicates that the freshly sorted ASC are more potent and could therefore perform better in a clinical setting. However due to the technical complexities that are associated with flow cytometry this isolation procedure is unlikely to translate easily to a clinical setting. Therefore we are currently assessing the utility of a magnetic-activated cell sorting (MACS) approach for ASC isolation. Here we use "negative selection" to label all non-ASC within the SVF with a novel cocktail of commercially available antibodies attached to magnetic microbeads. Our preliminary data indicates that this approach results in cells with high purity, high yields and high levels of differentiation potential. Therefore the MACS approach could offer a simple method to rapidly isolate potent ASC that can be easily translated to the clinic.

By understanding the molecular basis of differentiation potential we could use molecular tools to enhance the potency and clinical utility of ASC. To this end we have used microarray technology to analyse the differential expression of mRNAs and microRNAs between ASC with high (FACS sorted cells) versus low (purified by plastic adherence) differentiation potential. Data will be shown indicating that specific microRNAs are associated with high potency ASC. We observe that when these microRNAs are over-expressed in cells differentiation potential is significantly improved. This suggests that manipulation of the expression of specific microRNAs could be used to enhance the 'steminess' of ASC.

# S2: In search of the elusive tissue-resident stem cell: Using multicolour flow cytometry to enable characterisation of stromal cell populations in human dissociated tissue

Anna Brooks

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Mesenchymal stem cells (MSCs) are a diverse subset of multipotent precursors present in the stromal fraction of many adult tissues which have drawn intense interest from both clinical and basic research investigators, largely due to their therapeutic potential in regenerative medicine. To date, a large proportion of studies investigating properties of mesenchymal stem/stromal cells are instigated by isolating these populations using the classical plastic adherence method. This technique, although efficient, does not allow identification of the true, primary ex-vivo phenotype due to culture conditions influencing cell surface receptors. In addition, it is likely that that pool of cells isolated in this manner are also heterogeneous, or at least were propagated from a heterogeneous pool of adherent cells, and thus these cells can only, at best, be described as mesenchymal stromal cells due to their unknown origin. Much effort is underway to determine the cell surface marker signature of the true tissue-resident multi-potent stem cell to allow successful identification, isolation and characterisation. Multicolour flow cytometry is a powerful method for detecting and characterizing multiple cell populations simultaneously in a complex sample, such as dissociated tissue. We have optimised a 17-colour multicolour flow cytometry panel to characterise mesenchymal/pericyte/stem cell populations in human tissue, such as dermis (skin), stromal vascular fraction (fat), lymph nodes and liver. Using this panel we were able to find common vascular and stromal cell populations across all tissues investigated, whilst also detecting tissue specific differences. By characterising primary tissue we can start to get a better understanding of the true native phenotype of the stromal fraction, which will not only help identify their anatomical location in tissue, but also allow the isolation and purification of pure populations by flow cytometry for downstream analyses to facilitate the search for the elusive, true multi-potent stem cell populations.

## S3: Mesenchymal cell subsets in human tissues

Rod Dunbar

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Human mesenchymal Stem Cells (MSCs) are of wide interest due to their potential in cell therapy for a range of conditions. However the phenotypic and functional properties of MSCs remain poorly defined. In many assays, cells that fit the definition of Adipose-derived Stem Cells (ASCs) – one of the most accessible forms of "MSCs" – are very similar to other mesenchymal cells that can readily be grown from many human tissues, typically referred to as "cultured fibroblasts". In an effort to discriminate between mesenchymal cells with stem cell properties and those that are more differentiated, we have been using flow cytometry and immunofluorescence microscopy, coupled with functional assays on sorted cells, to survey the mesenchymal cell populations present in several human tissues. We find diverse mesenchymal cell populations in human tissues, although many of these populations have the ability to differentiate into adipocytes. However evidence of multi-lineage differentiation amongst these populations is weak, suggesting that populations that might reasonably be termed MSCs are rarer than previously appreciated. Our work points to a greater need to understand the phenotypic and functional properties of all human mesenchymal cell populations in order to predict the potential impact of the mesenchymal cells being administered in current clinical trials.

## S4: The role of transcription factors in cell lineage determination

John Gurdon

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Once a cell has embarked on a defined lineage, it very rarely, if ever, changes to a different lineage. Thus the stability of cell differentiation is a very important characteristic of cells that prevent abnormality through ageing, disease or other factors. Current work indicates to us that a site occupation by a transcription factor is extraordinarily stable and long-lived. Thus, transcription factor site occupation may contribute to the stability of cell differentiation.

## S5: Transdifferentiation in the zebrafish kidney

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Differentiation is fundamental to enabling different tissues to perform unique functions. Transdifferentiation occurs when a terminally differentiated cell either undergoes reversion back to a more progenitor-like state and then adopts a new fate (indirect) or commits to a new fate without an intermediary state (direct). In this study, we demonstrate a novel example of direct transdifferentiation in the zebrafish kidney. We show that a group of differentiated renal tubule epithelial cells undergoes conversion to an endocrine gland fate within the space of ~30 hours during larval stages. This fate conversion coincides with nuclear export of Hnf1b, which acts an important regulator of renal epithelial cell identity in the kidney. We also find that the Iroquois transcription factor *irx3b* prevents Hnf1b export, but this action is overcome by Notch. In order to be extruded from the tubule, gland-fated cells undergo Myosin II-mediated apical constriction, and this process can be inhibited by Blebbistatin treatment. During and subsequent to extrusion, gland cells retain epithelial status as they continue to express epithelial markers, such as *epcam*, and maintain E-cadherin labelling at their cell-to-cell junctions. Taken together, our results may demonstrate a rare example of direct transdifferentiation in a developmental system and provide a model for the embryonic control of cell fate changes and live cell extrusion.

# S6: Understanding how regeneration is regulated in a vertebrate model: the clawed frog Xenopus

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Tadpoles of the anuran amphibian *Xenopus laevis* can regenerate both their spinal cord containing tails and their developing limb buds almost perfectly. While the quality of limb regeneration undergoes a gradual ontogenic decline, tails regenerate right up until metamorphic climax (when the tail is lost and the animal assumes its adult form) except for a brief window known as the refractory period. *Xenopus* therefore provides a good model for both loss and gain of function regeneration experiments. Not surprisingly perhaps, many developmentally important growth factor signaling pathways are reactivated during the process of regeneration, and this is required for rebuilding the lost structures. Less is known about how the regenerative response is actually triggered by wounding or partial amputation of the limb or tail. The reactive oxygen species (ROS) hydrogen peroxide is produced as a very early response, and NADPH oxidase (NOX) inhibitors have been shown to block tail regeneration in *Xenopus* and geckos as well as fin regeneration in zebrafish (1-3). We are investigating the possible role of the transcription factor NF-kB, which can be activated by high levels of ROS, in this process.

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**S7: Immune Control of Regeneration**

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# S8: Trimethylation barriers to epigenetic reprogramming in mouse and cattle

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Radical manipulations, such as nuclear transfer (NT) cloning or induced pluripotent stem cell (iPSC) derivation can restore totipotency and pluripotency, respectively, in somatic cells. However, both reprogramming approaches remain inefficient. We hypothesized that trimethylation (me3) of lysines (K) in histone (H) 3 impedes somatic reprogramming. To test this idea, we engineered transgenic mouse lines for tetracycline-inducible (Tet-ON) expression of 1) the H3K9/36me3 demethylase KDM4B, fused to EGFP, and 2) its non-functional mutant, lacking a functional catalytic JMJC-domain[1].

In mouse embryonic fibroblasts (MEFs) derived from these lines, *Kdm4b* expression reduced H3K9me3 levels ~100-fold compared to non-induced controls. Through microarray analysis, mRNA-sequencing and qPCR validation, we identified several transcriptional targets of derestricted chromatin. In functional assays, KDM4B-MEFs reprogrammed six-fold better into cloned blastocysts and nine-fold better into iPSCs compared to non-induced donors. Mutant KDM4B-MEFs showed no functional differences between induced and non-induced cells.

We then generated bovine embryonic fibroblasts (BEFs) carrying the Tet- transactivator driven by a constitutive EF1α promoter. Tet-On BEFs were rejuvenated by NT and transfected with a Tet-responsive *PiggyBac*-transposase vector encoding functional murine *Kdm4b-Egfp*. Puromycin-selected stable clones were again rejuvenated by NT. Induced KDM4B-BEFs had ~5-fold and ~8-fold lower H3K9me3 and H3K36me3 levels, respectively, compared to non-induced controls. Serum starvation, a prerequisite for efficient bovine somatic cell NT, further decreased H3K9me3 and H3K36me3 levels ~6-fold and ~5-fold, respectively, resulting in a total ~30-fold and ~40-fold reduction, respectively. Induced KDM4B-BEFs reprogrammed marginally better into cloned blastocysts than non-induced controls ($P<0.05$). However, there was no difference in pregnancy establishment (at D35) and fetal development (at D90) between induced vs non-induced blastocysts (7/18 = 39% vs 6/18 = 35% and 0% vs 6%, respectively, n=2). We conclude that H3K9me3 impedes somatic cell reprogramming in mouse but not in cattle, indicating that this modification is not a universal roadblock for restoring cell potency.

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# S9: Retinol and ascorbate drive erasure of epigenetic memory and enhance reprogramming to naïve pluripotency by complementary mechanisms

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Naïve embryonic stem cells are characterized by genome-wide low levels of cytosine methylation - a property that may be intrinsic to their function. We found retinol/retinoic acid (Vitamin A) and ascorbate (Vitamin C) synergistically diminish DNA methylation levels, and in doing so, enhance generation of naïve pluripotent stem cells. This is achieved by two complementary mechanisms - retinol increases cellular levels of TET proteins (which oxidise DNA methylation), whereas ascorbate affords them greater activity by reducing cellular $Fe^{3+}$ to $Fe^{2+}$. This new mechanistic insight is relevant for the production of induced pluripotent stem cells used in regenerative medicine, and contributes to our understanding of how the genome is connected to extrinsic and intrinsic signals.

# S10: Double cytoplast nuclear transfer improves *in vitro* embryo development from mitotic embryo-derived pluripotent stem cells in cattle

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Producing multiple animals from one genomically selected embryo in a single generation would accelerate genetic gain in dairy cattle breeding. We previously converted bovine embryos into embryo-derived pluripotent stem cells ('ePSCs') using chemically-defined culture medium with two kinase inhibitors ('2i')[1]. Kinase inhibitor concentrations were titrated to further promote expression of pluripotency markers in bovine ('2i$^{PLUS}$' medium)[2]. Here we tested ePSCs for embryonic cell nuclear transfer (ECNT) and cloning of ePSC-derived cattle.

Following immunosurgical isolation from the inner cell mass of *in vitro* produced (IVP) blastocysts, adherent ePSCs were derived with an efficiency of 86±6% (blastocysts *N*=105, replicates *n*=5). After six days of feeder-free culture in 2i$^{PLUS}$, colonies were treated with 500nM nocodazole overnight. Immunofluorescence against phosphorylated histone 3 (P-H3) showed 40±2% of cells within a nocodazole-treated colony in metaphase (*N*=16, *n*=4) compared to 11±3% in DMSO controls (*P*<0.01). Within each colony, an average 43±13% of cells expressed the pluripotency markers SOX2 and NANOG (*N*=25, *n*=4). For ECNT, on average 27 mitotic ePSCs per colony (N=48) were harvested by sequential treatment in dispase, pronase, and trituration in $Ca^{2+}$/$Mg^{2+}$-free medium. Karyotyping revealed that 21% of ePSCs had 60 chromosomes, while 64% deviated ±5% from diploid (127 spreads, 28 colonies, *n*IVP=6). Mitotic ePSCs fused with cytoplasts at 76±13% efficiency and developed into blastocysts at 16±4% (*n*IVC=258, *n*=10), similar to NT with nocodazole-arrested fibroblasts (12±3%, *n*IVC=174, *n*=6). Double cytoplast ECNT ('ECDC'), whereby another enucleated oocyte was fused to the first reconstruct, increased blastocyst development to 24±5% (*n*IVC=574, *n*=14, *P*<0.01). *In vivo*, 21% of ECDC blastocysts established biochemical pregnancies at Day 21, but these were all lost by Day 60 (*N*=56, *n*=3). On average, one original IVP blastocyst genotype was thus multiplied into ~4 isogenic ECDC blastocysts. However, post-blastocyst development into viable fetuses was nil, probably due to genetic errors in the donor cells.

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## S11: Using a novel candidate human trophoblast stem cell population to unlock the secrets of placental organogenesis

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The placenta is essential for fetal growth and survival in utero, and inadequate placental development is associated with pre-eclampsia and intrauterine growth restriction. However, the placenta is also one of the least understood human organs, and our understanding of how cell function is compromised in pregnancy disorders is extremely poor. Different populations of a placenta-specific epithelial cell called trophoblasts are key to facilitate nutrient and gas exchange between the maternal and fetal circulations, ensuring healthy fetal growth. Trophoblast stem cells isolated from murine blastocysts have taught us a great deal about how different trophoblast lineages develop and function in murine placental development, but a 'true' human trophoblast stem cell model has yet to be established. Hoechst low side-populations are characteristic of stem cells from a variety of adult tissues. Therefore, we used the Hoechst side-population technique to isolate a candidate trophoblast stem cell population from first trimester placentae. Isolated side-population trophoblasts have >98% purity and have a distinct gene expression profile from mature trophoblast populations, including the expression of genes associated with pluripotency and murine trophoblast stem cells, making them a promising candidate stem cell population. More recently, side-population trophoblasts have also been isolated from term placentae, providing an exciting opportunity to compare normal and pathological placentae to understand the role that stem cells may play in the pathophysiology of these disorders. Side-population trophoblast survival is enhanced by culture with first trimester decidua and/or placenta-conditioned media, and cytokine array profiling of these media has identified targets to further enhance side-population trophoblast propagation. Enhancing the propagation capacity of side-population trophoblasts will allow us to undertake functional experiments to determine whether this candidate stem cell population can differentiate into all mature trophoblast lineages, and are thus a true population of human trophoblast stem cells.

## S12: Modelling kidney disease and injury with kidney organoids

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Advances in 'cellular reprogramming' methodologies now make it possible to convert virtually any cell of the body into a pluripotent stem cell. These so-called induced pluripotent stem (iPS) cells, with their potential to form any adult cell type, have the potential to greatly advance regenerative medicine. In addition, when iPS cells are generated form patients with genetic disorders, they provide a source of diseased cells to model the disorder *in vitro*. We have generated iPS cells from a patient with cystinosis, a rare lysosomal disorder caused by mutations in the *cystinosin* (*CTNS*) gene that encodes a cystine transporter. In its severest form, cystinosis causes damage to the renal tubules leading to kidney failure in the first decade of life if untreated. We have developed a method to convert iPS cells into kidney organoids, thereby giving us a new tool to model cystinosis and kidney injury. With this new breakthrough we hope to further our understanding of the pathogenesis of cystinosis and develop new treatments for renal injury.

## S13: Stem cell based functional genomics of human neurological diseases

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Induced pluripotent stem cells capture an individual's genetic make-up and, following differentiation into cell types of the brain, provide an attractive model system to perform functional genomics investigations into human brain diseases. We routinely generate iPSC from patients with a variety of neurological diseases including leukodystrophies, Ataxias and Down syndrome. We use CRISPR-mediated gene correction and/or CRISPR-engineered patient mutations in control iPSC combined with 2D and 3D (organoid) culture approaches to reveal disease phenotypes. We have amongst others used this approach to investigate genotype-phenotype relationships between the supernumary chromosome 21 genes in Down syndrome and early onset Alzheimers disease associated with trisomy 21. Our data challenge the classical beta-amyloid cascade hypothesis.

## S14: The Genetic Regulation of Human Neuroprogenitors

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The study of the genetic regulation of human neuroprogenitor cells is challenging. *In vitro* systems do not recapitulate conditions that are operating during embryogenesis *in vivo* and animal models are often insufficient because they lack structural similarity with the primate brain. To understand some levels of genetic regulation of these cells we have taken the approach of studying the genetics of a disorder of neuroprogenitor cell function, periventricular neuronal heterotopia (PH). PH is characterised by rests of grey matter lining the cerebral ventricles instead of being located at the surface of the cerebral cortex. The clinical corollary of this is broad, including seizures and intellectual disability. We have studied monogenic syndromes with this condition and identified new loci implicated in the function of neuroprogenitors including the cell adhesion molecules *DCHS1* and *FAT4*. We have also performed trio exome sequencing on a cohort of 72 unrelated affected individuals with this disorder and studied the mutational burden associated with the condition. We have noted that the study cohort has an excess of missense and nonsense mutations compared to the null hypothesis and that an elevated proportion of these mutations occur in mutationally-constrained genes. Finally networks of genes have been identified that pinpoint specific cellular functions associated with this disorder, most notably RNA processing.

# S15: Ventricular specific cardiomyocyte differentiation of mouse embryonic stem cells through modulation of molecular pathways

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Acute myocardial infarction diminishes blood flow to the heart leading to loss of large number of cardiomyocytes and causing tissue damage mainly in the left ventricle. In an effort to regenerate the lost cardiomyocytes, stem cells are gaining tremendous interest. Among the stem cells, pluripotent stem cells (PSC) are becoming popular due to their capability to proliferate and differentiate into any lineage, including cardiac lineage. Various protocols have been established to differentiate PSC into cardiomyocytes, however, all these protocols resulted in the development of a heterogeneous population of cardiomyocytes (atrial, nodal and ventricular). This is a major drawback because once transplanted to the left ventricle these nodal cells will generate unwanted cardiac arrhythmias.

In the current study, we designed a protocol with the aim to differentiate mouse embryonic stem cells (mESC) specifically to ventricular cardiomyocytes. By inhibiting Wnt signalling in the early stage, mESC should develop to cardiomyocyte lineage while inhibition of Retinoic acid signaling in the later stages should provide them a ventricular phenotype. To enhance the differentiation, ascorbic acid was added throughout the process. Gene expression of stage specific cardiomyocyte markers was validated by qPCR and protein expression was validated by flow cytometry and immunofluorescence analysis. The preliminary results show that the expression of all the cardiac markers, including the ventricular specific IRX4 and MLC-2V, are expressed at 14 days after treatments. The next step is to validate the function of the cells to understand the efficacy of the differentiated cells. The development of a specific differentiation protocol of PSC to ventricular cardiomyocytes will significantly contribute to the cardiac regeneration field.

# S16: A clinically relevant technique to rapidly isolate human Adipose-Derived Stem cells

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Mesenchymal Adipose-derived Stem Cells (ASC) can be isolated from human adipose tissue. These are abundant and easily obtained from the by-product of the minimally invasive surgery, liposuction. ASC are multipotent with the ability to differentiate into fat, bone, muscle and cartilage. Lipoaspirate can be digested into a heterozygous collection of cells known as the stromal vascular fraction, which is further isolated into ASC (characterised as CD34-, CD73+ and CD90+ cells). To date the main method to isolate these cells is via extended adherent culture for up to 28 days, however this leads to changes in the molecular properties of the ASC cells and therefore to changes in cellular behaviour. Our focus is on developing rapid and more clinically relevant isolation techniques to obtain ASC with maximum differentiation potential and cell yield. We are using a novel cocktail of antibodies in an immunomagnetic bead purification approach to rapidly isolate ASC. To determine the utility of this method we are comparing bead sorted cells to ASC sorted by fluorescence-activated cell sorting (which we consider as "gold standard cells", although this method is not clinically useful) and to cells isolated by traditional plastic adherence. The following are being assessed for each isolation method:

- Yields and purity.
- Differentiation potential into three distinct linages (adipocytes, osteocytes and chondrocytes)
- *In vitro* proliferation capacity
- Immunosuppressive activity
-

Preliminary studies indicate that we can purify high yields of potent ASC using our immunomagnetic bead method. Our data suggests that this is a useful method to rapidly isolate highly functional stem cells in a clinical setting.

## S17: Examining the molecular mechanisms underlying the differentiation potential of human adipose-derived stem cells

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Human adipose-derived stem cells (ASCs) are of interest to the field of regenerative medicine due to their multipotent nature and immunomodulatory capabilities. Extended periods of time in culture, up to 28 days, are required to obtain a pure population of ASCs for clinical use. We compared ASCs purified by fluorescence-activated cell sorting (FACS) to cells purified by the traditional plastic adherence cell culture method and observed that cultured cells exhibited a significant decrease in differentiation potential. RNA was harvested from both cell populations and analysed by microarray to identify differentially expressed microRNAs and mRNAs. Significant differences in gene expression were seen not only between day 0 and day 28 cells, but also between day 0 and day 3 cells. This is indicative of an almost immediate change in cell behaviour upon plastic adherence culture. RT-PCR was used to validate genes of interest in 3 new donors. Functional studies were performed on two candidate microRNAs which, based on their expression profile, may have a role to play in maintaining "steminess".

RT-PCR studies confirmed an increase in miR-21 expression and a decrease in levels of miR-378 in ASCs over time in culture. Decreased expression of a number of genes associated with differentiation was also observed, including KLF4, a transcription factor associated with stem cell potential. Inhibition of miR-21 and overexpression of miR-378 in late passage ASCs, which typically have low differentiation potential, resulted in increased differentiation potential when compared to control cells. These preliminary findings give insight into some of the molecular mechanisms underlying ASC differentiation potential. In the future these mechanisms could be harnessed to manipulate ASC in order to maintain their full potential even after periods of cell culture, so enhancing their efficacy in regenerative medicine therapies.

# S18: Towards CRISPR-Cas9 mediated replacement of the male germline in sheep

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Male germline transmission depends on spermatogonial stem cells (SSCs) which engender all spermatozoa. In mice, ablating the RNA-binding protein 'deleted in azoospermia-like' (*Dazl*) resulted in germ cell-deficient males. Following such genetic sterilisation, transplanted wild-type donor SSCs can colonize host testes, re-establish spermatogenesis and produce viable offspring. We aim to generate germline-disabled *DAZL*$^{-/-}$ sheep with a vacant SSC niche through somatic cell nuclear transfer (SCNT) cloning. These will be complemented with wild-type embryonic cells to restore the depleted germline in cloned chimeric animals.

We will employ the CRISPR-Cas9 system to disrupt *DAZL* through homology-directed insertion of a 6 base pair sequence, introducing a stop codon and *Taq1* restriction site. As targeting sequence for the Cas9 endonuclease, three guide RNAs (gRNAs) were designed based on the genomic *DAZL* sequence from primary male ovine embryonic fibroblasts (OEFs). Using an *in situ* fluorescence reporter system, we showed that gRNAs 1-3 cleave their target sequence in OEFs with 5%, 2% and 0.7% efficiency, respectively. Despite cleavage of an exogenous target sequence, no editing of the endogenous genomic locus was detected using mismatch cleavage assays. Furthermore, homology-directed repair was not detected after *Taq1* digest of the amplified region of interest. Finally, Taqman hydrolysis probes for both mutant and wild-type variants were used to quantify genomic DAZL genotypes within the transfected samples, with only the wild-type probe providing reliable amplification. Therefore despite an active CRISPR/Cas9 ribonucleoprotein complex within OEFs, editing of endogenous DAZL was undetectable.

For embryo complementation, OEFs were modified by using the *Sleeping Beauty* transposase to insert red fluorescent protein (RFP) under control of the constitutive CAGGS promoter. Two puromycin-selected cell clones were chosen based on uniformity and high level of RFP-expression. These lines will be used to produce SCNT donor embryos for aggregation with cloned *DAZL*$^{-/-}$ embryos, once available, and subsequent germline replacement.

## S19: Bespoke full-thickness human skin as a permanent wound solution

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Skin grafts are generally successful for treatment of superficial and partial-thickness burns; however, their success is limited for treatment of severe burns that require repeated grafting from multiple donor sites. Alternative grafting techniques have been developed but, while effective in the short-term, are susceptible to rejection, disease transmission, some are difficult to apply, and crucially are not permanent, therefore requiring autograft replacement at a later date. We aim to develop clinically safe methods for growth of full-thickness skin using autologous skin cells as a permanent graft solution for people with major burns. To achieve this, we are optimising techniques to expand freshly isolated cells from donated human skin as a monolayer in tissue culture flasks before being seeded onto synthetic 3D scaffolds for full-thickness skin growth. Firstly, we have determined the safest and most effective media for expanding keratinocytes in a clinical setting. In addition, the keratinocyte subsets responsible for *in vitro* proliferation and maintenance of full-thickness skin have been delineated using fluorescence activated cell sorting. We further tracked their molecular profiles in relation to distinct epidermal cell subsets *in vitro* by immunocytochemistry. These populations were primarily $CD29^{+}CD271^{-}$ and expressed cytokeratin proteins indicative of highly proliferative undifferentiated keratinocytes of the basal epidermal layer. This provided an understanding of keratinocyte precursors and led to the identification of small molecules that significantly enhance their *in vitro* expansion. Finally, we used this knowledge to modify and apply these methods to growth of full-thickness skin on a synthetic dermal substitute. Both dermal and epidermal layers were intact and displayed structures indicative of functional skin including extracellular matrix and stratified epidermis as determined by immunohistochemistry. These results provide a monumental step towards development of a permanent grafting solution for patients with severe burns.

# S20: Differentiation of human iPS cells into pancreatic beta cells

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Ever since iPS (induced pluripotent stem) cells were first successfully derived from embryonic stem (ES) cells by Takahashi and Yamanaka in 2006, the research in this field had expanded extensively. Scientists have been trying different combinations of defined factors and small molecules to create a directed route for iPS cells to further differentiate into mature cell types. There has been intense focus on generating pancreatic beta-cells which secrete insulin and are crucial for whole body glucose homeostasis as the lack of good human beta-cell models is a major limitation for diabetes research. In 2014, Pagliuca et al successfully generated glucose-responsive human pancreatic beta cells in vitro, by using small molecules in a feeder-free environment. Thereafter, we have been adapting to this protocol to generate human pancreatic beta-cells using two different iPS cell lines.

After two months of differentiation in a three-dimensional spinner flask environment, we have generated cell clusters that express specific pancreatic markers throughout the differentiation process. As expected, at initial stages we find early pluripotent markers such as OCT4 are highly expressed (as detected by both flow cytometry and immunostaining). Following the addition of different growth factors and small molecules, pancreatic lineage markers (such as PDX1) are increased and, mirroring known beta-cell development, subsequently decreased in the later stages when the late pancreatic progenitor marker NKX6.1 is expressed. The cells at the final stage are heterogenous, containing both insulin and C-peptide that are hallmark of beta-cells, but also trace amount of non-beta cell markers such as glucagon and somatostatin. Thus far, beta-cells we have produced from both the RIP and CRI1502 iPS cell lines have the capacity to secrete insulin in response to the membrane depolarizing agent KCl but not in response to glucose. Our research is now focusing on increasing the differentiation efficiency and investigating the possible defect of glucose sensing mechanism.

# S21: MiR-146a – role in T cell differentiation and apoptosis, and utility for assessing TNFi therapy response in rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is a debilitating autoimmune disorder characterised by chronic inflammation of the joints. TNFα inhibitors (TNFis) are effective at relieving symptoms but are coupled with adverse side effects, and 30% of patients do not respond to therapy. At present there are no robust biomarkers to rapidly assess the efficacy of TNFi therapy in patients, with current methods requiring a prior 3-4 months of treatment. T cells have been established as likely antagonists in RA, as those in patients display more activated and memory phenotypes compared to healthy individuals and are refractory to apoptosis. MicroRNA-146a (miR-146a) is aberrantly expressed in the CD4+ T cells of RA patients and its expression is positively correlated with TNFα levels *in vivo*. It is upregulated in memory T cells and evidence suggests that it suppresses apoptosis by downregulating FAF1 and FADD. Thus, miR-146a could be a key mediator of aberrant T cell function in RA.

We are using a lentiviral vector to over-express miR-146a in primary human T cells and investigating its effect on T cell differentiation and susceptibility to apoptosis. We are also investigating the effects of TNFα on miR-146a expression in naïve T cells. Naive CD4+ and CD8+ T cells were cultured in the presence of IL-7 and TNFα, plus or minus a TNFα blocking antibody. qPCR analysis showed that TNFα upregulated miR-146a expression in both naive CD4+ and CD8+ T cells. This effect is attenuated by TNFα blocking antibody in a concentration dependent manner. These findings add weight to the increasing appreciation for a role of CD8+ T cells in RA pathogenesis. They also advocate the potential of miR-146a levels as a biomarker that could provide a rapid clinical assessment of an RA patient's response to TNFi therapy, and expedite transition to more successful treatment options.

## S22: Zebrafish as a model for kidney regeneration

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Acute kidney injury (AKI) refers to the rapid loss of kidney function upon damage to the blood filtering tubules of the organ. Mammalian renal tubules are capable of repairing mild AKI via a process involving tubular epithelial cell dedifferentiation, proliferation and re-differentiation. However for reasons that remain unclear, this regenerative process can be derailed with some cells failing to undergo the re-differentiation step. Instead, these maladaptive cells produce pro-fibrotic cytokines and cause chronic kidney injury. A major challenge in the field has been the isolation and study of dedifferentiated tubular cells, largely due to the complexity of the mammalian kidney and a lack of tools to isolate cells during the repair process. We have overcome this hurdle using zebrafish, by developing various tools and assays to visualize and study the tubular repair process, including a novel transgenic line in which the proximal tubule cells are tagged with green fluorescence protein (*PT::EGFP*). Injection of the kidney toxin gentamicin mimics AKI and is followed by a regenerative response, which can be traced by the small molecule dye PT-Yellow. Using gentamicin-treated *PT::EGFP* fish, we have recently found that damaged proximal tubule cells show reduced GFP expression and increased proliferation indicating a dedifferentiated, regenerative state of these cells. To unravel the molecular mechanisms of regeneration, we have established flow cytometric sorting of dissociated proximal tubule cells into purified populations of “dim GFP” (=dedifferentiated cells) and “bright GFP” (=non-injured cells), which will allow us to compare the gene expression profiles by RNA-seq. A better understanding of the genes and signalling pathways operating in these proliferating cells during renal repair is essential in order to develop new pro-regenerative therapies in the future.

## S23: Using iPS cells and kidney organoids to model cystinotic kidney disease

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The lysosomal storage disease nephropathic cystinosis results from mutations in *CTNS*, encoding a cystine transporter, and initially causes kidney proximal tubule dysfunction followed by kidney failure. Modeling cystinosis in rodents and immortalized cell lines has been challenging due to a failure of these systems to fully recapitulate the human disease. As a result, the cause of the kidney failure in cystinotic patients remains uncertain. Here, we overcome these limitations by establishing cystinotic human induced pluripotent stem cells (iPSCs) and differentiate these cells into kidney organoids. We find that both *CTNS*-iPSCs and cystinotic kidney organoids recapitulate the classic characteristics of cystinosis including altered glutathione and cystine levels. Electron microscopy and staining with lysosomal marker Lamp 2 revealed enlarged lysosomes that were predominantly clustered around the nucleus in *CTNS*-iPSCs, compared to control-iPSCS. Furthermore, *CTNS*-iPSCs and organoids display reduced flux through the autophagy pathway, confirmed by increased numbers of autophagosomes to autolysosmes. Delayed flux can be rescued by exogenous expression of *CTNS*, confirming that it is the loss of cystinosin that affects autophagy. We believe this renewable source of cystinotic iPSCs, which can be easily differentiated into human kidney tissue, is an excellent model to decipher the cause of the kidney failure in cystinotic patients.

# Summary of Abstracts for the Poster Session Template

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| S15 | Ventricular specific cardiomyocyte differentiation of mouse embryonic stem cells through modulation of molecular pathways | Satthenapalli, R | Otago University |
| S16 | A clinically relevant technique to rapidly isolate human Adipose-Derived Stem cells. | Williams, E. J. | University of Auckland |
| S17 | Examining the molecular mechanisms underlying the differentiation potential of human adipose-derived stem cells | Iminitoff, M | University of Auckland |
| S18 | Towards CRISPR-Cas9 mediated replacement of the male germline in sheep | McLean, Z.L. | AgResearch Ltd, Hamilton |
| S19 | Bespoke full-thickness human skin as a permanent wound solution | Dunn, E | University of Auckland |
| S20 | Differentiation of human iPS cells into pancreatic beta cells | Shih, B.J.H | University of Auckland |
| S21 | MiR-146a – role in T cell differentiation and apoptosis, and utility for assessing TNFi therapy response in rheumatoid arthritis patients | Paynter, J.M | University of Auckland |
| S22 | Zebrafish as a model for kidney regeneration | Sander, V. | University of Auckland |
| S23 | Using iPS cells and kidney organoids to model cystinotic kidney disease | Hollywood, J. | University of Auckland |