CL1 Clinical Needs and TERM Solutions in Musculoskeletal Trauma

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Despite years of tissue engineering and cell therapy research in the field of musculoskeletal trauma, a very small percentage of treatments have made it into mainstream healthcare. As a result, there remain significant unmet clinical needs, which will require discoveries and innovations in basic, translational, and clinical research. The goal of this discussion will be to outline these needs, and establish a framework for further dialogue about tissue engineering solutions to address gaps in clinical care. Additionally, potential new therapies face significant barriers for successful implementation that include scientific, clinical, industrial, and regulatory concerns, which are all requirements for successful therapeutic translation. The discussion also will address these considerations, which when taken in the context of obstacles faced by industry and the scientific fields, will provide a more complete perspective of how teams need to work together on translational topics to bring any new treatment to the patient.

CL2 Modern operative cartilage treatment of the knee

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Over the last decade, regenerative treatment options have become the more routinely used techniques for treatment of traumatic cartilage defects, with predictable outcomes for their specific indications.

It is of utmost importance that the injury mechanism of the cartilage defects has to be understood. A combined treatment approach of the cartilage lesion itself and the underlying comorbidity is needed to reach sucessful outcome. The talk will reflect this and its importance for the clinical setup.

Small cartilage defects below 2.5cm2 should be treated with microfracture techniques. The outcome reveals initial clinical improvement with moderate detoriation of the clinical results after 3-4 years. The talk will address the techniques, the appropriate rehabilitation and potential recent developments to overcome these drawbacks.

For large chondral lesions in young patients, in particular, autologous chondrocyte implantation is the treatment of choice for restoration of joint health. This talk focuses on indications, results, and outcome predictors of autologous chondrocyte implantation in comparison to other regenerative treatment procedures and discusses improvement options and future perspectives for autologous chondrocyte transplantation. As research activities are increasing in the field of regenerative joint therapy, recent developments may help to overcome remaining limitations step by step.

Osteochondral defects are treated with osteochondral transfer or with autologous chondrocyte transplantation combined with bone grafting. The talk will show the decision making process for the one or the other technique. The potential use of cell free implants will be discussed.

In recent years treatment of early osteoarthritis came more and more into focus of orthopaedic research. In particular regenerative therapy options seem to have a high potential to fill the existing treatment gap for patients with early osteoarthritic changes. This talk will also focus on basic science, recent developments and available clinical data in the important field of operative regeneration procedures for treatment of chondral and osteochondral defects in early degenerative joints.

CL3 The Cardiocentro Ticino Experience on Cell Therapy Approaches in Patients with Ischemic Heart Disease

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Over the past decade, several clinical studies on cell-based therapies in patients with ischemic heart disease were conducted at the Cardiocentro Ticino in Lugano. The Swiss-AMI (Acute Myocardial Infarction) study was the first Swiss clinical trial in cell therapy in patients with AMI. The Cardiocentro Ticino, the University Hospital Zürich, the Inselspital Berne, and the Kantonsspital Lucerne participated in this multicenter, randomized, placebo-controlled phase-II trial. Two-hundreds patients with large, successfully reperfused AMI were randomized in a 1:1:1 pattern into an open-labeled control group and 2 groups receiving autologous bone marrow mononuclear cells administered intracoronarily either early (i.e., 5 to 7 days) or late (i.e., 3 to 4 weeks) after AMI. All bone marrow cells used in the trial were prepared in the cell factory of Cardiocentro Ticino. In this trial, intracoronary infusion of bone marrow mononuclear cells at either 5 to 7 days or 3 to 4 weeks after AMI did not improve LV function, as assessed using magnetiuc resonance imaging, at 4 and 12-month follow-up. In a distinct study (METHODS), the feasibility of percutaneous intramyocardial injection of bone marrow cells in patients with chronic ischemic heart disease was demonstrated. Alternative cardioprotective approaches currently under preclinical investigation at the Cardiocentro Ticino include the use of secreted extracellular vesicles, and particularly exosomes derived from human cardiac progenitor cells. These approaches have proven beneficial in animal models. They are based on the paracrine activities of cells and represent a potential cell-free alternative to cell therapy.

0001 Silk&Co.: Instructive materials for tissue engineering

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ECM is a key regulator of normal homeostasis and cells phenotype, playing a role in different physiological contexts (ex. wound healing, foetal healing, tumour,…).

For Tissue engineering, it can be considered a model for 3D scaffolds design, from a structural point of view but mainly for its active biological role and ability to dialogue with the surrounding cells.

While scaffold for TE should possibly replicate macroscopic properties of ECM, such as morphology, porosity, interconnectivity or even mechanical properties, additionally the materials they are made of should be able to activate biochemical dialogues with the surrounding environment, instructing cells to differentiate or driving their activity to perform their regeneration potential.

Natural materials and mostly natural polymers possess intrinsic bioactive properties, which in many cases can be exploited for the fabrication of tissue engineering matrices.

Examples that will be presented in this lecture are protein polymers of animal fibers, silk first, made by Lepidoptera, but also keratin, main constituent of wool and hairs. In these fibers, constituents' properties are arranged in hierarchical assemblies, their supramolecular structure imparting them high water and solvents stability, and chemical and ageing resistance. Processing and regeneration, i.e., dissolution and reconstitution, recovers materials with intrinsic biochemical cues, which have been demonstrated being favourable for the activation of preferential pathways inducing tissue regeneration.

The lecture will discuss processing and properties of these animal fibres derived proteins and methods for the fabrication of tissue engineering scaffolds, that have been used or proposed for several TE applications, such as bone, nervous tissue, cardiac tissue and so on.

Specific attention will be paid to the biological interaction of these materials with cells, and to their further functionalization to promote instructive pathways for cell differentiation and metabolic activity triggering.

0002 Biodegradable scaffolds for bone and cartilage tissue engineering

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Many biomaterials have been proposed to produce scaffolds aiming the regeneration of many tissues. We have a particular interest in developing systems combining natural polymers and synthetic biodegradable polymers. By proposing those systems for those demanding applications, we aim at obtaining biomaterial systems with enhanced properties namely mechanical properties, processability, cell-friendly surfaces and tunable biodegradability. Our biomaterials may be processed by melting routes (solvent-free) into devices with wide applications such as biodegradable scaffolds, films or particles and adaptable to many biomedical applications.

As an example of processing technologies, electrospinning has recently gained popularity as a simple and versatile technique to produce synthetic polymeric ultrafine fibers. This technique allows the production of non-woven meshes with fiber diameters in the nanometer range, which results in a high surface area-to-volume ratio and high porosity. Additionaly, these nanofiber meshes can mimic the extracellular matrix of human tissues and, therefore, can be used as scaffolds for Tissue Engineering (TE) applications. We have been developing such structures, enhancing its functionality by fine tuning the geometry of the collectors used, and allowing obtaining very special morphologies different than the random aligned structures typically obtained. Furthermore, we have been developing functionalization strategies for those meshes, enhancing their performance for a range of TERM-related applications. The properties of those meshes may also be optimized by various functional modifications further improving the biological performance of the nanofibre meshes.

Many sources of cells were considered for tissue engineering. Embryonic, iPS and adult stem cells are among the most promising to achieve the cell numbers required to have therapeutic relevance. The ethical and political constraints surrounding embryonic stem cell line derivation led most research efforts to concentrate both in iPS and in adult stem cells. We have been proposing adult stem cells from different sources for bone and cartilage tissue engineering applications.

This talk will review our latest developments using natural-based biomaterials and nanofibre meshes in the context of bone and cartilage tissue engineering applications.

0003 Chondrogenesis of human adipose-derived stem cells (hASCs) in 3D collagen scaffold containing decellularized bovine cartilage ECM fabricated via absorption stage printing process for cartilage regeneration

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This study is about the chondrogenic differentiation of human adipose-derived stem cells (hASCs) with various concentrations of decellularized bovine cartilage extracellular matrices (dCECM) (Table 1) embedded in collagen-based 3D structure. The collagen/hASCs/dCECM bioink was printed in 3D porous mesh shape via absorption stage printing process, which uses an absorbing stage and core/shell nozzle to dispense the bioink and crosslinker in an adequate manner at the same time (Figure 1). The printed scaffolds were cultured in media without any differentiation factors to see the effects of the dCECM on the chondrogenesis of hASCs. The chondrogenesis of hASCs was examined by measuring the amount of differentiation markers such as collagen type 2, aggrecan, and SOX9, via quantitative real-time PCR after 3 weeks of culture. Glycosaminoglycan (GAG) contents were also examined via alcian blue stain. Samples with dCECM showed higher GAG expression and chondrogenic differentiation of hASCs compared to control. This may be an interesting result in cartilage regeneration field since the chondrogenesis of hASCs in 3D culture has not been studied with cartilage ECM in absence of other chondrogenic differentiation factors.

Table 1. Various composition of bioinks

Bioink	Collagen (wt%)	dCECM (wt%)
Bioink 1	100	0
Bioink 2	95	5
Bioink 3	90	10
Bioink 4	80	20

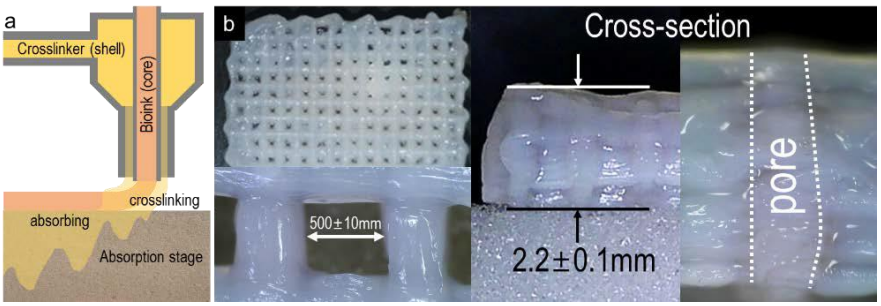

Figure 1. (a) Schematic of the absorption stage printing process and (b) the optical images of 3D collagen/dCECM (90/10) scaffold printed via absorption stage printing.

0004 Enhanced osteogenesis and angiogenesis in chitosan/gelatin scaffolds with controlled rheological properties

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Introduction: Chitosan/gelatin (Ch/Gel) composite scaffolds have been successfully used in tissue engineering applications showing an enhancement of the biological response due to their biocompatibility, biodegradability and low antigenicity. In this study we optimized the fabrication of porous Ch/Gel scaffolds by using various compositions of both compounds, and employing genipin vs. glutaraldehyde as crosslinkers. Moreover, we evaluated the rheological properties of the scaffolds, and assessed the in vitro and in vivo biological responses of pre-osteoblastic cells and Wharton's jelly (WJ) mesenchymal stem cells (MSCs) to validate their osteogenic and angiogenic potential, respectively.

Materials and Methods: Scaffolds were prepared and optimized at various compositions, using genipin or glutaraldehyde as crosslinker. The morphology of pores and pore size distribution was analyzed by scanning electron microscopy (SEM), the physicochemical characterization of scaffolds was performed by FTIR spectroscopy, swelling analysis and determination of the degradation rate. The scaffolds were characterized in a sensitive strain-controlled rheometer involving dynamic oscillatory measurements at 25 and 37 °C.

WJ-MSCs cultures for the in vitro angiogenic response were established from the inner lining of umbilical cords from physiological labours, and immunophenotypically characterized by flow cytometry. MC3T3-E1 pre-osteoblastic cell culture was used for the in vitro osteogenic assessment of the scaffolds. We performed the colorimetric assay PrestoBlue® for cell viability and proliferation, immunocytochemical staining for cell visualization by confocal fluorescence microscopy, cell morphology by SEM, collagen production in the ECM by a modified Sirius Red assay, examination of the CD31 marker expression by means of flow cytometry and confocal microscopy. Orthotopic osteogenesis was evaluated 8 weeks post-implantation in the femur of an adult male C57BL/6 mouse.

Results: We prepared Ch/Gel scaffolds at various compositions, with the 40%-60% Ch/Gel crosslinked with 0.1% wt glutaraldehyde showing the optimal morphology of interconnected pores and a homogenous pore size distribution with an average pore size of 100 μm. The scaffolds have a modulus of 3 kPa, indicate a weight loss of 48% after three weeks of degradation in PBS, support cell adhesion and viability, and significantly increase proliferation and infiltration of both cell types within the pores. Collagen production in the ECM is significantly elevated after 14 days in culture compared to the TCPS control. Histological analysis of in vivo samples retrieved after 8 weeks showed densely nucleated extracellular matrix surrounding the material and indicating calcium deposition.

Conclusions: Ch/Gel crosslinked scaffolds support adhesion, viability, proliferation, osteogenic and angiogenic differentiation in vitro and indicate the formation of new mineralized bone in vivo. Our results demonstrate a promising strategy for the application of the Ch/Gel scaffolds in vascularized bone tissue regeneration.

0005 clickECM - integration of click-groups into cell-derived human extracellular matrix to create ECM-based scaffolds and coatings

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The extracellular matrix (ECM) is a complex network of biomolecules which surrounds the cells in a human tissue. Primary cells are capable of producing an ECM in vitro which can be isolated after several days of culture. Because of its diverse functions and due to the high biological activity, the isolated ECM is a promising biomaterial for the use in tissue engineering and regenerative medicine The ECM is able to promote cell adhesion, proliferation, and differentiation in a tissue specific manner. However, the use of ECM as coatings or scaffolds is limited, e. g. due to minor mechanical stability. In our approach we used Metabolic Glyco Engineering (MGE) to introduce azide groups into the glycan structures of the ECM to create a variously deployable »clickECM«.

Histochemical and immunofluorescence analysis were performed in order to evaluate the incorporation of azides into this clickECM and to further characterize the biological composition of the ECM. We could show that MGE can be used to introduce azide groups into the ECM of human cells. This clickECM consists of glycans, collagens, and non-collagenous proteins whereby the ratio of these biomolecules is the same in clickECM and unmodified ECM.

To investigate the accessibility of the incorporated azides, substrates were functionalized with activated alkynes and clickECM was covalently immobilized via a copper-free click reaction resulting in a significantly increased coating stability compared to a conventional, physisorbed ECM coating. Adhesion, proliferation and differentiation on the clickECM were analyzed with different human primary cells. We could see that cells prefer the ECM compared to other substrates. Additionally, we couldn't detect any differences in cells' reaction on ECM or our clickECM.

We propose that our clickECM is a promising technology to e. g. generate coatings with an increased stability with high biological complexity or to introduce selected (bio)molecules into the clickECM to build up tissue-specific scaffolds for tissue engineering and regenerative medicine.

0006 Magnetically responsive tropoelastin hydrogel as a platform for soft tissue regeneration applications

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The natural polymer tropoelastin is a structural protein of ECM of tissues requiring elasticity as part of their function, including ligaments and tendons. Tropoelastin has an innate capacity of self-assembly into high-order structures, and together with elastic resilience, structural stability and bioactivity bring forth pleasant singularities in adopting it as a building block to fabricate hydrogels. Moreover, easy tailoring of properties can be attained via incorporation of specific components into the polymeric network, including magnetic nanoparticles (MNPs), which are beneficial for on-demand therapies. Thus, the main goal of this work consisted in developing a magnetically responsive tropoelastin (MagTro) hydrogel as a platform to study the response of tendon cells to a mechanical stimulus induced by application of an external magnetic field (EMF). For this purpose, to first produce hydrogels, a solution of recombinant human tropoelastin was first freeze-dried overnight inside a mould and then chemically cross-linked inside an open desiccator via vapour glutaraldehyde. Thereafter, MagTro hydrogels were obtained through *in situ* precipitation of MNPs by immersing tropoelastin hydrogels in $FeCl_2$ and $FeCl_3$ solution overnight and secondly by soaking them in NaOH. Hydrogels were then analysed morphologically by Scanning Electron Microscopy (SEM and Cryo-SEM). Enzyme-triggered degradation was studied after 72h at 37ºC in a human neutrophil elastase solution. Hydrogels exhibited a quick magnetic responsiveness to an EMF (Fig.1). Interestingly, MagTro hydrogels exhibited smaller pores as observed by Cryo-SEM. This feature can be tuned according to different soft tissue requirements by controlling different parameters of the fabrication process. Additionally, the release of tropoelastin into solution decreased, which suggests the formation of a surface coating of MNPs on tropoelastin network, protecting the hydrogel from a faster degradation. Preliminary results also indicate that cultured cells are viable and spread at the surface of the hydrogel. The application of an EMF to cell-laden MagTro hydrogels will be further investigated.

Overall, the streamlined fabrication of MagTro hydrogels was successfully attained and the hydrogel formulation represents a promising potential platform for soft tissue regeneration.

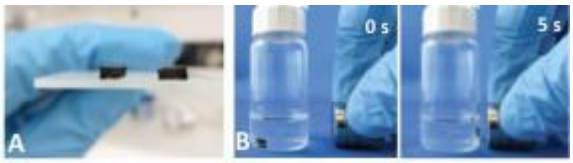

Figure 1. (A) MagTro hydrogels and (B) magnetic responsiveness to an EMF.

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0007 Standardized implant systems for murine and rodent bone regeneration studies

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The implant kits proposed opens new fields of interaction e.g. in molecular biology, where knockout techniques require the use of proper internal fixation in mice which represents the clinical situation. The lack of such systems triggered the ongoing development of RISystem. Systems used so far hitherto were not available at large and e.g. not standardized in stability or introduced uncontrolled loading.

For genetic studies with knockout technology the use of mice/rat is practically a prerequisite (Fig. 1.). Experimental studies of fracture healing require exactly defined, reproducible mechanical conditions. An approach to solving these problems using standardized technologies is reported here.

The methods hitherto available were regarded as open to improvement with respect to definition of the mechano-biological circumstances, standardization and/or simulation of conditions in human fracture treatment.

The goal of developing the new technologies for use in mice and rats was to provide several technologies of fracture or osteotomy fixation similar to the technologies used in humans avoiding mechanical artefacts and providing well standardized conditions. Absolute stability or different degrees of reversible instability should permit investigation of the effects of fixation stability on fracture healing and non unions under different genetic conditions achieved by knockout technology.

The availability of a range of fixation stabilities will permit investigation of both direct and secondary healing as well as mechanically induced, delayed healing and non union.

Micro surgical techniques are demanding but the results obtained so far by different institutions indicate that no major problems were encountered during handling.

The majority of the more 15’000 cases done at different institutions with the implant systems so far were performed using flexible splinting, compression technology and bridging of large defects.

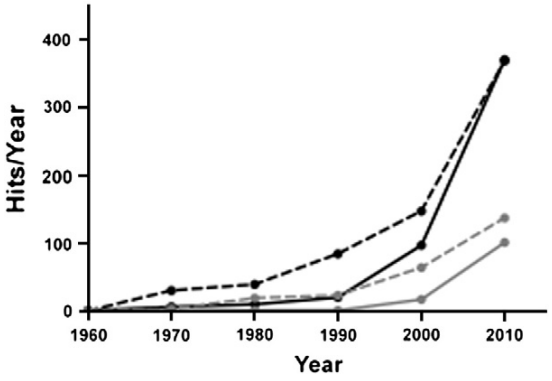

Fig. 1. Analysis of the number of publications listed per year using the Medline database, searched by keywords “bone repair and rats” (dotted black line) and “bone repair and mice” (black line) and “fracture healing and rats” (dotted gray line) and “fracture healing and mice” (gray line) from 1960 to 2010.

0008 Murine models of bone regeneration: increasing reproducibility and rigor

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Until the introduction of transgenic and knockout animal models, long bone regeneration studies utilized predominantly rabbit and dog models, and then by the late 1970s rat models were more heavily adopted. However, in the past several years, the utilization of mice (murine) for bone regeneration studies has surpassed the use of rats. The advantage of mice is their short generation time; the cost to purchase and house; reduced heterogeneity because of consistent genetic background; the ability to prospectively follow specific cell types using reporter transgenes; and perhaps most importantly the ability to study the impact of deletion or over-expression of specific genes in a temporally and spatially defined manner. However, mice have limitations. Their small size renders them challenging for production of reproducible bone injuries and there are concerns about the appropriateness of mice for modeling human disease.

We will present an overview of the variety of bone regeneration models in mice, discussing advantages and disadvantages of each. We will discuss a variety of factors that impact study outcome, and finally, we will discuss systematic approaches for decreasing variability and increasing reproducibility. As an example, modifications to fracture generation procedures in our own laboratory resulted in a 98.3% success rate in generating simple transverse fractures, with much greater consistency in the site of fracture. Rigorous design and evaluation approaches need to be taken to insure consistency.

0009 Design of an animal study - a stepwise approach

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Research using animals is a topic that rightly is under high public and scientific scrutiny. Such studies are lab- cost- and time-intensive. Therefore, it is as well ethically and scientifically crucial to design, execute, analyse and interpret experiments properly.

In order to conduct ethically and scientifically sound preclinical studies a stepwise approach is suggested and discussed:

As starting point, a well-defined, **clear research question** has to be addressed and a hypothesis formulated. Trying to answer too many questions in the same study is quite often an issue and may lead to non-conclusive results. At this point, alternative methods (e.g. computer models, in vitro models) have to be considered, if available. If an animal study has to be conducted to answer the research question, the **outcome variable(s)** should be defined in order that the research question can be answered. Research question, hypothesis and outcome variable will define **species, strain and model** to be used. It is important to keep this order so that the most relevant preclinical model is chosen for each research question. In each study, one or more **controls** (e.g. negative, sham and/ or positive) have to be included.

In order to specify the **number of animals per group**, it is essential to define what effect/ difference (i.e. effect size) is considered to be relevant and biologically/clinically meaningful, respectively. In other words, which effect size the investigator wants the experiment to be able to detect. Since the effect size is dependent on the standard deviation, there is a **need to control variation** and hence, a need for **standardization** and a well-defined study plan (i.e. housing conditions, feeding, health status, surgical technique incl. implant systems, anaesthesia and analgesia protocol, postop care).

Inclusion and exclusion criteria for animals involved in the study are defined and animals should be allocated **randomly** to the different groups. It might be needed to use randomized block design to take into account heterogeneity or variation within animals and/or research material over time. Last but not least, investigators should be **blinded** until the data is analysed to avoid any bias.

This general stepwise approach are explained and illustrated with examples of preclinical bone regeneration studies.

0010 Preclinical orthopaedic animal models: making them ethical, relevant and reproducible

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Animal-based research continues to provoke controversy in society. There is a growing realisation that many of the preclinical models on which we have relied may not be as predictive and reproducible as had once been assumed, making arguments about ethical cost-benefit much harder to defend. While there remains a critical role for animal studies in musculoskeletal research, the last decade has seen a move towards increasing regulatory oversight of the use of animals in research. This talk will present a framework for how to design and perform scientifically robust, clinically relevant and ethical animal studies. At the heart of this discussion will be the practical application of the fundamental tenets of the 3Rs – reduction, refinement and replacement – first proposed by Russell and Burch in 1959. However, arguments will be presented to consider more widespread acceptance of at least two more Rs – relevance and reproducibility – in the context of translational research. The goals of this presentation will be to challenge existing assumptions, to stimulate the audience members to critically evaluate research projects involving animals, and to provide examples of simple steps that can be taken to ensure scientific and veterinary best practices when using preclinical animal models in musculoskeletal research.

0011 Recent developments in collagen-based nanocomposite biomaterials

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Bionanocomposites constitute a versatile class of materials allowing for the combination of biological molecules with nanoscale materials so as to associate the biocompatibility and bioactivity of the former with the many physical (optical, magnetic, mechanical…) or chemical (reactivity, hydrophobic/hydrophilic balance) properties of the latter. This approach is particularly useful to improve or even provide new functionality to biohydrogels [1].

For instance, collagen-based materials are already widely used in many tissue repair applications. However, as such, they often suffer from poor mechanical properties while their large porosity and high hydrophilicity make them unsuitable as drug delivery systems.

In this presentation, we will show how the incorporation of silica nanoparticles within collagen hydrogels can provide a simple approach to address these limitations [2]. On the one hand, the mechanical behaviour of these nanocomposites was highly dependent on the silica particle size and surface chemistry as well as on collagen concentration. On the other hand, it was possible to achieve the controlled release of antibiotics [3] as well as protein delivery via in gel gene transfection [4]. These results also provide interesting insights into silica cytochemistry.

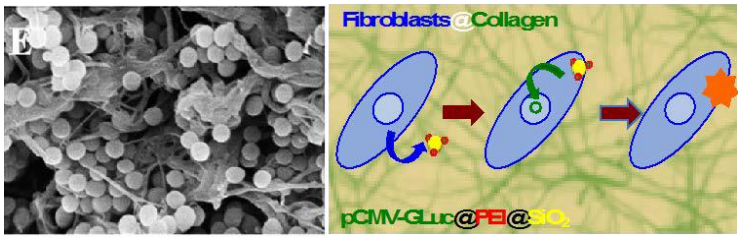

Fig. Collagen-silica nanocomposites and their use for in gel cell transfection [3,4]

Recent results on the extension of the nanocomposite approach to collagen associations with synthetic polymers and other biomacromolecules will also be presented.

0012 Visible light based micro-encapsulation of mesenchymal stem cells for osteoarthritis treatment

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Osteoarthritis (OA) is associated with the degeneration and limited capacity for regeneration of articular cartilage tissue. Current clinical therapies control the patients' symptoms in the short term, but do not address long-term regeneration, therefore strategies to engineer fully functional replacement tisse are desirable

Mesenchymal stem cell (MSC) have been used for cartilage regeneration due to their chondrogenic potential and immune-privileged status. The injection of the cells reduces invasiveness and lowers inflammation risk compared to to conventional surgery. However, several key challenges in maintaining cell viability, efficient chondrogenesis and retention within the local lesion limit current therapeutic potential.

Here, we present a strategy to address the aforementioned problems. Specifically, we utilized norbornene modified gelatin and a poly(ethylene glycol) crosslinker to construct a biocompatible culture environment via bio-orthogonal visible light induced click chemistry.

We applied a novel and cost-effective pipette tip-based microfluidic device to fabricate non-immunogenic protein microparticles containing encapsulated MSCs. The microparticle size and cell encapsulation density can be conveniently altered by simply adjusting the continuous phase flow rate. MSCs exhibited high viability post micro-encapsulation and also after 1 week in culture, demonstrating the biocompatibility of this micro-encapsulation technique as well as the feasibility of gelatin microparticles for long-term maintenance. In addition, numerous chondrogenic markers showed positive signal confirmed by immunostaining. Finally, qPCR was employed to evaluate the chondrogenic target gene expression in the microparticles in comparison to *in vitro* standard chondrogenesis pellet cultures. Here, MSCs cultured in microparticles with chondro-inductive supplement resulted in significantly higher upregulation of cartilaginous protein genes (Aggrecan, COMP and Col2A1) compared to the pellet culture, demonstrating the advantage of our microparticles in regard to supporting chondrogenesis. Furthermore, a more hyaline-like rather than fibrous-like cartilage tissue was identified in the microparticles based on further gene analysis.

In short, with our well-defined chemical composition and facile microfluidic approach, micro-encapsulated MSCs are expected to emerge as a promising therapeutic candidate for the treatment of OA.

0013 RNA extraction from peptide hydrogels enables gene expression analysis for 3D stem cell culture

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Human induced-pluripotent stem cells (hiPSCs) hold great potential for applications in tissue engineering and regenerative medicine, including: autologous cell therapies, drug screening and disease modelling [Hirschi et al. 2014 Biomed. Eng. 16:277]. However, a major challenge in unlocking their clinical potential is finding a fully-defined, xeno-free cultivation platform which is able to regulate hPSC adhesion but simultaneously supports their self-renewal and pluripotent nature [Fan et al. 2015 Stem Cell Rev. 11:96]. One potential avenue is to encapsulate stem cells in hydrogels [Tsou et al. 2016 Bioactive Mat. 1:39]. Of particular interest is a family of peptides based on the alternation of polar, hydrophilic and hydrophobic residues which self-assemble to provide both a tuneable and biocompatible platform [Mujeeb et al. 2013 Acta Biomat. 9:4609]. Optimising these peptide hydrogels for stem cell culture relies on our ability to understand how cells respond to changes in material composition. However, current methods of analysis have been optimised for 2D cell culture and are not compatible with cells encapsulated in different biomaterials. Since the biological response of cells to their surroundings is mirrored by changes in gene expression, we focused on optimising a method for isolating good-quality RNA from encapsulated cells which would then enable downstream applications such as qPCR. The aim was to apply this method to understand how changing the properties of these peptide hydrogels alters stem cell behaviour.

We investigated the extraction of RNA from four peptide formulations which vary in sequence length, net charge and charge distribution. In brief, we encapsulated HEK293 cells in all four peptide hydrogels and tested three different methods of RNA extraction: 1) precipitation-based extraction (Tri Reagent), 2) solid-state RNA binding to a silica membrane (Qiagen), or 3) a combination of both methods. We found that for all four peptide hydrogels the concentration of RNA was significantly lower than the cell-only control when using all three methods. We then demonstrated that our peptide binds to RNA preventing its extraction and that pre-enzymatically degrading all four peptide hydrogels significantly improved both the purity and concentration of RNA. The column based method (2) was found to be more suitable as we hypothesise that the strong binding of the RNA to the column competes with the peptide binding allowing the de-complexation of the RNA from the peptide. We also highlighted that minor differences in the peptide sequence can alter their susceptibility to enzymatic degradation. RNA extracted from all peptide hydrogels following pre-enzymatic treatment was able to act as a suitable template for qPCR, producing Ct values <20 for five different housekeeping genes. We then demonstrated that peptide hydrogels provided a platform which supports the survival and growth of hiPSCs during short-term 3D culture and showed how changing the mechanical properties of the hydrogel network influenced the pluripotency of these stem cells.

This works outlines an optimised protocol for RNA extraction from cells encapsulated in peptide hydrogels and uses it to provide accurate insight into how these hydrogels regulate stem cell behaviour.

0014 Oxygen mapping in 3D tissue engineering hydrogel constructs

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Tissue engineering (TE) approaches typically envisage the functional reconstitution of damaged tissue *in situ*. An adequate three-dimensional environment is therefore of vital importance for the cells associated to the scaffold material. Besides supporting the deposition of essential extracellular matrix (ECM), the biomaterial architecture may affect the nutrient and oxygen supply towards cells incorporated inside the construct. Due to the physical diffusion limit of oxygen (200 µm), an adequate oxygen supply is a major challenge on the design of TE constructs. The lack of sufficient oxygen may be detected by hypoxic areas within 3D scaffolds, which may affect the physiology of cells exposed to it. Accordingly, cell proliferation, gene expression and ECM deposition may be altered. In this study, we analysed the level of dissolved oxygen within collagen hydrogel scaffolds loaded with single as well as co-cultures of human adipose-derived mesenchymal stem cells (hAMSCs) and human articular chondrocytes (hACs). In addition, the oxygen data obtained was correlated with cell viability (LDH and Alamar blue) and proliferation (DNA via PicoGreen). Biomechanical measurements (Young's modulus) were made by the use of a nanoindenter. Gels with different stiffness were produced by adding hyaluronic acid (HA) in different concentrations. The Young's modulus data was also correlated to the oxygen levels within the 3D constructs. For the analysis of the oxygen levels *in situ*, an optical fibre-based micro sensor setup (MicroxTX) and a camera supported non-invasive optical sensor foil-based technique (VisiSens) were used. These complementary analytical tools enable the identification, localization, and temporal follow-up investigation of designated areas within TE constructs. Dependent on the seeded cell density, the *in vitro* cO_2 within the gels reached physiological ranges after 21d or 35d of culture. The lowest cO_2 was measured after 35d *in vitro*, featuring an oxygen level of 4.8 ± 1.3 %. Upon prolonged culture, a plateau-like status of the cO_2 around 8-9 % established, indicating a change in the physiological activity of the cells. Levels of dissolved oxygen increased significantly with increasing hACs percentage in the hAMSCs/hACs co-cultures (21d of culture). Single cultures of hACs featured a level of dissolved oxygen concentration up to 5.5% higher when compared to single hAMSCs cultures. Interestingly, adding HA to the gels resulted in a significant decrease in oxygen concentration. These gels were also characterized by higher Young's modulus. We conjecture that the low levels of oxygen in the Coll-HA composite hydrogels might be the result of poor oxygen diffusion due to higher stiffness of those gels. Our results demonstrated how the selection of biomaterials, mechanical properties of scaffolds and cell-cell interaction may impact the oxygen distribution in the final TE construct. Our data supports the importance of oxygen mapping within TE constructs and its correlation with cell viability and proliferation *in vitro*.

0015 In vitro and organ culture evaluation of chemically crosslinked hyaluronic acid hydrogels for nucleus pulposus repair

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Introduction: The ability of intervertebral discs (IVDs) to withstand cyclic mechanical loads is strongly linked to the confinement of nucleus pulposus (NP), a highly hydrated tissue with strong swelling potential. With degeneration, the confinement and/or swelling potential of the NP is diminished. Therefore, there is the need for hydrogels which (i) have high swelling potential, (ii) are capable of withstanding cyclic loads and (iii) support IVD regeneration. Herein, we evaluate the potential of chemically crosslinked hyaluronic acid hydrogels to address these goals.

Materials and methods: Hyaluronic acid crosslinked with 1,4-butanediol diglycidyl ether hydrogels (BDDE-HA) (degree of crosslinking, DS= 7.5-100%, 2.5% w/v) were prepared [1] and lyophilised. Swelling analyses were performed in DMEM-10% foetal calf serum. Swelling ratio was calculated as $(w_1-w_0)/w_0$, with w_1=wet weight and w_0=lyophilised weight. For in vitro studies, bovine nucleus pulposus cells (NPCs) were seeded on the lyophilised gels and cultured free swelling or in an agarose mold. Cell viability and matrix deposition were evaluated by lactate dehydrogenase/ethidium homodimer and safranin-O/fast green staining, respectively. Gene expression was quantified by RT-PCR. For whole organ cultures, bovine nucleotomized IVDs filled with NPC-seeded hydrogels were cultured under loading (0-0.2 MPa, 0.2 Hz, 1 hr/day) for 3 weeks.

Results and discussion: BDDE-HA swelling ratio was proportional to the extent of crosslinking and ranged from 40 to 120, which is comparable to young bovine NP tissue (~40). (Fig. 1a). Gels with DS 7.5 lost cohesion after day 7. NPC viability was maintained in BDDE-HA (Fig.1c), but cell loss was observed. IVD marker (collagen type I, collagen type II, carbonic anhydrase 12, keratin 19) expression in NPC was influenced by the degree of crosslinking with high ACAN and COL2 expression levels in BDDE-HA DS 15% (Fig. 1b). In organ culture, all hydrogels fully occupied the nucleotomized space following cyclic loading (Fig. 1d), attesting their ability to recover height following dynamic compression.

Conclusion: BDDE-HA hydrogels (i) have high swelling potential, (ii) are capable of withstanding cyclic loads in a nucleotomized disc and (iii) support re-differentiation of culture-expanded bNPCs, therefore these hydrogels have a strong potential for NP repair. **References:** [1] D'Este M, Renier D, US Patent 8,846,640.

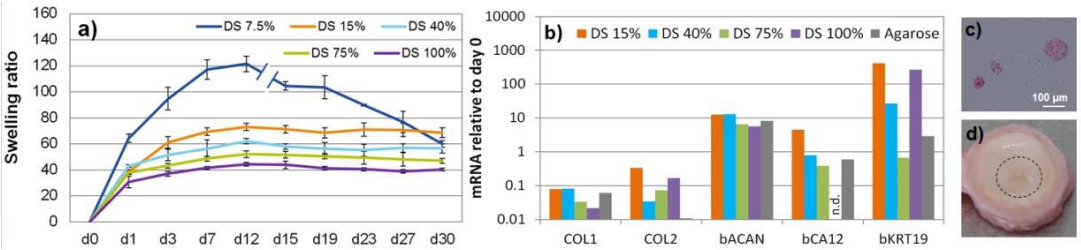

Fig.1: a) BDDE-HA swelling ratio, b) mRNA levels in NPC in BDDE-HA at day 14, c) Viable NPC aggregates in HA-BDDE (DS=15%) at day 7, d) transversal section of nucleotomized IVD filled with BDDE-HA (DS=15%).

0016 Tyramine-modified hyaluronan hydrogel for mesenchymal stromal cell encapsulation: cell viability and rheological properties

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Background: Regeneration of degenerative and post-traumatic cartilage defects using mesenchymal stromal cell (MSC)-laden injectable hydrogels provides a desirable approach in regenerative medicine. For this purpose, a tyramine-modified hyaluronic acid-based (HA-Tyr) hydrogel has been developed that covalently crosslinks in a horseradish peroxidase (HRP)-catalysed reaction in presence of hydrogen peroxide (H_2O_2). This study investigated the cytocompatibility and rheological properties of the MSC-laden HA-Tyr hydrogel and determined the bonding strength of HA-Tyr to native cartilage. **Methods**: Culture-expanded human bone marrow-derived (hBM)-MSCs were exposed to various concentrations of HRP (0.1–1.0U/ml) and H_2O_2 (50–1200µM) and cell viability was assessed using CellTiter-Blue after 24h and 96h of treatment. The same range of HRP and H_2O_2 concentrations was used to determine the viability of hBM-MSCs in 3D culture after encapsulation within HA-Tyr hydrogel discs (6mm x 1mm). Live/dead staining was analysed by confocal microscopy. The visco-elastic properties of HA-Tyr with and without cells were determined by rheological measurement. To measure the adhesive strength of cell-free HA-Tyr to native cartilage, push-out tests were performed using articular cartilage isolated from bovine stifle joints. Samples were processed for histological analysis before and after the push-out test. **Results**: In monolayer culture, all concentrations of HRP tested alone caused a moderate decrease in cell viability at both 24 and 96h, whereas H_2O_2 alone caused a dose-dependent decrease in cell viability with no recovery at 96h after exposure at 600 and 1200µM. Simultaneous treatment with HRP and H_2O_2 resulted in a dose-dependent decrease in cell viability with increasing concentration of HRP positively influencing cell viability (ED50 at 96h [µM H_2O_2]: HRP 1.0= 643.83, HRP 0.5= 563.40, HRP 0.1= 467.98). Rheological measurements revealed gel-formation (G"/G'> 1) in all formulations, with the exception of the lowest concentration (HRP 0.1+ H_2O_2 50µM). In 3D culture, live/dead staining demonstrated a homogenous cell distribution. hBM-MSCs appeared viable and round-shaped at 24h and began to spread after 7d. These data were confirmed by CellTiter-Blue, which revealed no significant differences and time-dependency in a specific dosage range of HRP (1–0.5U/ml) and H_2O_2 (150–600µM). Within this range the rheological properties of HA-Tyr can be precisely tailored (G' 100–900Pa). In addition, cell-free HA-Tyr (HRP 0.5U/ml+ 600µM H_2O_2) was observed, following Toluidine blue staining, in direct contact with the native cartilage after push-out test was performed. Biomechanical measurements revealed a bonding strength of 22.0± 3.09kPa, improved by chondroitinase ABC pre-treatment of articular cartilage prior to in situ crosslink (48.2± 11.7kPa). **Conclusions**: In a mid-range dosage of HRP and H_2O_2, viable hBM-MSCs can be encapsulated and maintained in a 3D environment. HA-Tyr can bind to native cartilage and its visco-elastic properties can be tailored even in presence of hMSCs. These features may be used to select the best conditions to influence the fate choice of MSC towards chondrogenic differentiation. Therefore, we propose HA-Tyr to be a promising candidate as cell carrier and bio-adhesive for cartilage defect repair.

0017 Novel bio-engineered dermo-epidermal skin grafts: a report on phase I clinical data and new experimental findings

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In the last 15 years, the Tissue Biology Research Unit (TBRU) in Zurich has bio-engineered innovative autologous skin grafts for the treatment of skin defects and abnormalities such as burns, chronic wounds, wounds caused by tumor resection and infections, (Marino et al, Sci Trans Med, 2014; Klar et al, Biomaterials, 2014; www.skingineering.ch/publications). Our skin grafts can reach up to 70 times the size of the original biopsy in a relatively short time (min 12 days for a dermal graft, 20 days for a dermo-epidermal graft). After transplantation, the bio-engineered autologous skin graft permanently remain on the patient and replace skin in its full thickness. Phase I clinical studies for one of our products (denovoSkin) are now completed. Results in terms of safety (and already efficacy) are very promising. Multicentric Phase II studies, will start in the second half of 2016. Orphan Drug Designation for the treatment of burn injuries has been reached for denovoSkin both in Europe and Switzerland.
Our present research focuses on the development of even more complex skin grafts which will comprise pigmentation (to match the skin color of the patient), and the establishment of a dermal network of blood capillaries (to fasten take and regeneration after transplantation). A corresponding product, Skin2+, is close to being used at the bed-side. Pre-clinical studies (including proof of concept and toxicological studies) have already been conducted. Establishment of the GMP production of Skin2+ is ongoing and within reach. First-in-man trials, on both adults and children, are foreseen for 2017.

0018 Translational platform to develop antibacterial cell therapies for wounds: three years of ambitions and challenges

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Management of burns belongs to “highly specialized medicine” in Switzerland and is concentrated on two National Burn Centers in Lausanne and Zurich. Along with improvements in fluid resuscitation and respiratory and nutritional support protocols that have considerably reduced the rate of early death after burns in our center, we are constantly investigating to reduce the morbidity after burns. We have been applying cell therapy techniques since 30 years by using cultured epidermal autografts and later on, biological dressings with progenitor skin cells. Cell therapies have helped us overcome the main surgical obstacle in burn treatment and for both temporary and permanent coverage of burn wounds. Meanwhile, nosocomial infections remain the major cause of morbidity and mortality in our patients and despite topical antimicrobials, early excision and immediate wound closure, the frequency of persistent burn wound infections due to hard-to-treat multi-drug resistant (MDR) microbes (e.g. Pseudomonas aeruginosa; PA) has increased. We have developed biological bandages, as a temporary dressing for burn wounds, with allogenic progenitor skin fibroblast cells (FE002SK2) on a biodegradable collagen matrix that have shown excellent healing properties. In the frame of a translational research platform, we aimed to improve and further evaluate these bandages and develop new generations of biological bandages with anti-bacterial properties in order to prevent PA infections along with enhancement of wound healing for burns. We focused on different elements of cell therapy; cells, delivery matrix and host tissue (burn wound microenvironment). We aimed to genetically modify cells in order to produce antimicrobial peptides and also embed delivery matrices with various antibacterial compounds that do not induce resistance in PA. We found that the compounds that we added to delivery matrix affected the viability and morphology of cells differently in the *in vitro* culture and in the final formulation. Therefore, choosing the antibacterial compounds to be added to bandages that would be safe to cells in vitro and in the bandage formulation was a major challenge. Moreover, the interactions between added compounds and the structure of delivery matrix affected their antibacterial efficiency and also toxicity. On the other hand, the host microenvironment (burn wound exudate) affected the PA growth, virulence and response for our biological bandages. Importantly, we found that the burn wound exudate affects that viability of our cell therapies and therefore a time window of application of biological bandages on burn wounds was determined and this should be optimized for overall patient care.

0019 Nasal chondrocytes for articular cartilage repair: update on clinical studies

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Purpose The introduction of autologous articular chondrocytes for the treatment of cartilage injuries in the knee has represented a breakthrough in the field of cartilage repair. However this strategy cannot achieve predictable and durable restoration of cartilage structure and function. We previously demonstrated that nasal septum-derived chondrocytes have a superior and more reproducible capacity to generate cartilage tissue and are compatible with environmental features typical of the injured knee. The primary outcome of this first-in-man clinical study was thus to test the safety and feasibility of implanting autologous nasal chondrocyte-based engineered cartilage for the repair of articular cartilage injuries.

Material and methods Nine patients with symptomatic, post-traumatic full-thickness cartilage lesions (2-8cm^2) on the femoral condyle/ trochlea were treated. Patients underwent nasal septum cartilage biopsy. Autologous nasal chondrocytes were isolated, expanded, seeded and cultured in collagen membranes (Chondro-Gide®, Geistlich) according to GMP regulations. After four weeks production, the engineered nasal cartilage grafts implanted via mini-arthrotomy. Patients were followed up clinically for serious adverse events/reactions (safety), radiologically by MRI (feasibility and graft stability, including delayed Gadolinium Enhanced MRI (dGEMRIC) for assessment of glycosaminoglycans content), and patient's self-assessed scores (IKDC and KOOS) (efficacy).

Results For every patient, it was feasible to manufacture cartilaginous grafts embedded in an extracellular matrix rich in glycosaminoglycan and type II collagen. Engineered tissues were stable through handling and could be secured in the injured joints. No adverse reactions were recorded and self-assessed clinical scores for pain, knee function, and quality of life were improved significantly from before surgery to 24 months after surgery. Radiological assessments indicated variable degrees of defect filling and development of repair tissue approaching the composition of native cartilage (the relative ΔR1 approaching ideal level of 1 (significant decrease from mean 1·61 [SD 0·43] at 6 months to 1·38 [0·38] at 24 months; p=0·0073). The water and collagen content of the repair tissue indicated a composition comparable with normal native cartilage.

Conclusion Our results demonstrate safety and feasibility of the method and preliminarily indicate that engineered nasal cartilage grafts can contribute to the repair of articular cartilage defects in the knee. The mean relative ΔR1 of 1.38 suggests that hyaline repair tissue can be achieved, possibly to a higher extent than MACT (2.18) or ACT. This study opens a new approach in biological cartilage regeneration, based on engineering of mature cartilage tissues using autologous nasal chondrocytes which has led to a phase II multicenter clinical study, which is receiving funding by the EU (Horizon2020 program).

Acknowledgments
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0020 Rupture of the annulus fibrosus: clinical presentation and current treatment options

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Disc herniation is frequent clinical consequence of ruptured annulus fibrosus with a life time prevalence of 1-3%. In case of prolonged pain and/or relevant paresis surgical treatment (discectomy) leads to satisfactory outcome in 75 – 80% of patients but a large proportion of these patients develop a recurrent disc herniation (10-18%) or accelerated disc degeneration with pain (25%) resulting in relevant re-operation rates of 15-20% in the long term follow up. The surgeons are confronted with the dilemma to perform an aggressive removal of disc material with the possible consequence of disc collapse or to remove only the herniated fragment with an increased risk of recurrent herniation.

In this overview lecture the current clinical indications and techniques for the repair of the annulus fibrosus will be presented. Techniques include direct repair with glues or sutures as well as partial replacement of the defect. The (limited) published data on outcome as well as possible complications and adverse long term consequences will be shown and critically discussed.

0021 Design requirements for repairing the annulus fibrosus

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The annulus fibrosus (AF) is a fiber-reinforced material consisting of collagen fibers oriented within an extracellular matrix. The fibers are oriented at ±30^{o} with respect to the horizontal plane, providing the disc with excellent resistance to twisting and bending. Disc herniation injury and annular tears increases strains on surrounding tissues and causes disc degeneration. There has been recent interest to develop engineered materials for AF repair, and these materials will need to recapitulate the mechanical behaviour of the native tissue under complex loading conditions. Our previous work used uniaxial and biaxial mechanical testing to characterize AF mechanical properties from healthy and degenerated discs. More recently we developed a method for evaluating failure mechanics of fiber-reinforced materials. The data from this study, along with previous work, provides a design framework for developing materials for AF tissue repair and regeneration.

AF tissue samples were acquired from caudal spine sections of skeletally mature bovines (age ~18 months). Rectangular sections were prepared using two razor blades to specify the initial specimen width, which was limited by the disc height. The front and back surfaces were microtomed to ensure flat-parallel surfaces. Then a razor blade was used to decrease the cross-sectional area at the mid-length, resulting in dog-bone-like specimen geometry. To evaluate the effect of biochemical composition on failure mechanics, glycosaminoglycans (GAGs) were enzymatically digested by soaking the tissue specimens in 0.125 U/mL of chondroitinase ABC. Monotonic and fatigue loading protocols were performed on healthy and degenerated specimens. Fatigue loading was performed at various stress amplitudes to create a stress versus cycles to failure curve (S-N curve).

Notching test specimens resulted in robust failure occurring at the mid-length. Under quasi-static loading condition (i.e. low strain rate), chABC digestion did not alter the toe or linear region modulus; however, chABC did significantly decrease failure stress and strain ($p < 0.01$). As expected, the Young's modulus increased with strain rate for both healthy and chABC digested specimens ($p < 0.05$). Interestingly, failure properties of chABC-digested samples were not significantly different from the control when loaded at a higher strain rate ($p > 0.15$). Although GAGs comprise of less than 15% of the AF's dry weight and the collagen fibers are a major contributor to sub-failure mechanical behaviour, the findings here suggest that GAGs may play a larger role in failure mechanics, especially at lower loading rates.

Fatigue testing of healthy AF specimens demonstrated a tertiary creep response, where rapid strain softening and catastrophic failure occurred in the tertiary region. Moreover, a nonlinear stress-cycle curve was defined that suggests an endurance stress at a value near 60% of the ultimate stress. Defining fatigue material properties for the healthy AF will be important for tissue engineering approaches that aim to recapitulate the mechanical function of the AF. In conclusion, the data from this study provide design criteria for researchers aiming to develop biological repair strategies for the AF.

0022 Identification of functional cell population in the annulus fibrosus for tissue repair and engineering

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Rupture of the anuulus fibrosus (AF) is one major cause of intervertebral disc (IVD) degeneration along with decrease of proteoglycan content in nucleus pulposus (NP). Annulus rupture induces herniation of the nucleus pulposus and alters IVD integrity leading to many spinal manifestations and secondary neural compressive pathologies. These facts support the rational of promoting basic research in in-depth knowledge of the AF.

AF is composed of cells derived from the mesenchyme, which is terminally differentiated towards chondrocytic cells that is far different from that of the nucleus pulposus cells. Thus, certain mesenchymal stem cells may be a suitable candidate. We focused on a transmembrane glycoprotein, CD146 (melanoma cell adhesion molecule [MCAM], MUC18, S-Endo121), expression of which gradually increased in hypoxic cultures of mouse AF cells. CD146 is known to be a putative MSC marker that correlates with high proliferative potential and capability for osteogenic, chondrogenic, and adipogenic differentiation. This is also known as a marker of endothelial cells and pericytes. We demonstrated gradual phenotypic change in primary mouse AF cell culture that was detectable as increase in number of CD146+ cells. This change was inducible rapidly by TGF β-1 and sorted CD146+ cells showed highly developed cytoskeletal organization with actin bundling. Significant upregulation in mRNA expression of SM22α in CD146+ cells corroborated that SM22α contributed to robust actin polymerization. Moreover, CD146 appeared to play roles in distribution of collagen type I to extracellular reason and formation of cell-cell junctions via CD146+ pseudopods. Such interactions among cell-cell and cell-matrix facilitated expression of highly contractile ability that was determined as strong shrinkage in cell-embedded collagen gels. Although, localization of CD146+ cells was limited in the outermost AF layer in the mouse IVD tissue, analysis of CD146 in vitro will provide us clue to regulate cellular differentiation towards flexible, connective tissue-like AF organization.

0023 Biological-basis for designing biomaterials for the injured and degenerated host

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Biomaterials are no longer considered innate structures and using functionalisation strategies tomodulate a desired response whether it is a host or implant is currently an important focus in current research paradigms. Fundamentally, a thorough understanding the host response will enable us to design proper functionalisation strategies. The input from the host response need to be weighed in depending on the host disease condition. In addition biomaterials themselves provide immense therapeutic benefits which needs to be accounted for when using functionalisaton strategies. Strategies such as enzymatic and hyperbranched linking systems, wehave been able to link biomolecules to different structural moieties. The programmed assemblyof biomolecules into higher-order self-organized systems is central to innumerable biological processes and development of the next generation of functionalized scaffolds. Recent design efforts have utilized a devolopmental biology approach toward both understanding and engineering supramolecular protein assemblies. Structural moieties have taken a variety of different forms such as nanofibers and nanoparticulate. This approach has resulted in functionalisation of micro and nanoparticles with biomolecules that include designed peptide motifs, growth factors and a multitude of gene vector systems. In addition, nature itself has abundant structural complexity that can be harnessed for targetted clinical applications. This talk will elucidate some of these ongoing strategiesin our laboratory that pertain to annulus fibrosus.

0024 Cell sources for intervertebral disc repair /regeneration

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Intervertebral disc degeneration, involving changes in the integrity of both the central nucleus pulposus and surrounding annulus fibrosus, is a significant cause of low back pain. Current treatments, both conservative and surgical, are aimed purely at relieving the symptoms without addressing the underlying causes, namely the aberrant cell and molecular biology and loss of tissue integrity. As such, efforts in the spine research community are being directed towards development of therapies aimed at alleviating pain through restoring cell populations and tissue function, with an emphasis on cell based regenerative and tissue engineering strategies. However, in order for these to be successful the phenotype of the intervertebral disc cells must be clearly defined, an appropriate cell source for implantation identified, together with suitable protocols to direct appropriate cell differentiation and formation of a functional matrix formation defined and optimised. Significant advances have been made in defining the phenotype of nucleus pulposus (NP) cells which has allowed assessment of discogenic differentiation of different cell types (specifically mesenchymal stem cells from different sources , but also induced pluripotent stem cells) to an NP-like cell and the development of specific differentiation protocols to ensure formation on an appropriate functional matrix. The presentation will focus on this work including the potential impact that the microenvironmental niche may have on implanted cell behaviour to illustrate the considerations that must be taken in to account when developing repair strategies for the annulus fibrosus.

0025 Human osteoinduction: Translating preclinical promises into clinical reality. The induction of bone formation by recombinant human transforming growth factor-β3 in pre-clinical and clinical contexts

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Reconstruction of full-thickness mandibular defects is a challenging endeavour despite major experimental surgical advances that have hitherto resulted in the rapid generation of novel molecular data on the apparent redundancy of molecular signals that initiate the induction of bone formation in primates. The enthusiastic advocation of bone morphogenetic proteins for therapeutic bone regeneration is now untenable. Alternatives to the failure of BMPs in clinical contexts are now required. Because of the prominent induction of bone by the recombinant human transforming growth factor-β3 (hTGF-β3) in the non-human primate *Papio ursinus*, full thickness segmental mandibular defects were surgically created in 6 clinically healthy animals and implanted with 125 µg hTGF-β3 per gram of allogeneic collagenous bone matrix. The hTGF-β3 induced unprecedented *restitutio ad integrum* of the newly generated and mineralized buccal and lingual plates by day 30. The induction of bone as initiated by hTGF-β3 is *via* the *BMPs* pathway with hTGF-β3 controlling the induction of bone by regulating the expression of *BMPs via Noggin* expression up-regulating endogenous BMPs and it is blocked by hNoggin, providing insights into performance failure of hBMPs. Physiological expression of *BMPs* genes upon implantation of hTGF-β3 may escape the antagonist expression of Noggin and other inhibitors whereas direct application of massive doses of hBMPs, representing a later by product step of the bone induction cascade as set by *TGF-β3* master gene in primates, sets into motion Noggin' antagonist action, as shown by the limited effectiveness of hBMPs in clinical contexts. The prominent induction of bone formation by hTGF-β3 by day 30 in both heterotopic *rectus abdominis* and orthotopic mandibular sites in *Papio ursinus* prompted translational research in clinical contexts. Patient 1: 8 year old male with right mandibular odontogenic myxoma requiring right hemimandible resection. Three month later the 11 cm long defect was implanted with 6 grams human demineralized bone matrix (DBM – pre-combined with 125 µg hTGF-β3 per gram of matrix) for a total dose of 750 µg hTGF-β3. Follow up for 5 years showed that aesthetic and functional developments have been good with scattered yet incomplete regeneration within the erstwhile mandibular defect site. Patient 2: 8 year old male with left aneurysmal bone cyst which required left hemimandible resection resulting in 11cm defect. Reconstruction with 12 grams (10grams loaded with 250 µg hTGF-β3 with addition of further 2 grams of unloaded hDBM) for a total dose of 2500 µg hTGF-β3) with 4 years follow up showing by CT complete regeneration of the mandibular defect with an ossicle duplicating mandibular anatomy and mandibular bone trabeculation with regeneration of the avulsed coronoid process. The implementation of controlled clinical trials will confirm whether these isolated reports of 250 µg hTGF-β3 per gram of matrix will become the novel required newly implemented morphogen' dose in clinical contexts. The rapid induction of bone formation by hTGF-β3 together with

TGF-β1,TGF-β3, *BMP-2*, *BMP-3*, *BMP-7*, *Osteocalcin* and *Runx2* up-regulation but with down-regulation of *TGF-β2*, angiogenesis and capillary sprouting are the novel molecular and morphological data of the induction of bone formation in humans.

0026 Bone remodelling imaging in a human-based *in vitro* co-culture model - A proof of concept

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INTRODUCTION: Osteoporosis is a major disease characterized by low bone mass and structural deterioration leading to an increased fracture risk. To advance the development of therapeutics for osteoporosis, it is imperative to use models that allow the evaluation of the structural changes in bone that occur during the bone remodelling process. Human-based osteoblast (Ob) and osteoclast (Oc) *in vitro* co-culture studies are attractive, because they are close to natural conditions and might allow to elucidate the complex interactions between bone-forming (Ob) and bone-resorbing cells (Oc). A few 3D co-culture models of human mesenchymal stem cells (hMSC) and human monocytes (hMn) have been investigated, but they rely only on the assessment of both cell activities at gene and protein expression level. So far, a model that integrates i) the culture of cells in a 3D mineralized extracellular matrix and ii) monitoring the changes of the structure and the density of the mineralized matrix over time has not been developed, yet. **AIM:** To establish a human hMSC/hMn co-culture model to study and monitor bone remodelling processes using micro-computed tomography (micro-CT) *in vitro*. **METHODS:** hMSC or a co-culture of hMSC/hMn were seeded on pre-mineralized and decellularized engineered bone-like tissues and cultured for up to 35 days in a spinner flask bioreactor at 70rpm. hMSCs were cultured with osteogenic media (OG) to induce Ob differentiation. The co-culture of hMSC/hMn was cultured with OG and growth media containing receptor activator for nuclear factor κ B Ligand and monocyte colony stimulating factor to induce both, Ob and Oc differentiation. Dynamic morphometric parameters were then evaluated based on sequential micro-CT scans. **RESULTS:** A 70.6% decrease in mineralizing surface (fig. A) accompanied by a 198.8% increase in bone resorption rate (fig. B) was observed for the hMSC/hMn co-culture compared to hMSC culture. In line, the registered 3D images obtained by micro-CT showed high bone resorption (blue) and lower bone-like tissue formation (orange) in the hMSC/hMn co-culture (fig. D) compared to hMSC culture (fig. C).

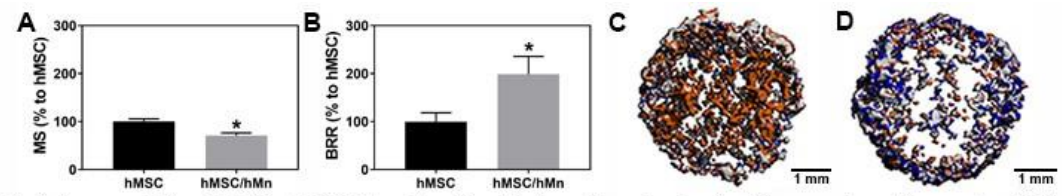

Fig. 1. Bone remodelling in hMSC and hMSC/hMn culture. Quantification of dynamic mineralized tissue morphometric parameters (A, B). 3D images of the registered micro-CT scans at day 21 and day 35 showing newly formed (orange), resorbed (blue) and constant (grey) mineralized tissue in hMSC (C) and hMSC/hMn (D) cultures.

DISCUSSION & CONCLUSIONS: These results are a proof of concept for an *in vitro* human bone remodelling model. The model allows the monitoring of bone resorption and bone formation non-invasively with micro-computed tomography. This study opens a new way to investigate human bone physiology *in vitro* and represents an innovative approach for a high-throughput testing of new treatments for bone diseases while reducing animal experiments.

0027 In vivo safety and efficacy testing of a thermally triggered injectable hydrogel, loaded with hydroxyapatite nanoparticles, to promote repair and regeneration of bone defects

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AIMS: We have previously reported the development of a synthetic Laponite® crosslinked pNIPAM-co-DMAc hydrogel (L-pNIPAM-co-DMAc) loaded with hydroxyapatite nanoparticles (HAPna), which can be injected via narrow gauge needle (26G) into bone to deliver and induce oseogenic differentiation of human mesenchymal stem cells (hMSCs), without the need for additional osteogenic growth factors *in vitro*[1]. This synthetic hydrogel addresses several clinical requirements in one biomaterial: it is cytocompatible in the liquid state and thus enables the safe and homogenous encapsulation of cells and HAPna; the fabrication technique utilised does not require additional chemicals to be added for gelation or clean up and the hydrogel undergoes gelation at body temperature; thus could utilise the patient's own MSCs and be injected directly into the target site to provide a simplified treatment strategy for bone repair or augmentation. This study investigated the delivery, integration and biocompatibility of this hydrogel *in vivo* following injection into a rat femur defect model. We additionally investigated the ability of this hydrogel to augment bone regeneration *in vivo*, both with and without MSCs and HAPna, to evaluate the efficacy of this treatment approach for the repair and regeneration of bone matrix.

METHODS: Pre-set hydrogel constructs were subcutaneously implanted into Wistar rats alongside sham operated controls following 6 weeks, implantation site and organs were histologically assessed for potential toxicity, inflammation or biomaterial redistribution. In addition, blood samples were harvested and full blood analysis perfomed. To evaluate the ability of L-pNIPAM-co-DMAc hydrogel systems to augment bone regeneration *in vivo*, ex-breeder wistar rats, with reduced capacity, were utilised a single bur hole was created in the shaft of the femur and filled with liquid hydrogel or left void as a control. Four experimental groups were investigated: hydrogel without HAPna; acellular hydrogel with HAPna; incorporated with rat MSCs and HAPna or sham operated Following 4 weeks, the defect site and organs were extracted for histological and immunohistochemical examination to determine biomaterial integration, wound repair and inflammatory response as well as micro-computed tomography (Micro-CT) to assess bone mineralisation.

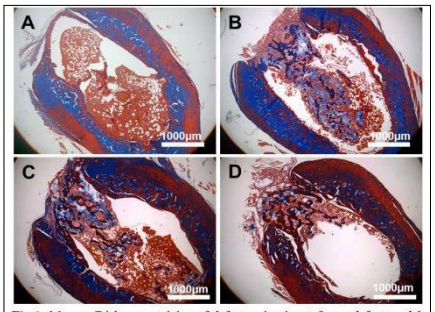

Fig 1: Masson Trichrome staining of defect region in rat femur defect model after 4 weeks. (A) Sham operated control. (B) defect filled with hydrogel + Rat MSCs+ HAPna, (C) acellular hydrogel + HAPna and (D) acellular hydrogel without HAPna. *Scale bar 1000µm.*

RESULTS: No inflammatory reaction, organ toxicity or systemic toxicity was seen in any animal. Where liquid hydrogel was injected into the mid shaft of the femur, rapid solidification was observed and the hydrogel was histologically shown to be maintained within the defect region. Within the defect region integration of the hydrogel with surrounding bone tissue was observed without the need for delivered MSCs; native cell infiltration was also seen and bone formation was observed within all hydrogel systems investigated. No significant difference in the bone volume, as determined by micro-CT, was observed between hydrogel injected defects and controls however increased collagen deposition, was observed where hydrogel with HAPna was injected, which was further enhanced by incorporation of rat MSCs (Fig 1).

IMPACT: The hydrogel was shown to be biocompatible, able to integrate with surrounding bone tissue and promote increased deposition of early markers of bone formation. The low viscosity nature of the L-pNIPAM-co-DMAc hydrogel, enables its delivery directly into the target site, where it can fill both micro and macro fractures to provide a scaffold between adjacent surfaces of bone tissue and thus provide initial mechanical support, facilitate the migration of native cells to aid tissue integration as well as promote the osteogenic differentiation of transplanted stem cells to regenerate a functionally integrated bone matrix. This system could potentially provide safe and efficacious bone regeneration for the repair of small bone defects as well as clinical cases of osteoporosis.

0028 Preclinical testing of vascularized bone tissue engineering in a large animal model

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Until now, the gold standard of treating large-scale and poorly vascularized bone defects remains the transplantation of autologous vascularized bone, however, accompanied by a range of donor site morbidities. Apart from the three tissue engineering key elements scaffold, cells and growth factors, there is an urgent need of a functional vessel network within the bone transplant to ensure optimal support with nutrients and oxygen.

For generation of large, axially vascularized and transplantable bone we developed the sheep arteriovenous (AV) loop model. Endothelial progenitor cells (EPC) and mesenchymal stem cells (MSC) were isolated from sheep blood and bone marrow, respectively, and characterized (flow cytometry, real-time PCR, functional analyses). Cells were seeded on two clinically approved bone substitutes (easy-graft™ CRYSTAL / NanoBone® block) and incubated in a bioreactor for 3 weeks. Different concentrations of bone morphogenetic protein 2 (BMP-2) and vascular endothelial growth factor A (VEGF-A) were evaluated in 2D co-culture. In ongoing experiments NanoBone® block is combined with EPC/MSC, with or without growth factors and placed with the AV loop into a titanium chamber for prevascularization. After 4 weeks the construct is transplanted into a sheep tibia defect and connected to local vessels. Control groups include a non-vascularized scaffold and autologous bone. Evaluation is performed using 3D imaging (MRI, CT, micro-CT, x-ray) and histology after 12 weeks of implantation.

EPC were positive for typical angiogenic markers and showed functional angiogenic properties (tube formation, sprouting, uptake of acLDL-DiI) (Fig. 1A). MSC could successfully be differentiated into different cell types (osteogenic, chondrogenic, adipogenic) and expressed typical stem cell markers. Both bone substitutes showed optimal biocompatibility in the bioreactor setting. With addition of 50ng/ml BMP-2 and 20ng/ml VEGF-A osteogenic and angiogenic marker expression was significantly enhanced. The sheep tibia defect could successfully be established. In the autologous control group 3D imaging techniques revealed an ongoing vascularization in the defect area, but without bony bridging confirmed by histology. We developed a custom-made titanium implantation chamber individually adapted in size and shape according to the sheep's leg (Fig. 1B). In current analyses the AV loop group is evaluated and compared to the clinical gold standard and the non-vascularized scaffold group.

This large animal AV loop model successfully demonstrates the in vivo generation of large and axially vascularized, transplantable bone tissue. The basis sheep tibia defect model will serve as for further preclinical testing and can make it possible to optimize current therapies for critical bone defects leading to faster healing without creating significant donor site morbidities.

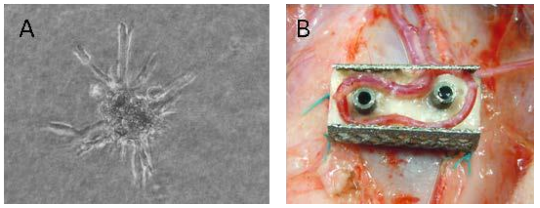

Fig. 1: (A) EPC sprouting, (B) AV loop sheep in titanium chamber

0029 Site-directed immobilization of BMP-2 and noggin inhibitory peptides onto collagen i based recombinant peptide microspheres for bone regeneration

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Bone regeneration requires concomitant processes of osteogenesis and neovascularization. The use of new materials, cells and growth factors has been therefore in focus of bone tissue engineering. In this study, we want to covalently couple Bone Morphogenetic Protein 2 (BMP-2) variants onto collagen I based recombinant peptide (RCP) microspheres in a site directed manner and test their osteogenic potential *in vitro* and *in vivo*. Based on the problems observed in all the approaches developed to incorporate growth factors into biomaterials, such as surface adsorption or encapsulation within polymeric matrices, we decided to immobilize the growth factor BMP-2 site-directly to collagen beads by the use of “click chemistry”. This method potentially overcomes the drawbacks connected with surface absorption and encapsulation, such as the need of large amounts of BMP-2 and poor release-dose control. For this purpose, we created a BMP-2 variant through site directed mutagenesis, comprising one unique non-natural amino acid substitution in each chain of the mature polypeptide, allowing a site-specific coupling by a specified coupling chemistry.

Apart from the physical and chemical aspects connected to the immobilization, we also focused on improving the growth factor´s bioactivity by capturing the BMP antagonist Noggin. BMPs perform their pro-osteogenic effect by binding to and oligomerizing of membrane receptors. This can be hampered by a variety of intracellular but also extracellular antagonists, such as Noggin. Noggin binds to BMPs and interferes with their ability to induce receptor dimerization. Here we identify peptide sequences through phage display method and analyze them for their Noggin-binding characteristics and their potential to inhibit BMP-2 mediated biological responses. Both tools, the covalently coupled BMP-2 and the peptides, which might act as Noggin "deflectors", might produce an innovative biomaterial with superior bone healing properties.

0030 A composite matrix containing various ratios of hydroxyapatite doped with strontium for bone tissue regeneration

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The effective reconstruction of large bone segments remains a major unsolved problem in the clinical field, evident in cases of severe trauma, cancer treatment or maxillofacial reconstructive surgery. Because of the limitations of autologous bone grafts clinicians attempt to prefer the use of synthetic or natural biomaterials that promote bone tissue regeneration and vascularization of the tissue in a cell-free and growth factor free approach. The aim of our project is to develop an injectable osteoinductive and osteoconductive composite polymer composed of doped-strontium hydroxyapatite (HA) dispersed within a polysaccharide-based scaffold for promoting both vascularization and bone formation; The objectives of this work are i) to synthesize and characterize HA doped by strontium with different ratios of strontium (Sr) ii) associate these nanoparticules with polysaccharide-based scaffolds, iiI) analyze *in vitro* the cellular responses of mesenchymal stem cells (hMSCs) to this matrix and then, iv)evaluate *in vivo* the fate of this composite polymer in both ectopic (subcutaneously in mice) and heterotopic sites (femoral condyles in rats). HA particles were synthesized with different ratios of Sr (0%, 8%, and 50%, (w/w)). X-ray diffraction (XRD), Inductively Coupled Plasma (ICP), and particle size analysis (Nanosizer™) were used to characterize these particles. Particles were dispersed at different ratios (10%, 20%, and ≥ 90%) within a pullulan-dextran based matrice (Autissier, 2010), Electronic scanning microscopy Back Scattering Electron microscopy (ESEM-BSE) and ICP were used to characterize the composite scaffolds. Cell viability and metabolic activities of hMSCs were investigated and osteoblastic gene expression was studied by RT-qPCR. Matrices containing these different particles were then implanted subcutaneously in mice and in the femoral condyles in rats. Explants were analyzed by Micro-Computed Tomography (micro-CT), by histology(Masson's trichrome staining) and by Immunohistochemistry(CD31 immunostaining)

XRD analysis revealed the carbonated hydroxyapatite structure of both non-doped HA and Sr-doped HA. ESEM-BSE confirmed the distribution of the HA particles within the matrices. ICP analysisshowed an increase of Sr concentration within the matrix with Sr substitution. *In vitro* studies revealed that Sr-doped HA matrices stimulate the expression of osteoblastic markers, compared to non-doped HA matrices. Subcutaneous implantation demonstrated the formation of a mineralized tissue, with an optimal ratio of 20 % of HA particles within the matrices. Histological analysis revealed osteoid tissue in contact to the material and a significantly higher percentage of tissue mineralization induced by the presence of 50 % of Sr. Immunostaining of CD31 showed that the 50% Sr-HA matrices also promote the formation of neovessels within the tissue. Osseous implantations also highlight the importance of Sr substitution by increasing significantly the mineral content in presence of 50% Sr-HA matrices, compared to undoped matrices or to 8 % Sr.-HA matrices. In conclusion, the ability of this injectable composite scaffold to promote tissue mineralization, bone formation and vascularization is promising. This new matrix could represent a cell-free alternative to the autografts for the regeneration of large bone defects.

0031 Application of pro-osteogenic cell-derived vesicles

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Bone fractures present a rapidly growing worldwide medical and socioeconomic burden that is putting tremendous strain on healthcare-systems. Current treatment approaches have significant limitations; autologous grafts cannot meet demand, allogeneic bone lacks bioactive factors, growth factor-based (e.g. BMP-2) approaches are expensive and have been linked with serious side-effects, and seemingly promising cell-based approaches are frequently prevented by insurmountable regulation, ethical and economic issues. Consequently, there is a considerable need to devise new methods for the generation of large volumes of bone without associated patient morbidity.

Here a novel solution is proposed that delivers all the advantages of cell-based therapies but without using viable cells. By harnessing the regenerative capacity of extracellular vesicles (EVs) that are naturally generated during skeletogenesis we can produce a scalable acellular therapy to meet demand.

Pre-osteoblast murine cells (MC3T3-E1) were cultured in media containing serum that had been purified of EVs. Media was collected from cells every 2 days and EVs isolated using differential ultracentrifugation. Use of atomic force microscopy, dynamic light scattering, and nano-particle tracking analysis confirmed EVs were approximately 150 nm in size. ImageStream flow cytometry was used to demonstrate the presence of membrane lipids and the tetraspanin transmembrane protein CD9.

The therapeutic application of isolated EVs was assessed by adding them to mesenchymal stem cell (MSC) cultures. A significant increase ($p<0.05$) in alizarin red calcium staining (day 14) and the expression of alkaline phosphatase (days 3, 7, 14) were observed in MSC cultures containing EVs. Elemental maps produced using micro-X-ray fluorescence revealed substantial more calcium and phosphorus co-localisation compared with mineralisation induced by the current gold standard, bone morphogenic protein-2.

In conclusion, this study demonstrates the potential use of EVs as a novel therapy for hard tissue regeneration.

0032 Biomimetic materials for corneal regeneration as alternatives to donor transplantation

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Collagen is the main structural component of the cornea and therefore is a good choice for use as implants to replace damaged or diseased corneas. Our current goal is to review the uses of collagen and its analogues as pro-regeneration template in treatment of corneal blindness. Collagen derived from animal or human cadaveric sources, however, suffer from batch to batch heterogeneity and carry a risk of pathogen transmission. These problems are circumvented by using recombinant human collagen (RHC). We have shown that cell-free cornea implants made from RHC can stimulate the regeneration of corneal epithelium, stroma and nerves in patients without the need for immune suppression. In patients with severe pathlogies such as chemical burns or previous graft rejections, addition of a synthetic phosphorylcholine network to the RHC allowed for implants to perform well in these adverse environments and affect stable restoration of corneal integrity in patients with corneal ulcers and surface erosions. Nevertheless, like native collagen, RHCis large, relatively inert, chemically intractable biopolymers, leading to the development of a range of collagen-like peptides (CLPs) or collagen mimetic peptides (CMPs) as collagen analogs. We compared the versatility and functionality of such analogs to that of full-length collagen as scaffolds for promoting regeneration in vivo in mini-pig and rabbit models. We also showed that CLP-based hydrogels affected cell differentiation and regeneration by cell-substrate and cell-cell communication through extracellular vesicles.

0033 Repopulating decellularized porcine corneas with human corneal fibroblasts

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Introduction: There is a serious donor shortage for corneal transplantation to treat patients suffering from corneal disorders. Biomaterials developed for corneal tissue engineering need to be transparent, have sufficient strength to be sutured and promote regeneration once implanted. Decellularized corneas are drawing attention in the tissue engineering field as they match native mechanical strength and maintain most of the micro architecture within the tissue. While several studies have examine methods of decellularizing cornea, few have examined methods of recellularizing them. Hence the aim of this study was to compare different approaches for recellularizing decellularized corneal scaffolds.

Methods: 8-mm diameter porcine corneal buttons were decellularized using 1% Triton X-100, 0.5% SDS, DNAse and RNAse. DNA, sGAG and collagen were analysed using histological staining and biochemical assays. Three recellularization methods were examined using human corneal fibroblasts. Method A involved embedding of decellularized corneal scaffolds in cell seeded collagen hydrogels. Method B involved injection of cells directly into scaffolds. Method C involved seeding of cells in small volumes onto scaffolds. Post-seeding, scaffolds were cultured for 21 days in serum-containing medium in wells covered with anti-adhesive Teflon. After culture, scaffolds were wax-embedded, sectioned and stained with DAPI to assess recellularization.

Results: Decellularization was confirmed by the almost complete removal of DNA and the absence of nuclei present after DAPI staining. For Method A, on average, 16 cells could be counted as migrating from the surface of the scaffold, in a range of 67-703 µm, with a median of 201 µm (Fig 1A). For Method B, the injected cells stayed in place and did not migrate further out of the injection site (Fig 1B). On average, 37 cells could be counted as migrating from the surface of the scaffold or the injection site. Method C resulted the best recellularization method (Fig 1C). Corneal fibroblasts repopulated the acellular scaffolds, migrating in a range from 31 to 613 µm the median being 190 µm. Cells migrated more into the anterior stroma, mimicking the native cornea. When cells were directly seeded on the posterior stroma, they would stratify but not migrate into the stroma.

Discussion: Seeding of cells directly onto decellularized corneas appears to be the best method to enable recellularization to occur. Further studies will focus on the optimization of the recellularization process with the aim of generating functional corneal graft for transplantation. Research is supported by ERC STARTING GRANT [EyeRegen - 637460]

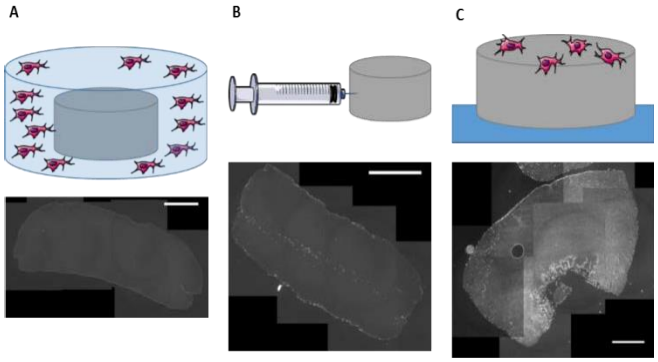

Figure 1. Recellularization of decellularized cornea schematics and histological DAPI stained

0034 Tissue-engineered Descemet's stripping endothelial keratoplasty*, in vivo* proof of concept in sheep

Karl Brown[1], Jean-Pierre Scheerlinck[2], Hong Zhang[1], Berkay Ozcelik[3], Penelope McKelvie[4], Greg Qiao[3], Mark Daniell[1]

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Aim. To conduct a pre-clinical trial of a tissue-engineered Descemet's stripping endothelial keratoplasty (TE-DSEK). Assessment criteria: implant is able to be implanted via a minimally invasive procedure, lack of toxicity and immunogenicity, biodegradation of the scaffold, and abatement of oedema in a model of corneal endothelial dystrophy.

Methods. Tissue-engineered corneal endothelium (TCE) was manufactured as previously described (Ozcelik et al. 2014). Briefly, ovine corneal endothelial cell (CEC) monolayers were cultured to confluence directly on an novel ultra-thin (50 µm) poly(ethylene glycol)-based hydrogel film (PHF). Culture conditions were adapted from those used clinically for cultivated limbal epithelium transplantation (CLET) (Mariappan *et al.*, 2010). Donors and recipients were outbred Merino-Dorset ewes of 12-14 months of age. A model of corneal endothelial dystrophy was created by surgical removal of CEC from a 7 mm diameter area of the central cornea. The TE-DSEK procedure was essentially the same as an existing surgical technique: Descemet's stripping automated endothelial keratoplasty (DSAEK). Negative control animals received a PHF without CEC, or a TCE not placed over the endothelial wound. Animals were observed for at least 21 days post surgery and scored for inflamation, corneal clarity, and oedema on a validated *pro-forma* (Williams et al 1999). Oedema was rated 0 – 4, with 0 being no oedema and 4 being maximally thick. Post mortem whole eyes were subjected to haemotoxylin-eosin histology.

Results. TCE was sufficiently robust for implantation by a DSAEK-like minimally invasive procedure. No evidence of toxicity or immunogenicity was observed in both clinical observations and histological sections. The PHF was completely degraded before day 21. Allogeneic TCE was non-toxic and non-immunogeneic for >20 days (n = 13). In animals receiving TCE the PHF completely degraded in <21 days. In test animals the TCE abated oedema (final score 0 or 1, 70% n = 10).

Impact. There are shortages of donor corneas in many nations. A TCE consisting of CEC cultured on a PHF may be able to replace donor tissue for endothelial keratoplasty. TE-DSEK could be readily adopted by ophthalmologists who are familiar with DSAEK, an equivalent surgical technique using donor tissue.

0035 Response of corneal stromal cell behaviour to variations in substrate stiffness

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Corneal blindness due to injury or disease affects approximately 10 million people worldwide. Due to a shortage of healthy donor tissue for transplantation, methods for regenerating corneal tissue are becoming more attractive as alternative therapies. It is well documented that the proliferation, migration and phenotype of many cell types are affected by the stiffness of their surroundings. In this study, we aim to investigate the influence of the mechanical environment, specifically substrate stiffness, on the growth, morphology and gene expression of human corneal stromal cells. Of particular interest are the physical factors which promote a healthy keratocyte cell type.

Using blends of two commercially available polydimethylsiloxane (PDMS) elastomer kits, cured polymer substrates with elastic moduli ranging from 12 kPa to 3 MPa were achieved. Samples were coated with a thin layer of collagen I to promote cell adhesion. Contact angle measurements were taken before and after protein coating, with a decrease in angle indicating a significant increase in hydrophilicity of the PDMS. Phase contrast imaging was used to visualise differences in morphology of cells seeded onto the substrates, while vinculin, actin and DAPI staining were employed to view cytoskeletal structure under confocal microscopy. Cells on softer substrates displayed increased proliferation, as determined by Presto Blue assay, with a notably spread and elongated fibroblastic morphology. Stiffer substrates induced increased cell aggregates, with a more dendritic, keratocyte-like shape visible. Gene expression of cells was also monitored by qPCR, with observed upregulation of the keratocyte marker ALDH3A1 in cells seeded on less compliant substrates.

The results of this study demonstrate that corneal stromal cells respond to the stiffness of their surroundings, with a higher stiffness resulting in cells most resembling a native keratocyte phenotype. This information can be used to enhance the culture environment for corneal stromal cells and improve the design of scaffolds for cornea tissue engineering.

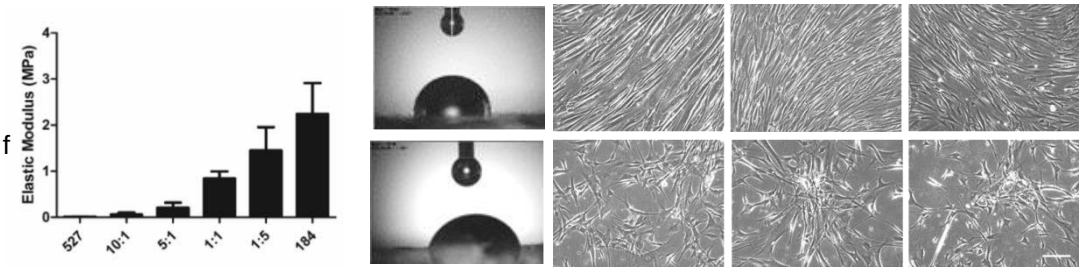

Figure 1. Left: Elastic modulus of PDMS blends determined by tensile testing.

Centre: Contact angle before (i) and after (ii) collagen coating.

0036 The tissue-engineered human cornea: a model to study corneal wound healing

Sylvain Guérin[1,2], Camille Couture[1,3], Pascale Desjardins[1,2], Gaëtan Le Bel[1,3], Karine Zaniolo[1,2], Richard banzin[1,2], Lucie Germain[1,3]

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Several models of wound healing have been developed in order to investigate the corneal mechanisms of reepithelialization and to screen for growth factors susceptible to stimulate an adequate healing response. Although very useful because of their ease of use, cell monolayers *in vitro* models however suffer from the lack of epithelial-mesenchymal interactions and the limited epithelium thickness. In addition, studies of corneal wound healing in animal models are very expensive and inter-individual variability among animals is inherent to *in vivo* experiments. We succeeded in tissue-engineering human corneal substitutes (hTECs) that show appropriate histology, expression of basement membrane components and integrins. The method is based on culturing endothelial and corneal epithelial stem cells from the limbal area in flasks that are used to produce a corneal substitute by the self-assembly approach where human corneal stromal fibroblasts, cultured in the presence of ascorbic acid, secrete and organize their own ECM. Besides being devoid of any synthetic materials, corneas tissue-engineered by our self-assembly approach exhibit a well-developed stratified epithelium that expresses differentiation markers (such as keratine 3), a stroma and a well-organized basal membrane. When mechanically damaged (using a biopsy punch), this fully human, tissue-engineered cornea, produced from living fibroblasts and untransformed human corneal epithelial cells, mimics many aspects of the reepithelialization process including cell migration, proliferation and the restoration of a stratified epithelium.

We recently used our hTECs to investigate genes whose expression is altered during wound healing. Gene profiling analyses were performed by microarrays and further validated by qPCR and Western blot for many of the deregulated genes. Expression of many MMPs-encoding genes was shown to increase in the migrating epithelium of wounded corneas. Most interestingly, analysis of the microarray data revealed important alterations in the expression of a few mediators from the PI3K/Akt pathway (marked increase in PIK3CD, AKT3 and HSPB3 in the wounds) in response to the ECM remodeling taking place during wound healing of hTECs. Profiling signal transduction-activated kinases and mediators on proteome arrays revealed that most of the mediators that become either phosphorylated (such as Akt kinases, WNK1 and P53) or dephosphorylated (such as CREB and STAT5) in the wounded hTECs belong to the PI3K/Akt pathway. Interestingly, whereas wound closure was severely reduced by inhibition of Akt with GDC-0068, blocking activation of CREB with C646 while simultaneously enhancing activation of AKT with SC-79 considerably accelerated wound closure in our hTEC model.

Our completely biological hTEC is therefore very promising as a wound-healing model that will help dissect the mechanisms involved in the corneal reepithelialization process.

0037 3D printed recombinant collagen type III (RHC III) scaffolds and their potential for corneal tissue engineering

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Introduction**:** Corneal visual impairment is the 4th cause of blindness globally (5.1%), according to the WHO[1], and currently the only treatment is surgical intervention using a human donor cornea, a procedure known as penetrating keratoplasty. The greatest challenge facing the treatment of corneal visual impairment is the global lack of donor corneas. The use of 3D printing in tissue engineering (TE) is a way of producing customized scaffolds, offering a tantalising prospect in the treatment of corneal visual impairment. This work studies the potential of 3D printing for producing recombinant collagen type III (RHC III) scaffolds for corneal tissue engineering.

Methods: RHC III scaffolds were formed by nebulizing a 3.4mg/ml RHC III solution in 10mM HCL and accelerating it onto a substrate using nitrogen gas. Layers were formed with assumed thickness of approximately 3µm and repeated 2, 10, or 29 times to achieve thicknesses of up to 90µm. Via this method, patterns were imparted on the sample surface in the direction of printing, and spiral patterns were used. Collagen scaffolds were then crosslinked using EDC [1-ethyl-3-(3-diaminopropyl)carbodiimide] and NHS (N-hydroxysuccinimide) at a molar ratio of 1:1. The thickness of 10- and 29-layer samples was measured using Optical Coherence Tomography (OCT). Corneal MSCs were isolated through collagenase digestion (4 hours) of the stroma. Cells were cultivated in DMEM + 10% FBS. First passage MSCs were seeded onto the collagen scaffold samples consisting of 2 layers (n=4), 10 layers (n=4) and 29 layers (n=4) of RHC III at a density of 100 cells/sample. For controls, cells were seeded on glass (n=3) and plastic (n=3). At day 23, scaffolds were processed for immunocytochemistry (n=9) and inspected via Scanning Electron Microscopy (SEM) (n=3).

Results & Discussion: OCT thickness measurements showed a mean thickness of 28 ± 9µm for 10-layer samples and 87 ± 23µm for 29-layer samples. Light microscopy showed that corneal MSCs proliferated on all printed RHC III scaffolds, regardless of the number of layers. SEM showed the circular print pattern of the RHC III. With immunocytochemistry, we observed collagen type III, and MSCs were observed throughout the samples, indicating penetration into the RHCIII along with superficial growth, which was also observed. The MSCs cytoskeleton stained positive for phalloidin, and we observed alignment of the superficial cells following the direction of the collagen in the SEM images.

Conclusions: These results demonstrate that 3D printing can be used to produce RHC III scaffolds which are suitable as a substrate for cultivating corneal MSCs.

[1]World Health Organization, *Causes of Blindness and Visual Impairment,* http://www.who.int/blindness/causes/en/, accessed 30/11/2016.

0038 Towards clinical translation and commercialisation of living engineered neural tissue for peripheral nerve repair

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Tissue engineering offers exciting possibilities for generating living cellular constructs to replace the nerve autograft, which remains the clinical gold standard for repairing long gap peripheral nerve injuries. Such a construct would likely include therapeutic cells, biomaterials, cell and tissue level architecture and quite possibly other bioactive small molecules. This approach is considerably more complex from a practical manufacturing and regulatory point of view than current nerve repair products such as tubular conduits or decellularised tissues.

Engineered neural tissue (EngNT) is a living aligned cellular biomaterial that mimics the columns of regenerative Schwann cells (Bands of Büngner) in the nerve autograft using therapeutic cells organised within a collagen matrix [1]. Previous work showed that EngNT can be constructed using autologous cells [2,3] and allogeneic cells [4] and that regeneration support was achieved in preclinical models. This presentation will focus on the pathway to commercialisation and clinical translation of EngNT as an example of a potential nerve autograft replacement that would be classified as an advanced therapy medicinal product (ATMP).

0039 Strategies and considerations for the commercialisation of cell and gene therapies for nervous system injury

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Cell and gene therapies are at the forefront of the current approaches that aim to re-establish function following nervous system injury. Nervous system damage can have a major impact on the patients' quality of life and often results in a permanent loss of function. Over 1 million people are affected by spinal cord injury caused by trauma annually[1]. Cell based therapies may offer a novel and promising approach to nervous system repair.

While there have been scientific advances and demonstration of safety in numerous clinical studies there have been limited clinical breakthroughs so far and as such limited investment. As a result this cell therapy sector, unlike others such as immunotherapies, has upcoming challenges to overcome during the commercialisation of potential neuroregenerative therapies.

Cell and gene therapy products present scientific challenges which are well recognised. However one less understood challenge may be the potential to commercialise the product and ensure timely reimbursement. Commercialisation relies on many factors including the correct adoption of strategies that ensure that all risks are considered. These risks should be mitigated in a planned and structured way, feeding into an appropriate business model. Here we outline the challenges associated with commercialisation of therapies for nervous system injury (Fig. 1) and using worked examples, discuss the routes to mitigating these.

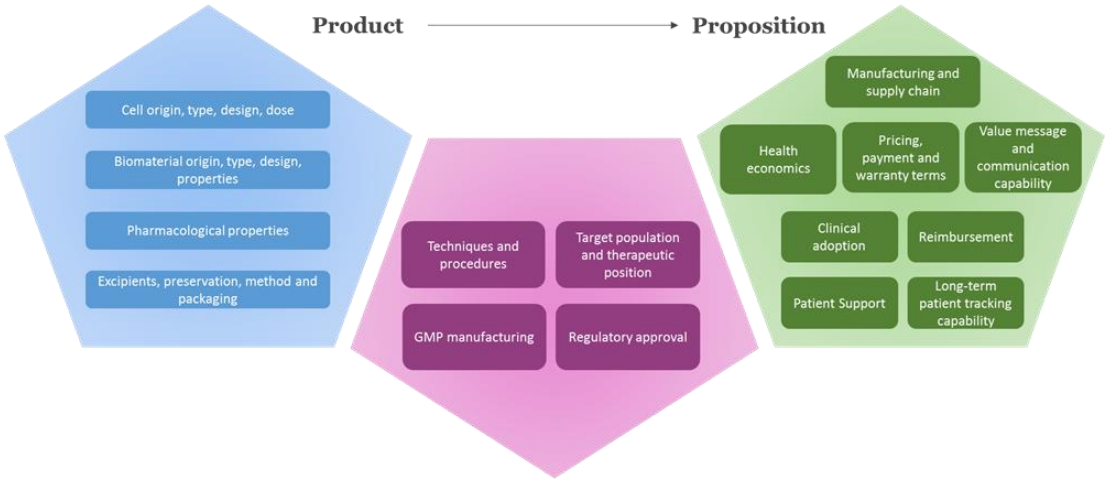

Figure 1. Considerations for the commercialisation of neuroregenerative therapies.

0040 Implantation of human amniotic membrane ameliorates recurring perineural adhesions and intraneural scarring in a rat sciatic nerve fibrosis model

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Inflammation, fibrosis and painful adhesions with the surrounding tissue ("internal scars") represent major complications associated with peripheral nerve surgery. Despite improvements in surgical techniques and postoperative rehabilitation programs, clinical symptoms, such as restricted mobility and chronic pain, often reappear due to recurring adhesions. One effective method to avoid adhesions could be the use of a physical barrier to the surrounding tissue which should be easy to handle, biodegradable and reduce scar formation and adhesion to a minimum without interfering with wound healing. Although several new therapeutic approaches have been tested, they could, in most cases, not sufficiently hinder the recurrence of symptoms.

In this study we aimed to reduce recurring fibrotic adhesions between the sciatic nerve and the surrounding tissue by implantation of human amniotic membrane (hAM), the innermost layer of the fetal membranes. Because of its extraordinary flexibility and with a thickness of less than 0.05 mm, hAM could be the ideal candidate for a biological adhesion-preventing barrier. Also, it is known to exhibit anti-inflammatory and anti-fibrotic properties and has shown anti-adhesive effects as a mesh coating for hernia defects, without causing adverse immune reactions. We tested hAM using a novel rat model for the induction of intraneural fibrosis and perineural adhesions in female Sprague Dawley rats (n = 54). Three weeks after generating severe neural damage, the nerve was re-exposed and wrapped with hAM in our treatment group, or left untreated as a control. The reformation of perineural adhesions was analyzed at 1, 4 and 12 weeks post-implantation during re-exposure using an adhesion score in a blinded manner. Also, quantification of perineural and intraneural fibrosis was performed as well as overall structural analysis via (immuno-)histochemistry. Calculation of the sciatic functional index after gait analysis was used to determine differences in nerve function among the experimental groups at different time points.

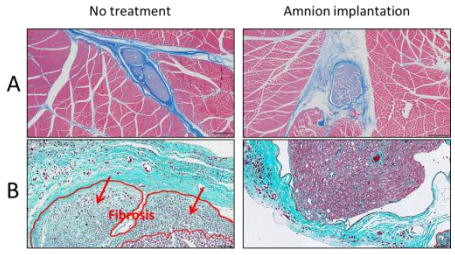

Fig 1. Histological analysis 4 weeks after external neurolysis reveals major differences among the experimental groups. (A) Cross sections stained with CAB of amnion-treated rats show loose connective tissue only in contrast of untreated controls, which demonstrated severe adhesions surrounding nerve and infiltrating the muscle bed. (B) Representative sections of nerves stained with Masson's trichrome for collagen exhibit large amounts of intraneural fibrosis, whereas the neural structure after amnion implantation appears recovered.

As a result, no significant differences could be detected at the early time point. However, from 4 weeks on, major improvements in the extent of perineural and intraneural scarring as well as functional recovery were determined in the hAM-treated group, which persisted until the end of the observation period. Moreover, immunohistological analysis at 4 weeks post-implantation implies accelerated nerve regeneration following amnion implantation.

In conclusion, the implantation of human amniotic membrane represents a very promising therapeutic approach for the prevention of recurring perineural adhesions and the amelioration of intraneural fibrosis, hopefully leading to a better quality of life and alleviation of neuropathic pain.

0041 Development of sterilisation strategies for acellular nerve grafts

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Introduction A process has been developed to remove the cells from porcine peripheral nerves using low concentration sodium dodecyl sulphate. It is hypothesised that acellular nerve could be used surgically as a scaffold to repair peripheral nerve injuries. This project aims to determine the effect of sterilisation methods (Peracetic acid; γ-irradiation; E-beam; Ethylene oxide & Supercritical CO_2) on the biological and mechanical properties of acellular peripheral nerves.

Methods A decellularisation process was optimised to remove all native cells from porcine femoral nerves. DAPI and H&E staining together with DNA quantification was carried out to verify cell removal. Histological staining (Sirius Red & Millers Elastin), antibody labelling for collagen 4, laminin and fibronectin, tensile slow strain rate (10 $mm.minute^{-1}$) to failure testing and contact cytotoxicity testing were carried out to establish baseline data. Acellular nerves (n = 30) were sterilised using 0.1% (v/v) peracetic acid or γ-irradiation (25 kGy). The effects of sterilisation were determined by histological staining, antibody labelling, and mechanical characterisation.

Results Following decellularisation there was a lack of visible nuclei and whole cells (DAPI and H&E) and the DNA content was 16.78 (+/- 10.34) $ng.mg^{-1}$, well below the 50 $ng.mg^{-1}$ limit generally considered as appropriate verification of effective decellularisation. Acellular nerves were found to be biocompatible, and retained structural and mechanical properties similar to native nerves. There was a significant reduction (33 %) in the ultimate tensile strength of acellular nerves treated with peracetic acid compared to native tissue, whereas both acellular nerves and γ-irradiated acellular nerves showed no significant differences. Nerves sterilised using γ-irradiation retained ultrastructural features well compared to nerves treated with peracetic acid, which showed some disruption histologically (particularly to the structure of the endoneurium). Antibody labelling demonstrated the presence of collagen 4 in the endoneurium and perineurium of native and acellular nerves; however, it was not visible in acellular nerves treated with peracetic acid. Collagen 4 labelling was positive but reduced in intensity following γ-irradiation.

Discussion Peracetic acid sterilisation appeared to alter the mechanical properties of acellular nerves, and cause structural and chemical alterations in the endoneurium. The endoneurium provides mechanical support and chemotrophic guidance to individual neurons. Collagen 4, laminin and fibronectin are key components of the endoneurial basement membrane, facilitating the attachment of Schwann cells and neuronal regeneration. The loss of specific collagen 4 staining may be due to complete removal/degradation of the molecule, or changes in surface chemistry preventing antibody binding. The observed alterations may therefore negatively impact upon the ability of the graft to promote neuroregeneration across a defect. As γ-irradiation appeared to cause only a slight reduction in collagen 4 staining intensity, and no significant changes to the mechanical properties or ultrastructure were observed, it may represent a suitable sterilisation method for use with acellular nerves.

0042 Adipose derived stem cells and their extracellular vesicles as therapeutics for peripheral nerve repair

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Introduction: Peripheral nerve injury is a common form of trauma, affecting annually more than one million people worldwide, seriously compromising their quality of life. Such nerve injuries cause chronic disability that leads to substantial work leave and health care expenses. Therefore, a pressing need exists to develop the next generation of therapeutic intervention for this type of serious injury, and devising innovative structures for neurological repair is the overall goal of our research. We combine stem cell technology with tissue engineering in order to create structures that mimic the key features of a nerve autograft (the current gold standard for repair), without the disadvantages of donor-site morbidity and limited availability of donor tissue. Adipose tissue is used as a source of stem cells to provide the growth support normally elicited by endogenous Schwann cells. To date however, there is very limited evidence showing that the stem cells directly form de-novo nerve tissue, suggesting that they modulate the injury microenvironment via their secretome. In this study, we hypothesised that secreted extracellular vesicles play a role in stem cell enhancement of axon regeneration.

Methods: Adipose derived stem cells (ASC) were stimulated with a mix of factors (basic fibroblast growth factor, platelet derived growth factor-AA, neuregulin-1 and forskolin). Using a precipitation and low-speed centrifugation protocol the extracellular vesicles were isolated from the medium of the ASC cultures and also from primary Schwann cells. The conditioned media or concentrated vesicles were applied to neurons in vitro and computerised image analysis was used to assess neurite outgrowth. Total RNA and proteins were also purified from the extracellular vesicles and various neurogenic mRNA and microRNAs were identified using qRT-PCR.

Results: Nanoparticle tracking analysis showed that the modal size of the vesicles was 140nm and the preparations expressed characteristic exosomal markers CD63 and HSP70. Both conditioned media and vesicle preparations obtained from the ASC and Schwann cells significantly enhanced neurite outgrowth in vitro. The RNA cargo included GAP43, Rac1, RhoA and Tau mRNAs and several microRNAs including miR-18a, miR-182 and miR-685 all of which were up-regulated by the in vitro stimulation protocol. Vesicular RNA transfer into the neurons was confirmed using SYTO RNASelect Green Fluorescent Dye. Pharmacological modulation of receptor/raft-mediated endocytosis has been used to dissect the relative importance of the various proposed mechanisms of vesicle/exosome uptake and the consequences for biological activity.

Conclusions: These results suggest that the isolated extracellular vesicles are sufficient to replicate the activity of living stem cells, and they might therefore be a useful adjunct in tissue engineering strategies for peripheral nerve repair. Our future research will examine the combination of extracellular vesicles with biomaterials in order to create a biologically active nerve conduit to stimulate axon regeneration.

0043 The impact of autologous nerve graft phenotype and extracorporeal shockwave therapy on peripheral nerve regeneration

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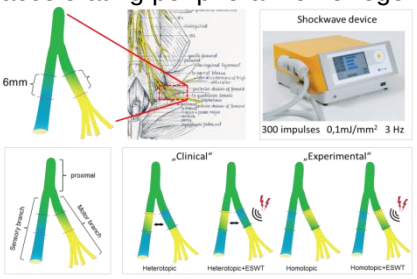
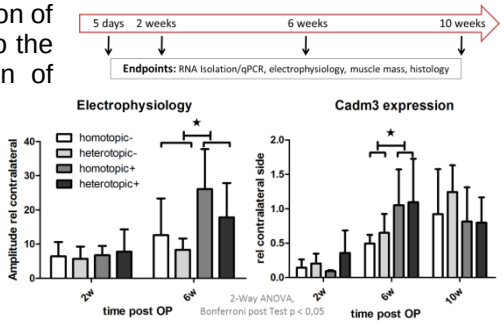
Introduction: Peripheral nerve injuries are common and a frequent cause of hospitalization displaying a major burden to patients and social health-care systems. Although regeneration after autologous nerve transplantation has been the target of scientific curiosity since the beginning of modern medicine, not much progress in accelerating this tedious process has been made. A possible explanation could be the experimental model chosen. Most research groups use the sciatic nerve defect as a model for autologous nerve transplantation, dismissing the influence of phenotypically different nerve grafts on regeneration. We thus hypothesize that this mismatch has a negative influence on motor axonal regeneration and that extracorporeal shockwave therapy (ESWT) can ameliorate this effect. Our first aim was to establish a modified femoral nerve defect model reflecting the phenotypical difference of transplanted autologous nerve grafts in the clinic. Second, we aim to evaluate the effect of ESWT, which has been shown to be one of very few treatment options accelerating peripheral nerve regeneration, in this model.



Methods: Adult male Sprague Dawley rats were divided in groups of at least 8 animals. A 6 mm autologous nerve transplantation was performed using either homotopic (matched) or heterotopic (mismatched) grafting. The treatment groups received ESWT directly after wound closure. Regeneration was evaluated functionally, histologically, and by qPCR.

Results: Motor nerves show less than 50% expression of pro-proliferative markers (Ki67, p75) in early stages of neuronal regeneration than sensory nerves. Furthermore, electrophysiological as well as histological evaluations indicate slower regeneration of motor axons in the heterotopic setting when compared to the homotopic grafting. ESWT increases expression of markers for re-myelination (Cadm3) and homeostasis (TrkB) up to 100% 6 weeks after injury in both groups, indicating amelioration of negative effects of phenotypical mismatch.



Conclusion: This study shows that ESWT is able to accelerate peripheral nerve regeneration in a successfully modified femoral nerve model which reflects the clinical reality after autologous nerve transplantation. Hereby, providing support for the use of ESWT after surgical repair of peripheral nerve injuries.

0044 Biomimetic surfaces with glycosaminoglycans - from control of adhesion to cell differentiation and anti-inflammatory activity

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Glycosaminoglycans (GAG) represent a group of polysaccharides providing high affinity binding sites for proteins that regulate adhesion, growth and differentiation of cells or which interact with cells directly. Due their inherent bioactivity, relatively high thermal and chemical stability, the presence of functional and charged groups allowing covalent or physical bonding and cross-linking, GAG represent highly interesting biomaterials.

The covalent modification of biomaterial surfaces with GAG like chondroitin sulfate, heparin and hyaluronan was achieved here by the oxidation of GAG to generate reactive aldehyde groups that allow reactions with amino groups (i) and by thiolation (ii) that permits their direct immobilization on gold, photochemical binding to vinyl groups, disulfide bond formation and others. Protein adsorption and fibroblast adhesion studies showed that a lower degree of such functionalization did not impair the bioactivity of GAG significantly, which was evident by the ability of activated GAG to bind proteins like fibronectin and also to support the adhesion of fibroblasts. Beside the covalent also a physical immobilization of GAG with layer-by-layer technique was applied here to generate multilayers using collagen I (Col I) as polycation and oxidized or native chondroitin sulfate (CS) or hyaluronan (HA) as polyanions. Multilayer made of HA/Col I supported the chondrogenic differentiation while multilayers made of Col I/CS supported osteogenic differentiation of mesenchymal stem cells. It was also shown that multilayers composed of GAG as polyanions are useful for the uptake and release of growth factors like BMP-2. Hence, such bioactive surface coatings made of GAG can be designed to tailor the microenvironment of stem cells to direct their differentiation into the desired phenotype.

A further application of surface coatings from GAG is based on their anti-inflammatory activity, which was evident by reduction of activation of macrophages indicated by reduced formation of multinucleated giant cells, beta integrin expression and release of pro-inflammatory cytokines, which was particularly evident when heparin was used. Hence, GAG may be also useful to modify the surfaces of implantable sensors and other biomedical devices to avoid fibrosis and subsequent failure of the device.

Acknowledgements

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0045 Injectable gelatin-hyaluronic acid hydrogels for articular cartilage regeneration

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Introduction.

Gelatin-hyaluronic acid hybrid hydrogels have been proposed as matrices for articular cartilage regeneration due to their ability to mimic the extracellular matrix of this tissue. Enhanced chondrogenesis of encapsulated chondrocytes has been demonstrated when these natural origin polymers are combined. The aim of this work is to explore if these systems are able to promote chondrogenesis when mesenchymal stem cells are encapsulated, and if this potential is maintained without the addition of growth factors.

Materials and methods.

Injectable hydrogels were synthesized by grafting tyramine onto gelatin and hyaluronic acid using N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride and N- hydroxysuccinimide and further crosslinking solutions containing different amounts of both tyramine conjugates by enzymatic reaction with horseradish peroxidase and hydrogen peroxide. Gelation time and mechanical stiffness of the hydrogels were studied by rheometry. Enzymatic degradation was evaluated in vitro in solutions containing a mixture of 10 U/mL hyaluronidase and 3 U/mL collagenase. Human bone marrow mesenchymal stem cells (BM-hMSCs) were cultured for 14 days in chondrogenic and growth medium. Cell proliferation was recorded by MTS and cell differentiation by the expression of aggrecan, glycosaminoglycans (GAGs), collagen II and SOX9.

Results and discussion.

Rheological measurements showed an increase in the storage modulus (G´) when the HA content was increased from 172 Pa to 789 Pa. Enzymatic degradation showed faster degradation for hydrogels with more Gel in their composition and increased swelling capacity when 20-30% degradation was reached, indicating bulk degradation. The hydrogels rich in HA promoted rounded morphology of BM-hMSCs and chondrogenic differentiation in growth medium, demonstrated by the higher secretion of aggrecan and GAGs than pure gelatin. Chondrogenesis was promoted in all the systems when chondrogenic medium was used. Although pure HA seemed to promote chondrogenic phenotype, it is not the best matrix for cartilage tissue engineering as it does not promote cell proliferation.

Conclusions.

Injectable extracellular matrix inspired hydrogels of gelatin and hyaluronic acid promote mesenchymal stem cells proliferation and chondrogenic differentiation without the addition of growth factors being good candidates for articular cartilage regeneration.

Acknowledgments.

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0046 Advanced nanofibrous scaffolds combined with stem cells for the development of effective devices and therapies

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Among the various possible embodiements of Advanced Therapies and in particular of Tissue Engineering the use of temporary scaffolds to regenerate tissue defects is one of the key issues. The scaffolds should be specifically designed to create environments that promote tissue development and not merely to support the maintenance of communities of cells. To achieve that goal, highly functional scaffolds may combine specific morphologies and surface chemistry with the local release of bioactive agents.

Many biomaterials have been proposed to produce scaffolds aiming the regeneration of a wealth of human tissues. We have a particular interest in developing systems based in biodegradable polymers. Those demanding applications require a combination of mechanical properties, processability, cell-friendly surfaces and tunable biodegradability that need to be tailored for the specific application envisioned. Those biomaterials are usually processed by different routes into devices with wide range of morphologies such as biodegradable fibers and meshes, films or particles and adaptable to different biomedical applications.

In our approach, we combine the temporary scaffolds populated with therapeutically relevant communities of cells to generate a hybrid implant. For that we have explored different sources of adult and also embryonic stem cells. We are exploring the use of adult MSCs, namely obtained from the bone marrow for the development autologous-based therapies. We also develop strategies based in extra-embryonic tissues, such as the perivascular region of the umbilical cord (Wharton´s Jelly).

This talk will review our latest developments of natural-based biomaterials and scaffolds in combination with stem cells for advanced biomedical devices and therapies.

0047 The effects of polyhydroxyalkanoates on insulin release from pancreatic cells

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Islet transplantation has emerged as one of the ways in which advanced Type1 Diabetes can be treated. One of the main challenges of Islet transplantation is the loss of Islets after transplantation. Seeding pancreatic cells in polymer scaffolds has been shown to increase the likelihood of survival post transplantation[1]. Polyhydroxyalkanoates (PHAs) are intracellular energy storage polymers synthesized by Gram-positive and Gram-negative bacteria. Based on the hydroxyalkanoate backbones, there are two kinds of PHAs- short chain length (scl) and medium chain length (mcl) with 3-5 and 6-16 carbon atoms in the monomer backbones respectively. Scl and mcl-PHAs have different physical properties with scl-PHAs being generally more brittle and hard and mcl-PHAs more elastomeric in nature. The ease of modification, biocompatibility and biodegradability make PHAs suitable candidates for pancreatic tissue engineering[2]. This work aimed to evaluate the suitability of varying PHA structures as potential scaffolds for Islet transplantation.

Short-chain-length PHAs (scl-PHAs) and medium-chain-length PHAs (mcl-PHAs) were produced by bacterial fermentation using *Bacillus cereus* and *Pseudomonas sp.* respectively. The polymers were thoroughly characterised with respect to molecular structure, mechanical and thermal properties. 2D PHA films, 2D Poly-(L-lactic acid)-PLLA films and 3D scaffolds were produced using the solvent casting/moulding method. Cell viability and insulin release assays were performed on the PHA and PLLA scaffolds using clonal pancreatic BRIN-BD11 beta cells. In comparison with the positive control (tissue culture plate), cells seeded on the mcl-PHAs neat and 3D structures had statistically significant increases in viability ($p<0.0001$). All other PHA structures showed no significant difference. In comparison with PLLA, all PHA structures except mcl-PHA 3D scaffolds showed statistically significant ($p<0.0001$) increase in insulin release from BRIN-BD11 cells seeded on them. SEM images of cells after insulin release tests confirmed that the cells seeded on mcl-PHA films exhibited a higher degree of clustering (similar to native Islet cells), as compared to the other substrates. Hence, this work confirmed that PHAs are promising candidates for improved pancreatic tissue engineering.

0048 Sustained release of nanoliposomal sodium nitrite and growth hormone from biomimetic collagen coating improves endothelialization

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Introduction: Endothelium is the perfect natural blood compatible surface which secretes various substances affecting platelet adhesion and aggregation. Therefore, creating a functional lining of endothelial cells on vascular grafts or blood-contacting parts of medical devices might increase the biocompatibility of these devices. Improved endothelialization might be obtained by using biomimetic coatings that allow release of biologically active molecules. We aimed to test whether incorporation of growth-inducing nanoliposomal growth hormone (nGH) and anti-thrombotic nanoliposomal sodium nitrite (nNitrite) into collagen coating of silicone tubes enhances endothelialization by stimulating endothelial cell proliferation and anti-thrombotic function.

Material and Methods: First nNitrite and nGH were prepared by using the thin-film hydration technique. Collagen solution blended with nNitrite and/or nGH, was co-immobilized on the internal surface of acrylic acid (AAc)-grafted silicone tubes. The release of nitrite and/or GH from the surface-modified silicone tubes was determined, and the effect of the released molecules on endothelial cell proliferation and prostaglandin I_2 (PGI_2) release, as well as on platelet adhesion were assessed.

Results: nNitrite-nGH-collagen coating decreased the water contact angle from 102° to 48°. After 120 h incubation, 58% nitrite and 22% GH of the initial amount of sodium nitrite and GH in nanoliposomes were gradually released from the nNitrite-nGH-collagen coating. After 6 days, endothelial cell confluency in the absence of surface coating was 22%, with collagen coating 74%, and with nNitrite-nGH-collagen conjugate coating 83-119%. The release of anti-thrombotic prostaglandin I_2 from endothelial cells was stimulated after nNitrite-nGH-collagen conjugate coating by 1.7-2.2-fold compared with collagen coating.

Conclusion: Our data shows improved endothelialization and blood compatibility using nNitrite-nGH-collagen conjugate coating on silicone tubes suggesting that these coatings are highly suitable for use in vascular grafts or blood-contacting parts of biomedical devices.

0049 Tissue-Engineered Substitutes with Stem Cells: From the Bench to the Patient

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The LOEX centre from Laval University is specialized in stem cells and the production of tissue in the laboratory for fundamental research as well as clinical applications. The self-assembly approach of tissue engineering was designed to elaborate complex living substitutes. The ability of dermal fibroblasts to produce and organize their own extracellular matrix (ECM) is used to produce tissue-engineered dermis (TED). Tissue-engineered skin (TES) is obtained by culturing skin epithelial cells on TED, without exogenous ECM or scaffolds. Although different methods are used to culture epithelial cells from the skin, not all of them allow the preservation of stem cells. The TED natural ECM favors long-term preservation of stem cells, facilitate vascularization and innervation, and provides adequate mechanical properties to these living three-dimensional TES. TES are also useful model to study the pathophysiology of diseases such as psoriasis. Furthermore, autologous TES can be produced with this technique for the treatment of skin wounds. It allows the healing of chronic wounds (ulcers). Moreover, TESs provide permanent autologous grafts for severely burned patients. No significant contraction was observed in vivo after grafting. The presence of the ECM promoted a good healing and skin suppleness after grafting. TES presented a well-organized epidermis and stem cells were settled and maintained in the basal layer after grafting. A complete basement membrane with numerous hemidesmosomes was observed indicating a cohesion between the dermis and epidermis. The integrity of the transplanted TES persisted over time (2 months to 8 years follow-up) with no defect in epidermal regeneration and no significant contracture. Minimal hypertrophic scars were only observed between the TES. These living substitutes comprising cells and extracellular matrix possess the capability of expanding with the child's growth. We conclude that the TES produced by this approach is a promising skin substitute for resurfacing full-thickness skin injury given its functional characteristics: minimal contraction after grafting and promotion of long-term tissue regeneration. Moreover, other organs such as bladder, blood vessels, heart valves and cornea are developed using the self-assembly approach of tissue engineering.

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0050 New thermoresponsive materials for cell printing and delivery

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We have been working on news classes of particulate materials that undergo phase transitions around body temperature. These materials switch from suspensions into porous solids as a result of changes in colloidal stability or transient changes in polymer blend glass transition.

The setting mechanism of these new materials is very gentle to cells because there is no change in chemical composition or rapid fluctuations in local temperature or solute concentration. We therefore propose they have many applications in biofabrication of cell seeded scaffolds because the material can be printed as a liquid that converts to a solid within seconds or minutes.

These thermoresponsive materials have some unusual properties because they form by particle aggregation rather than chemical or physical polymer crosslinking. The materials have high porosity and can be designed with virtually all pores greater than 100 micron in diameter.

This talk will present the underlying physical chemistry that is the foundation for a new class of materials and our work to exemplify their use in regenerative medicine and controlled drug delivery applications.

0051 Submerged drop-on-demand 3D bioprinting of tailored cell-laden hydrogel blends

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Bioprinting is a powerful technology to build-up cell-laden hydrogels in a three-dimensional, tissue-like arrangement. While most groups working on this topic use fused layer manufacturing/fused deposition modeling (FLM/FDM) techniques, the drop-on-demand (DoD) technique exhibts additional advantages.

It will be shown in the talk that even complex three-dimensional structures can be build-up using the DoD bioprinting technique. This is possible by a patented technique of dropwise printing under perfluorocarbon (PFC). PFC is a liquid of very high density, which mechanically supports the printed hydrogel droplets until the gelation process is completed. No blending occurs between the cell-laden hydrogel droplets and the surrounding PFC due the high hydrophobicity of the support liquid. Collagen is very cell-supporting due to its RGD sequences. However, pure collagen constructs cannot be build-up, not even with the submerged PFC technique, because of the slow and complex gelation characteristics of collagen. We have shown that both, 3D printability on the centimeter scale and a high viability of embedded human mesenchymal stem cells (hMSC) can be achieved by tailored hydrogel blends. Mixtures made of collagen/alginate and collagen/agarose are of special interest in this context. Futhermore it was shown that the stiffness of the different hydrogel blends significantly influence the differentiation behavior of the embedded stem cells. Additionally we could show that capillary tube formation can be induced by co-culturing human umbilical vein endothelial cells (HUVEC) and hMSC using tailored collagen-based hydrogel blends.

It can be concluded that three-dimensional cell-laden constructs on the centimeter scale can be build-up by a drop-on-demand bioprinting technique using tailored hydrogel blends which enable differentiation of stem cells and vasculogenesis in vitro.

0052 Technologies for biofabrication

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Biofabrication combines principles of engineering, biology, and material science and holds the promise to change the toolbox for many biotechnological disciplines. Recently, in the context of tissue engineering and regenerative medicine applications, we have clarified the definition of biofabrication as *"the automated generation of biologically functional products with structural organization from living cells, bioactive molecules, biomaterials, cell aggregates such as micro-tissues, or hybrid cell-material constructs, through Bioprinting or Bioassembly and subsequent tissue maturation processes." [1]*. This new definition includes the fabrication of scaffolds with hierarchical structural properties or smart surface properties within the realm of bioprinting. When progressing from single tissues to organ regeneration, bioprinting is already showing promising routes to develop functional 3D *in vitro* models that can be used to test drugs or new therapies for specific diseases. In this more educational presentation, a few examples in literature from the biofabrication community are presented to highlight the current state-of-the-art and discuss the challenges that we are still facing to move from 3D *in vitro* models to functional organ replacements.

0053 Thiol-ene cross-linked bioinks with controlled nanoparticle release behaviour

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Biofabrication is a young and dynamically evolving field of research [1]. It aims at the automated generation of hierarchical tissue-like structures from cells and materials through Bioprinting or Bioassembly. This approach has the potential to overcome a number of classical challenges relating to organization, personalized shape and mechanical integrity of generated constructs.

Although this has allowed achieving some remarkable successes, it has recently become evident that the lack of variety in printable hydrogel systems is one major drawback for the complete field [2]. Aside of printability, the control over post-processing properties is one important issue that may be addressed by supplementing the ink with drug-loaded nanoparticles.

This contribution will focus on printable hydrogels based on thiol-ene cross-linking [3] as alternative to the often used free radical polymerization to stabilize printed hydrogel structures with high resolution and reproducibility. When charged molecules such as hyaluronic acid are supplemented, electrostatic interactions can be used as tool to control the behavior of nanoparticles that are plotted together with the hydrogel precursor solution, so that consecutive release profiles can be adjusted.

0054 Regenerative frontiers of smart bio-inspired self-assembling macroporous bioreactors: Beyond morphogens and stem cells

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Regenerative medicine and tissue engineering are the grand multidisciplinary challenges of molecular, cellular and evolutionary biology requiring the integration of tissue biology and experimental surgery to explore how to trigger *de novo* morphogenesis of tissues and organs in man. The use of human recombinant bone morphogenetic proteins, at an inflexible high concentration singly delivered by potentially immunogenic matrices to induce bone in clinical contexts has been unsuccessful and it is now untenable. The emerging novel question of biomaterials science is whether biomaterial scientists and molecular biologists alike can assemble self-initiating biomimetic matrices that *per se* initiate *de novo* the induction of bone formation without the exogenous application of osteogenic soluble molecular signals of the TGF-β supergene family. The novel and exciting concept of bone tissue engineering is to develop *smart* biomimetic matrices that in their own right do initiate the induction of bone formation. Coral-derived bioreactors obtained after hydrothermal conversion of coral reef genera into hydroxyapatite do spontaneously initiate the induction of bone formation when implanted in the *rectus abdominis* muscle of the non-human primate *Papio ursinus*. The unique *connubium* of geometrically modified surfaces by osteoclastogenesis, Ca^{++} release, angiogenesis, stem cell differentiation with expression and secretion of both angiogenic and osteogenic soluble molecular signals set into motion the construction of the morphogenetic gradient of the self-assembling *smart* macroporous bioreactors. Osteoclast-driven functionalized nano-patterned topographies with Ca^{++} release re-program somatic stem cells to initiate *de novo* bone formation. Blockage of osteoclasts and Ca^{++} ions by the biphosphonate zoledronate and the Ca^{++} channel blocker verapamil hydrochloride respectively, yielded minimal if any induction of bone formation. This has highlighted the critical role of osteoclastogenesis in priming the macroporous surfaces to release Ca^{++} that together with angiogenesis initiate the intrinsic induction of bone formation. Treatment of the bioreactors with doses of recombinant human transforming growth factor- β_3 (hTGF-β_3) dramatically change the equation of the soluble and insoluble *connubium* of the morphogenetic bioreactor rapidly initiating the induction of bone formation. Implantation of coral-derived constructs with or without doses of hTGF-β_3 set into motion the expression of *Runx2* and *Osteocalcin* and of several profiled *bone morphogenetic proteins*' and *TGF-β* genes that set into motion the bone induction cascade. The primary differentiating events invocating the induction of bone formation by untreated macroporous bioreactors develop within the macroporous spaces after cell migration and differentiation within the macroporous spaces with lack or minimal *BMP-2* expression within the surrounding adjacent muscle. In 250 µg hTGF-β_3/treaded bioreactors, the adjacent muscle shows *BMP-2* up-regulation, relating to the temporo/spatial rapid induction of bone formation at the periphery of the implanted substrata only, with lack of bone formation within the macroporous spaces.

0055 Hydrogels for 3D cell culture

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In addition to the classical scaffold-based tissue engineering, a scaffold-free strategy, relying on the directed tissue self-assembly with tissue spheroids or microtissues as building blocks, has been developed within the last decade [1]. Despite much success, this approach also proved to have certain drawbacks and faces several challenges on the way to its widespread adoption. In the meantime, there is a growing consensus that an alternative – third strategy, based on the integration of tissue spheroids with conventional solid scaffolds, could be a potential optimal solution [2].

A representative example of the third strategy in tissue engineering is a recently reported lockyball construct - a tissue spheroid encaged within a highly porous microscaffold [3]. Lockyballs support bottom-up modular tissue assembly, since the size and the porosity of microscaffolds promote spheroid fusion, while providing high initial cell density. At the same time the microscaffolds material can be varied independently of the tissue spheroid, enabling adjustment of the mechanical properties of the construct and its degradation profile. In addition, microscaffolds can be designed to contain functional elements, such as hooks allowing interlocking with neighboring microscaffolds. Two-photon polymerization (2PP) technique, based on localized crosslinking of photopolymers induced by femtosecond laser pulses, was used by our group to produce the first lockyballs [4]. Due to a high spatial resolution provided by 2PP, this technique is most perspective for fabrication of microscaffolds with size is on the order of the spheroid diameter (100-500 µm) [5].

In this contribution we review the recent reports supporting manifestation of such development. The main principles, distinguishing elements and current challenges of this emerging third tissue engineering strategy will be discussed.

0056 3D printing of polymers: one concept, a plethora of materials and applications

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The development of scaffolds using 3D printing still represents a rapidly evolving field. A large variety of polymers can be printed including polyesters and hydrogel building blocks (gelatin, pluronics, …), … The number of (commercially) available devices and techniques that enable 3D printing is growing very rapidly as well.

In the present work, we want to overview various approaches in which 3D printing has been researched in our laboratories.

A first application will cover orthopaedic implants. Printing of biodegradable polyesters has enabled us to develop patient-specific scaffolds (i.e. menisci). As the selected polymers are biodegradable while lacking cell-interactive properties, a covalently bound surface decoration of the developed scaffolds was realized using gelatin. The applied surface modification technologies could be successfully transferred from 2D surfaces to 3D scaffolds as evidenced by a variety of surface characterization techniques as well as cell culture studies.

Using the cell-interactive component of the above mentioned application, the development of a 3D porous scaffold composed of gelatin was investigated. For this purpose, bifunctional gelatins were developed containing both cross-linkable methacrylamide moieties (to avoid dissolution after printing at physiological conditions, cfr UCST-behaviour) as well as hepatocyte-binding galactose ligands (targeting the asialoglycoprotein receptors). The scaffolds developed were shown to hold the potential to act as liver-cell embedding environment and might in the long run serve the purpose of regenerating part of a diseased liver or act as drug screening tools.

In addition to the selected polyesters and gelatin derivatives, our research activities have also focussed on applying acrylate end-capped urethane-containing polyethers for 3D printing purposes. The versatility of this material includes the fact that both direct and indirect 3D printing techniques can be applied. Furthermore, cross-linking of the obtained hydrogel scaffolds can be realized in the absence of a photo-initiator and in the solid-state. By selecting the polymeric building block in between the acrylate end-groups, scaffolds with varying properties can be developed ranging from hard to soft, from hydrophilic to hydrophobic.

With the present contribution, we hope to contribute to the ever evolving and intriguing field of 3D printing and boost the cross-disciplinary discussion among engineers, chemists, cell- and molecular biologists, medical doctors, entrepreneurs and investors.

Acknowledgement

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0057 Bioceramic 3D printing for osteoporotic bone regeneration

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Osteoporosis is a systemic skeletal disease characterized by low bone mass and bone quality, resulting in a high risk of fractures in accidents. Once fractures occur, bone substitutes are often required. However, a high incidence of implant failure for these substitutes is reported because of the lower regeneration capacity of osteoporotic bones compared with that of normal bones. Customized bone substitute designs for osteoporotic bone tissue regeneration with a function of controlling bone metabolism are consequently necessary. In this study, bioceramics based bone scaffolds were developed with phytochemicals for use in treating osteoporosis. Phytochemicals, especially phytoestrogen (PE) molecules are responsible for biological functions, including osteogenic, anti-osteoclastogenic, and anti-adipogenic effects. The bioceramic based scaffolds were fabricated by a three-dimension (3D) printing. The PEs could be incorporated directly into the ceramic printing matrix because our ceramic printing process does not require further heat treatment after the 3D printing fabrication. This original process was highly effective in producing bioceramic scaffolds with good biofunctionality and performance. This process could be applied to fabricate various types of bioceramic scaffolds with biofunctional materials, such as osteoblast cells, drugs, and proteins. The bioceramic scaffolds with PEs resulted in a significant increase in osteoblast cell proliferation and differentiation and in decreased osteoclast cell proliferation and differentiation activity. In vivo study with rat calvaria defect model also supported clear positive effect of PEs on bone tissue regeneration. All results indicated that PEs in bioceramic scaffolds plays an important role in both enhancing bone formation and suppressing bone resorption. Consequently, this technology promises great potential in osteoporotic bone tissue regeneration.

0058 Poly (vinyl alcohol) based gels for cells encapsulation - design considerations

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The encapsulation of cells within hydrogels is a promising technology for the treatment of many diseases and defects. Our overarching hypothesis is that the ideal encapsulation system will be comprised of both synthetic and biological polymer to capitalise on the inherent benefits of both polymer types. One key design element is the covalent incorporation of the biological molecules into the synthetic hydrogels to ensure that both polymers persist in the longer-term. Our research group utilises poly (vinyl alcohol) (PVA) as the base synthetic material, and has developed numerous approaches for the covalent incorporation of the biological molecules. Several different approaches will be discussed.

We have shown that pure PVA gels, regardless of functional group and polymerisation technique, support limited long-term survival of encapsulated cells. The addition of heparin molecules into the methacrylate based gels resulted in >90% viability for fibroblasts and insulin producing cells at 28 days post encapsulation. In a phenol based system, visible light was combined with ruthenium and SPS, and was shown to generate very reactive radical species. Gelatin and sericin incorporated within hydrogels were shown to protect the cells by scavenging the detrimental radicals. Sericin (1%) successfully promoted cell survival during the encapsulation process. A higher concentration of gelatin was required to have similar protective effects. Longer term studies showed that the encapsulated cells were only able to spread and form cell-cell contacts when both sericin and gelatin were combined in the gels.

In conclusion, there are numerous approaches to fabricating biosynthetic gels, however the benefits and drawbacks of each scheme must be considered. While relatively short term cell survival is an important indicator, future research must focus on the long-term effects on cellular function.

0059 Bioengineered stem cell niches for regenerative medicine and study of disease

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The classical paradigm of tissue engineering involves an integrated use of human stem cells, biomaterial scaffolds (providing a structural and logistic template for tissue formation) and bioreactors (providing environmental control, and dynamic sequences of molecular and physical signaling). This “biomimetic” approach results in an increasingly successful representation of the environmental milieu of tissue development, regeneration and disease. Living human tissues are now being engineered from various types of human stem cells, and tailored to the patient and the condition being treated. A reverse paradigm is now emerging with the development of platforms for modeling of integrated human physiology, using micro-tissues derived from human iPS cells and functionally connected by vascular perfusion. In both cases, the critical questions relate to our ability to recapitulate the cell niches, using bioengineering tools.

This talk will discuss the biomimetic approach as the common underlying principle for tissue engineering, and some recent advances in regenerative medicine, modeling of disease, and drug screening applications. To this end, we will discuss the critical questions in bioengineering cell niches in: (i) regenerative medicine (engineering of clinically sized human bones and whole lungs), and (ii) study of disease and drug screening (microphysiological platforms with interconnected human tissues derived from the patient’s iPS cells).

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0060 Testing anticancer efficacy of genetically engineered T cells in a 3D-microfluidic based preclinical platform mimicking tumor niche

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The critical role of the tumour microenvironment (TME) niche in adoptive cell-mediated cancer immunotherapy is now widely recognized among immunologists and pathologists. 3D microfluidic multicellular assays with real-time imaging and precise control over spatiotemporal parameters are poised to help elucidate the roles played by each cell type involved in this complex domain. However, the potential of cancer therapeutics is still limited in understanding the relationship among the TME components in the niche.

Adoptive transfer of T cell receptor engineered T cells is a promising approach for the elimination of various carcinoma including HBV-related liver tumours. Common to all adoptive therapy procedures involving the introduction of new T cell receptors, the cell engineering pipeline is laborious and time consuming due to the use of viral vectors for gene delivery, but protocols utilizing mRNA electroporation have shortened the process. However, preclinical testing of such engineered T cells still relies primarily on 2D cell assays that does not recapitulate the complex 3D niche and biochemical environment of the liver. To address this limitation, we developed a microdevice-based cellular assay capable of evaluating the physical interaction, cytotoxicity and the production of secretory factors by the engineered T cells when it encounters liver tumour cells in 3D space under different environmental conditions. Utilizing this approach, we were able to determine the different cytotoxic potential of activated and resting T cells engineered to recognize HBV-antigen expressing liver tumour cells. We also determined that while the liver niche, like hypoxic and inflammatory conditions, have little effect on the cytotoxic activity of engineered T cells against disseminated liver tumour cells, the presence of inflammatory cytokines IFN-γ and TNF-α drastically accentuates the cytotoxicity against liver tumour aggregates. These studies demonstrate the practical application of an easily customizable microdevice-based assay to evaluate the functional efficiency of T cell receptor engineered T cells under conditions potentially encountered in the liver of patients.

0061 Multifunctional biomaterials to modulate the behaviour of progenitor cells and to enhance skeletal muscle regneration

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Skeletal muscles have a robust capacity to regenerate, but beyond a certain injury severity threshold this endogenous regeneration potential proves insufficient. Recent studies have elucidated multiple intrinsic mechanisms of skeletal muscle repair, and identified various cells types and bioactive factors which modulate the process of muscle regeneration. Traditionally, tissue engineering strategies for muscle repair have focused on substrates that promote myogenic differentiation of transplanted cells. Since the failure of direct myoblast delivery in earlier clinical trials, current efforts include biomaterial-based strategies aiming at the local delivery of progenitor cells, growth factors, or a combination thereof to support endogenous regeneration cascades.

The success of such stem cell therapies hinges on the ability of transplanted cells to engraft, differentiate, and repopulate injured tissues. Employing an in vivo injury model that reflects clinical scenarios, we recently demonstrate that bone-marrow derived Mesenchymal Stem/Progenitor Cells (MSCs) can orchestrate the regeneration of severely traumatized skeletal muscles by regulating the function of muscle progenitor cells in a paracrine manner, without undergoing differentiation. This paracrine effect can be significantly enhanced by utilizing a multifunctional biomaterial acting as a synthetic niche that conditions adherent MSCs with recombinant growth factors. The reported strategy offers an effective alternative to frequently employed bolus administration, where high cell doses are required to compensate for massive cell death and poor localization at the site of injury.

In the proposed talk, we would like to focus on muscle repair and present a condensed summary of studies using biomaterials to modulate cellular function and improve endogenous regeneration. We also like to outline promising future trends in the field of muscle regeneration involving a deeper understanding of the endogenous healing cascades and utilization of this knowledge for the development of multifunctional, hybrid, biomaterials which modulate the function of the delivered progenitor cells and thereby enable muscle regeneration even under compromised conditions.

0062 Fluorescence live detection of protein nuclear import in mesenchymal stem cells adhering to the "nichoid" nanoengineered culture substrate

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INTRODUCTION: Mesenchymal stem cells (MSCs) are multipotent and self-renewing. When in their physiologic niche, MSCs are exposed to mechanical stimuli controlling their stemness and differentiation. 3D structural synthetic niches fabricated by two-photon laser polymerization are promising tools to mimic natural niches. We developed a 3D synthetic niche culture substrate (or "nichoid") of micrometric lattice size, made of SZ2080, a zirconium/silicon hybrid photoresist. The nichoid maintains pluripotency genes switched on and differentiation genes switched off in MSCs culture[1]. We hypothesize that the mechanical constraints imposed to cells in the nichoid regulate the nuclear pore structure thus the import fluxes of transcription factors (TFs) involved in cellular fate reprogramming. Literature already confirms a correlation between cell deformation and TFs localization and activity[2], but nothing is known about variations in TFs nuclear import fluxes in function of cell/nuclear deformation.

AIM: To design and purify fluorescent TFs involved in MSCs differentiation in order to dynamically assess their live nuclear import into MSCs cultured in the nichoid substrate compared to a flat culture condition.

METHODS: In order to label TFs with a fluorescent probe and at the same time promote a cell membrane crossing system, we purified a variant of green fluorescent protein (GFP) with 30 negative surface charges (-30GFP)[3]. The negative protein surface is recognized by cationic lipid-based transfection formulations and internalized into cells following DNA-like standard transfection procedures. We produced three recombinant proteins containing (-30)GFP alone or C-terminally fused to MyoD or Ascl1, transcriptional activators of myogenesis and neurogenesis, respectively. We transduced the recombinant proteins into rat bone marrow-MSCs cultured on a flat substrate or in the nichoid substrate hosted into Nunc® Lab-Tek® II chambered coverglass. Protein transduction was mediated by Lipofectamines MessengerMAX Transfection Reagent (ThermoFisher). Finally, the nuclear import was assessed by fluorescence confocal microscopy.

RESULTS and DISCUSSION: The recombinant proteins (-30)GFP (251 aa, 28.2 KDa), (-30)GFP-Ascl1 (511aa, 55 KDa), and (-30)GFP-MyoD (597 aa, 64.3 KDa) were purified in a µMolar scale. For each product, the excitation and emission wavelengths were 485 nm and 512 nm, respectively. We achieved the best transduction efficiency (40% of seeded cells) at 50% cell confluence. Live assessment of transduced MSCs by confocal microscopy demonstrated recombinant protein membrane crossing and nuclear localization after 3 hours in both 3D and 2D cultures. This preliminary set-up is suitable to follow nuclear fluxes of the recombinant TFs in living cells with live fluorescence techniques, such as FRAP and correlation spectroscopy.

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0063 Three-dimensional bone marrow niche model supporting long-term multiple myeloma culture and immunotherapy interventions

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Bone marrow niches are essential in supporting hematopoiesis and hematologic malignancies, such as multiple myeloma. The progression of multiple myeloma depends on signals and cell-cell interactions provided by the surrounding bone marrow niche. A 3D model of the bone marrow niche is needed to support the culture of primary multiple myeloma *in vitro*. Once established, such a model can be used for both fundamental and translational research.

This study analyses the usability of a previously developed 3D bone marrow niche model, capable of culturing primary multiple myeloma cells for up to 28 days, as a platform for the testing of immunotherapies. The tested immunotherapy consists out of αβT-cells reprogrammed to express tumour-specific Vγ9Vδ2 γδTCRs (TEGs). TEGs are capable of eliminating a wide variety of tumour cells through metabolic cancer targeting, including multiple myeloma cells. However, it is not evaluated yet whether these cells are active in the physiological environment of human bone marrow. Their targeted response has also mainly been evaluated using cell lines instead of primary cancer cells.

Primary multiple myeloma cells (n=6) were cultured in the presence of multipotent mesenchymal stromal cells (MSCs) and endothelial progenitor cells (EPCs) in a 3D environment and allowed to form tumour aggregates. Both TEGs and mock transduced T-cells were added to the cultures, analysing their ability to: (1) migrate through the hydrogel, (2) exert a killing response towards the cultured multiple myeloma cells, and (3) display off-target effects on the supporting stromal microenvironment, all using confocal microscopy.

The added TEGs were capable of migrating through the 3D culture, exerting a killing response towards the primary MM cells in 5 out of 6 donor samples after both 24 and 48 hours. Such a killing response was not observed when adding mock T cells. The supporting stromal microenvironment was unaffected in all conditions after 48 hours.

In conclusion, the previously developed 3D bone marrow niche model allows the study of novel immunotherapies on primary cancer cells. The model could be used to screen general effectiveness of immunotherapies, analyse both on- and off-target effects, and discriminate between responders and non-responders for personalised therapies.

0064 The degree of sulfation of sulfated glycosaminoglycan mimics controls binding of fibroblast growth factor and subsequent cell processes

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Glycosaminoglycans (GAGs) are key factors in various molecular and physiological processes. In particular, it has been shown that the modification of sulfation codes, or sulfation arrangements of GAGs affect developmental processes and numerous diseases in the brain. Sulfation codes have been specifically shown to be responsible for the differential binding to growth factors. However, few mechanisms that regulate the action of sulfated GAG molecules have been elucidated. Understanding the role of sulfated GAGs will enable the development of engineered biomimetic sulfated substrates that would enhance growth factor binding and ultimately be used in the treatment of neural diseases/injuries.

In the current work, alginate was prepared with different sulfation densities. Binding of fibroblast growth factor (FGF) to the sulfated biomimetic materials free in solution was first assessed with ELISA. Biotinilated alginate sulfate was then used to engineer sulfated nanofilms presenting various sulfation densities via streptavidin-biotin interactions. The build-up of the sulfated films and subsequent FGF binding were assessed *in situ* using quartz crystal microbalance with dissipation monitoring (QCM-D). FGF binding was then validated quantitatively via ELISA and qualitatively via immunostaining. Finally, the morphology and growth of adipose derived stem cells was evaluated on the different substrates by cytoskeletal staining followed by ImageJ analysis.

Binding of growth factors to sulfated mimetic GAGs was found to be dependent on the degree of sulfation. Using ELISA, we show that the binding of the growth factor to sulfate GAGs in solution significantly increased by increasing the degree of sulfation. This effect was also validated with QCM-D, ELISA and immunostaining measurements when sulfated molecules were bound to substrates whether in layer-by-layer films or via biotin-streptavidin interactions. Moreover, sulfation caused drastic changes in cell behaviour where increaed sulfation inhibited cell spreading and promoted longer philopodia. Cell size analysis indicated that cells on highly sulfated substrates could better maintain their stemness when compared to substrates with a lower sulfation density.

The ability to prepare sulfated substrates with controlled sulfation levels has strong implications in the biomedical field. In particular, it can be used to induce different levels of growth factor binding and subsequently result in differential effects on cells seeded on these substrates.

0065 Using the extracellular matrix to deliver growth factors and immunomodulatory signals

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Growth factors and stem cells have obviously a great potential in regenerative medicine. Yet, numerous clinical trials based on growth factors and stem cells have failed to show sufficient safety or effectiveness. Our poor understanding of how growth factor and stem cells are controlled by the tissue microenvironment in which they are delivered is probably one the main reasons underlying their modest translation to the clinic.

The extracellular matrix (ECM) is well known to play a critical role during tissue repair and regeneration. Especially, the ECM tightly regulates growth factor presentation and signalling. One recent strategy we have proposed to improve growth factor efficiency is to use endogenous ECM as the delivery system for growth factors, rather than delivering growth factors with exogenous biomaterials. To achieve controlled release of growth factors by endogenous ECM, we have fused growth factors to domains exhibiting very strong affinity (super-affinity) for endogenous glycosaminoglycans and ECM proteins. For example, in rodent models of impaired wound healing and bone regeneration, we have shown that low doses of super-affinity growth factors delivered topically or with a fibrin matrix lead to enhanced repair and regeneration, while wild-type growth factors are inefficient.

The immune response to tissue injury is also recognized as a key modulator of the tissue healing process. Thus, controlling the immune system to promote tissue repair and regeneration is an attractive option. Particularly, the regenerative functions of stem cells and growth factors most likely depend on the immune microenvironment in which they are delivered. Recently, we have shown that the regenerative capacities of mesenchymal stem cells (MSCs) are inhibited by the inflammatory cytokine interleukin-1 (IL-1). We found that the innate immune response to bone injury via the IL-1 receptor 1 (IL-1R1) signalling axis impairs bone regeneration as well as the regenerative capacity of MSCs. Mechanistically, we revealed that IL-1R1/MyD88 signalling impairs the regenerative functions of MSCs by inhibiting the Akt/GSK-3*β/β*-catenin pathway. By using a fibrin-based cell-delivery system that we functionalized with a specific inhibitor of IL-1R1 signalling, we could significantly improve MSC-driven bone regeneration in rodents. Similarly, we have found that IL-1R1 signalling diminish the pro-regenerative effects of growth factors such as BMP-2 and PDGF-BB, and we engineered a variant of IL-1 receptor antagonist (IL-1Ra) presenting super-affinity to ECM proteins. Co-delivering low doses of PDGF-BB and BMP-2 with super-affinity IL-1Ra could significantly improve bone regeneration driven by BMP-2 and PDGF-BB, while growth factors delivered alone were ineffective.

0066 Extracellular vesicles from human cardiac progenitor cells: role in cardioprotection

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Cell transplantation is a promising approach for cardiac repair after myocardial infarction (MI). Injection of Cardiac progenitor Cells (CPC) or Bone Marrow Mesenchymal Cells (BM-MSC) resulting in a limited decrease in infarct size and functional improvement. However, only a few cells survive on the long run and can be detected months after cell transplantation. This suggests transplanted cells may act by stimulating endougenous cell mechanisms through secreted factors, rather than by direct cell differentiation along cardiovascular lineages: the so-called paracrine hypothesis. A key component of paracrine secretion is exosomes (Exo). These membrane vesicles are secreted upon fusion of intracellular multivesicular compartments with the plasma membrane. Exo have recently emerged as an important intercellular communication vehicle. Using transmission electron microscopy (TEM), we have shown that human CPCs release Exo. Particle tracking analyses using NanosightTM technology showed that Exo preparations purified from culture media conditioned by CPCs largely consisted of particles in the size range of Exo. Expression of Exo markers (TSG101, CD63, Alix) was demonstrated by Western blot analyses. We also have shown that both culture media conditioned by CPCs and purified Exo-CPC, but not Exo released from normal dermal fibroblasts (Exo-NHDF), are cardioprotective and angiogenic *in vitro*.

In vivo, Exo-CPC, but not Exo-NHDF, injected into the infarct border zone reduced scar, increased viable mass and vessel density, and improved global heart function at 1 and 4 weeks after MI in rats Exo-BMC was less cardioprotective than Exo-CPC, although it exhibited a nonsignificant trend towards functional benefit.

Conclusion: Exo fully accounts for paracrine cardioprotective effects exerted by CPCs and BM-MSCs. On a same patient-basis, Exo-CPC were superior to Exo-MSC in this regard. Thus, Exo-CPC may represent an attractive cell-free therapeutic approach for MI.

0067 Mesenchymal stem/stromal cells recruitment from the cartilaginous endplate: a new perspective for IVD regenerative strategies through ECM remodeling

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The IVD degenerative process is characterized by a deregulation of cell catabolic/anabolic activity and cell death, resulting in loss of native extracellular matrix (ECM) components and water. Repopulating the IVD with cells that could revert this process and recover the tissue homeostasis would be a major achievement.

Mesenchymal Stem Cell (MSC) recruitment from the cartilaginous endplate (CEP) towards the IVD can be promoted by a chemoattractant hydrogel[1]. Herein, we investigate the role of hyaluronan-poly(N-isopropylacrylamide) (HAP) with stromal derived factor-1 (HAP-SDF) and the CEP-recruited MSCs in repair/regeneration of IVD. For this purpose, an IVD model of nucleotomized bovine discs (cavity) was used[1]. Human bone marrow-derived MSC (hMSC) were seeded on top of the CEP[1,2] and different groups of IVD were compared at distinct time points (7, 15, 21 days): 1) Intact; 2) Cavity (C); 3) C+hMSC; 4) C+HAP-SDF+hMSC and 5) C+HAP-SDF. Metabolic activity (rezasurin assay), cell proliferation (DNA content) and ECM deposition/distribution (histology;DMMB, immunohistochemistry/ immunofluorescence for aggrecan (Agg)/ collagen (Col) type I and type II were assessed.

hMSC fluorescently labelled with CM-Dil Dye migrated from CEP towards the IVD and remained viable, being detectable even after 21 days in culture. The presence of HAP-SDF triggers the recruitment of a higher number of cells at earlier time points. At day 21 recruited MSCs were present in all the IVD tissue, however they increased significantly the deposition of both Col type II and Agg, only in the inner part of IVD (the nucleus pulposus) and not in the outer annulus fibrosus.[2] The result was independent from the presence of HAP-SDF.

Still, the chemoattrantant delivery system seems to have a role in early time points by enhancing Col type II expression at day 15 in the C+HAP-SDF+hMSCs group when compared to hMSCs treatment alone.

This work demonstrates that hMSC migration through CEP can be an alternative route for hMSC-based therapies, and suggests that a chemoattractor-delivery system may accelerate ECM remodeling in the IVD.

0068 Mesenchymal stem/progenitor cell heterogeneity in the endogenous synovial intimal and sub-intimal compartments

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Human synovial mesenchymal stem/stromal cells (MSCs) represent a promising cell source for cartilage repair. However, precise definition to isolate and characterize endogenous synovial MSCs has not yet been established. Hence this study aims to address the gap in knowledge about the phenotypic characteristics of endogenous synovial MSCs. Markers reacting specifically with most or a sub-population of freshly isolated MSCs from synovium were identified and used for FACS sorting. The sorted cells were cultured, immunophenotyped and chondrogenically differentiated. The anatomical localization of the different MSC subsets in the synovium was verified by immunohistochemistry. Our results demonstrate that a combination of CD45, CD31, CD73 and CD90 can isolate two distinct MSC subsets in the primary synovium. These MSC subsets did not express CD45 or CD31 but expressed CD73 and a sub-population of $CD73^+$ cells additionally expressed CD90. $CD45^-CD31^-CD73^+CD90^-$ MSCs were significantly more chondrogenic than $CD45^-CD31^-CD73^+CD90^+$ MSCs. Interestingly, these MSCs had distinct anatomical localization; $CD73^+CD90^-$ cells were found in the intimal layer lining the joint cavity whereas $CD73^+CD90^+$ cells were located in the perivascular region of sub-intima. Most of primary MSC specific markers were expressed only in sub-intimal MSCs and not in intimal MSCs. Furthermore, reduced chondrogenic ability of $CD73^+CD90^+$ cells could be reversed by the addition of BMP2, showing discrete chondrogenic factor requirements by distinct MSC subsets. In summary, we introduce markers that can isolate distinct MSC sub-populations in synovium. Our study provides first evidence of existence of phenotypic and functional heterogeneity in two subsets of anatomically distinct synovial MSCs.

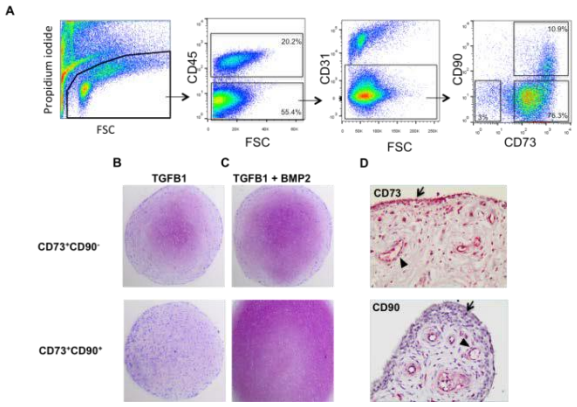

Fig. 1. A) Synovial cells were stained with CD45, CD31, CD73 and CD90, gated on propidium iodide negative live cells, followed by gating on CD45-CD31- subset, then analyzed for expression of CD73 and CD90. Sort windows were set as shown in the CD73 vs CD90 plot and cells were sorted with BD FACS Jazz. B &C) After FACS sorting, cells were cultured and subjected to chondrogenic differentiation. (Thionin staining). *Note that the reduced chondrogenic ability of CD73+CD90+ cells can be reversed by the addition of BMP2.* D) *Both CD73 and CD90 are expressed on perivascular MSCs (marked by arrowhead). MSCs in the lining intimal layer (marked by arrow) express CD73 and are negative for CD90*

0069 Exosomes to enhance diabetic wound healing

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Exosomes are vesicles with a diameter of 50-200nm originated in Multivesicular Bodies (MVBs) that contain bioactive proteins, lipids and RNA [1]. It has been shown that exosomes collected from peripheral blood stem cells (CD34+ cells) mediate the paracrine effect of progenitor cell transplantation for therapeutic angiogenesis [2], replicating the pro-angiogenic activity of CD34+ cells in vitro and in vivo [2], and suggesting that exosomes may be an alternative to stem cell transplantation for therapeutic angiogenesis. In the current work we have evaluated the therapeutic effect of exosomes isolated from mononuclear cells in diabetic chronic wounds. Exosomes were isolated from the conditioned media of human umbilical cord blood mononuclear cells (hUCBMNC) by sequential ultra-centrifugation and characterized by DLS, Zeta potential, TEM and expression of surface markers. hUCBMNC exosomes were found to enhance several biological processes *in vitro*, namely endothelial and keratinocyte survival under ischemic conditions, fibroblasts proliferation, fibroblast and keratinocyte wound healing rate in a scratch assay and endothelial tube formation in Matrigel. The miRNA composition of the exosomes was further studied by RNA deep sequencing and qRT-PCR and the most important miRNAs validated by functional *in vitro* tests. Importantly, the therapeutic effect of UCBMNC exosomes was confirmed in non-diabetic and diabetic mouse models to enhance the wound healing kinetics. This is the first work to show at molecular and cellular levels the effect of exosomes in the context of wound healing.

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0070 Circulating healing (CH) cells as effectors of endogenous tissue repair

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Restoration of damaged tissues through the activation of endogenous progenitor cells is an enticing therapeutic option. Indeed, the development of strategies to potentiate and prolong the body's own endogenous repair capacity is a subject of widespread interest and high impact for the field of regenerative medicine. In this context, a deep evaluation of the intrinsic cellular properties of stem/progenitor cells as well as of the mutually interactions with injury-induced environments is of critical importance. Recently, we reported that a newly identified sub-population of circulating stem/progenitor cells, named Circulating Healing (CH) cells, endowed with a unique therapeutic role in the bone fracture healing process was present in the peripheral blood (PB) of healthy mice. Specifically, CH ($Lineage^{neg}CD45^{neg}$) cells were demonstrated to be reactive to the injury signals, as the ones generated by a bone fracture, that direct their specific recruitment to the lesion site with consequent engraftment and differentiation in bone and cartilage tissue-specific cells.

Here, we implemented the characterization of these circulating progenitors by means of the identification of two distinctive and previously unrecognized cell-surface markers, Bone marrow stromal cell antigen-2 (BST2 or CD317) and Lysophosphatidic acid receptor-1 (LPAR1). Their characterizing expression in CH cells has been revealed by global transcriptome analysis of FACS-purified populations from healthy mice, compared to other precursor cells characterized by varying stemness degree and confirmed by Real Time-PCR. By flow cytometry we confirmed that CH cells expressed the BST-2 antigen, not only allowing an efficient isolation by positive selection but also enabling a more specific enrichment of these circulating progenitor cells ($CD317^{pos}$ CH cells). More importantly, $CD317^{pos}$CH cells derived from Red Fluorescent Protein (RFP)-transgenic mice and systemically injected into syngeneic wild-type mice 20 hours after femoral fracture migrated and engrafted in wounded tissues, maintaining the capacity to actively participate in the fracture healing process. Moreover, taking advantage of a chimeric mouse model, the bone marrow compartmental origin of PB-derived $CD317^{pos}$ CH cells was demonstrated.

Regarding the interactions between $CD317^{pos}$ CH cells and the injury-induced environment, valuable insight might reside in the LPAR1 cell-surface protein here identified. Indeed, the latter mediate most of the effects of the bioactive phospholipid LPA, whose secretion by activated platelets in an inflammatory microenvironment could regulate progenitor cell proliferation and migration. Indeed, when RFP^{pos} $CD317^{pos}$ CH cells were intravenously injected in syngeneic WT mice 40 days post-femoral fracture, they lost the homing capacity toward this injury environment characterized by inflammation resolution. These preliminary results could indicate that LPA/LPAR1 axis could work as a damage response signal responsible for the specific CH cell recruitment to the lesion site.

In conclusion the presented data provide not only the identification of useful and functional markers but also a starting point to design new therapies targeting CH cells for the treatment of bone defects.

0071 Cell homing and intervertebral disc regeneration - lessons from organ culture studies

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Cell based therapies have frequently been described for repair or regeneration of the intervertebral disc (IVD) and have already been used in some clinical studies for treatment of discogenic back pain. In particular, intra-discal delivery of bone marrow derived mesenchymal stem cells (MSCs) shows great promise, due to their differentiation potential and their regenerative and anti-inflammatory effects. In recent years, homing of MSCs into degenerative IVDs has been identified as an endogenous repair mechanism with potential to be activated or enhanced as an alternative to cell injection.

Whole IVD organ culture systems represent attractive models for investigations into cell homing phenomena and effects of cell based therapies. Thereby IVDs from large animals such as bovine or ovine resemble the human situation more closely than IVDs from small animals. To induce a degenerative condition in an organ culture model, various combinations of bioreactor-guided high frequency/high amplitude mechanical load with stabbing of the annulus fibrosus or exposure to growth media with low glucose content can be applied. Such measures generally lead to increased expression of catabolic marker genes, release of cytokines and chemokines, and accelerated cell death. The chemokines secreted by such induced-degenerative IVDs play an important role for the recruitment of stem or progenitor cells. Experimental data confirm the feasibility of organ culture models to study cell migration through the endplate towards the IVD.

Similarly, large animal IVD organ cultures can be adopted to study the fate of MSCs after implantation into induced-degenerative IVDs. It has been shown that the differentiation and the efficacy of implanted MSCs depend on the loading and culture history of the host IVD. Furthermore, the cell carrier material plays a crucial role for both the behaviour of encapsulated MSCs and the response of the IVD to MSC treatment. In bovine IVD nucleotomy models, a thermoreversible hyaluronan-based hydrogel demonstrated a favourable differentiation environment for human MSCs, whereas fibrin and fibrin-hyaluronan hydrogels showed good integration with the native IVD tissue.

While such acute responses can reliably be simulated, the limitation of organ culture systems is that human disc degeneration can only partially be reproduced. Similarly, assessment of repair processes remains challenging in organ culture models, since long term studies are required to demonstrate structural matrix restoration. Nevertheless, recent data using the bovine whole IVD model indicated that MSCs that migrated through the endplate towards nucleotomized IVDs could increase the expression of matrix proteins in the IVD. Furthermore, homed MSCs stimulated the production of anabolic factors, providing indirect evidence of an ongoing repair process.

In conclusion, large animal IVD organ culture models are valuable for investigating cell homing and repair processes, whereby long term cultures also allow assessment of matrix remodelling.

0072 Bovine intervertebral disc organ culture under compressive and torsional loading

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The talk summarizes concepts of biomedical engineering, which aim to develop physiological-relevant *ex vivo* 3D tissue or organ culture models for the human intervertebral disc (IVD). From the lab bench's perspective, the current research is to improve our understanding of how forces interact with the highly specialized IVD cells. From the patient's bedside perspective, the identification and understanding of the causes for IVD failure as seen in human patients, would be the ultimate goal. Many studies used coccygeal bovine IVDs, which are derived from the easy-accessible elongation of the spine. Strenghts and weaknesses of this animal model are shortly summarized in terms of biomechanical, biochemical and cellular properties. Further, details on the protocols on how to prepare the IVDs and the cartilaginous endplates prior culture are discussed based on recent improvements, such as the culture medium.

Increase of torsional freedom has been associated with progressing IVD degeneration in patients in a magentic resonance imaging study [1]. In rat tail models, *in vitro* and *in vivo,* it could be demonstrated that torsional loading may cause injury to the disc at excessive amplitudes that are detectable biologically before biomechanically manifestation [2]. Nevertheless, the importance of torsion for the IVD in organ culture models remains obscure. Thus, in order to tackle the role of torsion, a customized two-degree-of-freedom bioreactor has been designed. Recent research with coccygeal bovine IVDs comparing low static compressive force (20N) and three different torsional angles (0° ± 2, ± 5 and ± 10° at 0.1Hz) revealed that torsion significantly increased cell viability compared to the compression only group [3]. Furthermore, in another study [4] with 8h high loading and 16h resting with no load, it was found that the combination of compression (i.e. 0.6 ± 0.2MPa sinoidal loading and torsion 0 ± 2° both at 0.2Hz = "twisting") compared to either pure compressive loading 0.6 ± 0.2MPa at 0.2Hz or pure torsional loading (0 ± 2°) at 0.2Hz significantly decreased cell viability in the centre of the IVD, i.e. the nucleus pulposus. This detrimental effect could be confirmed by modifying the duration of the twisted loading stimulus from 2, 4 to 8h of continuous dynamic axial rotation of 0 ± 2° that was applied at a frequency of 1Hz, superimposed on a static compressive load of 0.2MPa during the torsional loading period[5].

Enzyme-driven or mechanical overloading of the IVD were proposed to mimic human IVD degeneration. Finally, these *ex vivo* organ culture models are attractive to test the feasibility of novel "smart" biomaterials for IVD repair in combination with stem cells under simulated physiological conditions. Especially, for experimental work of annulus fibrosus damage the application of twisted motion could be clinically relevant.

0073 Intervertebral disc cell and tissue systems for in vitro investigation of degenerative mechanisms and regenerative treatments.

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Intervertebral disc degeneration, a major cause of back/neck pain, continues to be a disease whose pathogenic mechanism is poorly understood and for which we have no curative treatments other than replacing it. To understand the disease and to test treatment strategies, scientists and engineers commonly use in vitro and in vivo models. With increasing levels of complexity, the models can capture the interactions necessary to study all facets of the disease and treatment as it interacts with the local and systemic environment. However, these models tend to be difficult to control and monitor at the molecular level necessary to understand important cellular and sub-cellular interactions. For such detailed investigations, in vitro cell and tissue cultures are often used. Here, the challenges to create such systems for the unique physico-chemical environment of disc cells is presented and the advantages and disadvantages of these model systems compared to those of higher complexity are discussed and illustrated with examples.

It is well known that disc cells are sensitive to their physico-chemical environment. Such parameters as pH, osmolarity, oxygen and glucose can affect their anabolic, catabolic and inflammatory behaviour. Similar to other cells, they are also sensitive to their matrix environment and the mechanical conditions that they experience. They must be able to attach to substrates like they do with their matrix and in doing so maintain natural shape and equilibrium of their cytoskeleton. In the nucleus pulposus, the matrix is soft and isotropic creating a hydraulic hyperosmotic environment, but in the annulus fibrosus, the matrix is highly anisotropic and stiff deforming the cell in a complex and heterogenous manner. Furthermore, the magnitude, frequency and duration of mechanical strain/stress can stimulate the cells to behave diversely. Some of these factors are easier to control in simple monolayer or hydrogel cultures, but the interaction with the natural matrix is often lost. In fact, some of the physical environments, such as osmolarity, attachments and complexity of deformation are governed by the matrix and can stimulate strong cellular reactions such as inflammation, de-differentiation or even apoptosis. Such interactions are easier to maintain with tissue cultures, but then maintenance and application of the physical environment and mechanical stimulation is more difficult.

The main advantage of in vitro cell and tissue cultures is the control of conditions that can be achieved with high fidelity and the ability to monitor cellular reactions and to even perturb at the cell or intra-cellular level. Another advantage is the availability of diseased human cells and tissues rather than those of other species that may have dissimilar disease process or regenerative mechanisms. However, the main disadvantage is the lack of interactions with adjacent tissues and systems, such as for the study of inflammation and pain. Finally, as we do not completely understand the disease process, re-creation of it, especially in cell cultures remains challenging.

advantages and disadvantages of these model systems compared to those of higher complexity

0074 Modelling intervertebral disc injury and degeneration using organ culture

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Human intervertebral disc (IVD) degeneration is a source of back pain because of the specific structural and compositional alterations that occur. IVD degeneration accumulates over years and therefore correlates with aging, yet specific degenerative defects may be considered distinct from aging.

IVD organ culture models have been used to identify how specific structural defects and chemical challenges can affect structure, biomechanics, and cellular behaviors of IVDs. Local IVD defects from needle puncture can result in localized cell death and strain concentrations that induces organ-level changes in IVD biomechanics. Loss of nucleus pulposus pressurization from puncture or glycosaminoglycan loss affects axial biomechanical axial behaviors with particular sensitivity in the neutral zone. Larger AF defects induce larger local structural and biomechanical disruption, and on the organ level these alterations can induce increased torsional range of motion. AF delamination requires asymmetric compression overload to separate annular layers and induce broad structural disruption.

IVD degeneration involves a chronic inflammatory condition. IVD cells and surrounding tissues produce pro-inflammatory cytokines to initiate a healing response. Organ culture models have been used to demonstrate how pro-inflammatory cytokines can be transported into the IVD and induce permanent catabolic shifts. As a result, the low cell density, hypoxic conditions and limited nutritional environment of IVDs limits its capacity for healing.

Human IVD organ culture models are an important innovation since it is impossible to simulate IVD degeneration, which occurs over decades of life in humans, with acute injuries or chemical alterations in animal models. Human IVDs are a limited source with relative small sample size and high biological variance, and as a result thoughtful consideration of controls must be exercised, selection of controls must be given thoughtful consideration.

IVD organ culture models of injury can provide important insights into the pathophysiology of IVD degeneration. Once injury and degeneration is induced, IVD organ culture models can provide systems to screen potential therapies. IVD model systems are particularly good at maintaining control over experimental conditions and can address large animal scaling issues, however, they limited in that they do not involve systemic factors.

0075 Bioprinting: challenges to commercialization of academic research. The story of organovo

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This talk will present the story behind the first commercial bioprinting company, Organovo. Each attempt to commercialize academic research has its own challenges. In the case of bioprinting, a paradigm shifting innovation at the time, the pitfalls and hurdles were above the average. The talk will briefly overview and the science underlying Organovo's extrusion bioprinting technology, the process to the establishment of the company, its evolving business model, the beginning of the commercial operation and the path to the Initial Public Offering (IPO). Special emphasis will be put on applications of the technology that have resulted in commercial products, such as the ExVive human tissues (liver and kidney proximal tubule), already in use for drug toxicity essays by several major pharmaceutical companies. We will also briefly overview the present status of the commercial bioprinting space. It is hoped that the lessons from this story will provide useful input to others in the field.

0076 Bioprinted kidney model for nephrotoxicity assessment

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Bioprinting is a promising technology to produce three-dimensional (3D) tissues in shapes not possible with standard tissue engineering approaches. In this way the native complex tissue organization can be reproduced.

Nephrotoxicity is a major cause for potential drugs not being marketed. The problem is the lack of suitable in vitro test systems to assess kidney toxicity. Current test systems include either animal experimentation and 2D cell culture approaches which are nowadays highly questionable for their physiological relevance and predictivity.

The goal of our project is to develop an in vitro human nephrotoxicity test system with two tubular structures inside a hydrogel that represent the proximal tubule (first part of the nephron in the kidney) and a blood vessel adjacent to it to allow communication between the two vessels. The tubular structures are then seeded with the corresponding cell types and flow is applied to cultivate the tissue under physiological conditions.

First, we developed a printable and cell-compatible photo-polymerizable gelatin-methacrylate-based bioink that allows strong cell adherence of both cell types. We used the human proximal tubule epithelial cell line (PTEC) HKC-8 and the human umbilical vein endothelial cell line (CI-HUVEC). In order to generate the tubular structure a sacrificial polymer was printed into the bulk bioink and later removed by cooling, when pluronic was used or heating when gelatin was used. The 500 µm (Ø) tubular structures were then seeded with the two cell types. First, the cells were cultured overnight under static conditions to let the cells adhere. Afterwards, flow was applied and steadily increased up to 1 dyn/cm^2. Both cell types adopted to the flow conditions and remained attached to the tubule walls. The HUVECs were aligning to the flow direction. The cell seeded tubules were cultured for more than 7 days under perfusion. The PDMS-based perfusion device was developed in house containing a mold to print the tubular structures inside and also harboured the luer-lock connections to easily perfuse the tissue. In order to assess the PTEC's functionality glucose and phosphate transporters were analysed in monolayer, static cultures. Both transporters are in vivo strongly Na^+-dependent, which we could only detect for the phosphate transporters and not for the glucose transporters (SGLT1, SGLT2) in the cell line. Also the receptor-mediated endocytosis of FITC-labelled BSA was only detectable with OK (opossum-kidney) cells under static, monolayer cultures and not with the human HKC-8 cell line.

We will verify whether the culture under more physiological conditions in the novel flow system enhances the functionality of the HKC-8 cell line compared to the static monolayer culture. Furthermore, we also evaluate the potential of the human RPTEC/Tert1 cell line to be incorporated into this tissue culture system. The final goal is to provide a functional assay to analyse drug effects on receptor-mediated endocytosis in the proximal tubule.

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0077 Patient-specific implant made of degradable poly(trimethylene carbonate) – hydroxyapatite implant for bone repair: in vitro and in vivo evaluation

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INTRODUCTION:

Stereolithography (SLA) allows for unmatched accuracy and precision in the fabrication of scaffolds for tissue engineering. Using such advantageous technique, poly(trimethylene carbonate) (PTMC) based resin loaded with nano-hydroxyapatite (nHA) were recently produced to create implants[1,2]. In this study, we evaluated how the implant composition influences its osteogenic property under *in vitro* and *in vivo* condition.

METHODS:

PTMC-methacrylate resin mixed with nHA at 0, 20 and 40% w/w were prepared and used for films and scaffolds fabrication using SLA. *In vitro*, human bone marrow stromal cells (hMSCs) were seeded on films and cultivated for 4 weeks in osteogenic media and differentiation was assessed by quantification of alkaline phosphatase activity (ALP) and by mineral deposition using alizarin red staining (ARS). *In vivo* experiment was conducted by creating 4 calvarial defects of 6 mm Ø on rabbits and the defects were either left empty (control group) or PTMC and PTMC/nHA at 20 and 40% w/w scaffolds were inserted in the cavities. Following 6 weeks of implantation, osseointegration was assessed by X-ray and by histology (Giemsa-Eosin staining).

RESULTS:

In vitro hMSCs were able to attach and to proliferate similarly in all biomaterials and expressed high ALP and ARS when cultivated on PTMC 20 and PTMC 40. *In vivo*, the incorporation of 20 and 40% of nHA in PTMC significantly increased osseointegration of the implant compared to PTMC only.

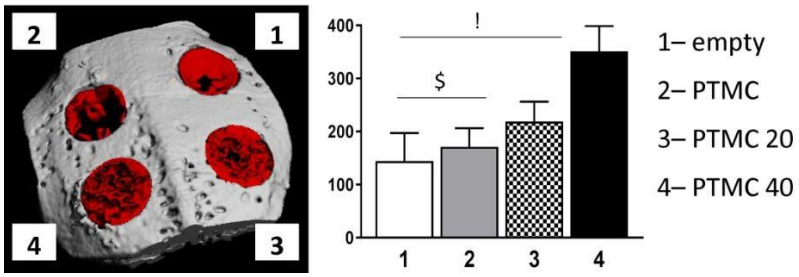

Figure: In vivo illustration of bone formation after 6 weeks in the different groups (new bone is shown red) and tomography quantification (expressed in mg HA/ccm).

DISCUSSION & CONCLUSIONS:

For the first time, we reported the fabrication and characterization of PTMC/nHA-based SLA scaffolds for bone repair. This composite biomaterial exhibited excellent biocompatibility and osteopromotive effect. This study opens the field of patient-specific implants made of PTMC/nHA fabricated using additive manufacturing.

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0078 Photo-curable Thiol-ene Gelatin Based Hydrogel bio-inks for 3D-biofabrication of cartilage

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A major challenge in biofabrication of hydrogel-based materials lies in designing a versatile macromer that has both relevant properties as a bioink and the capacity to be cell-instructive, supporting cartilage tissue formation [1]. This study aimed to tackle this problem by utilising a versatile photo-click thiol-ene chemistry, where a new 3D-printable gelatin based bioink (allyllated gelatin; GelAGE) was cross-linked by low molecular weight and octafunctional PEG-based thiols, and the resulting gels and the effect of the different mesh sizes where evaluated for incorporation of bioactives and cellular functionality.

Gelatin was reacted with allyl glycidyl ether (AGE) and 1:6 NaOH:AGE at 65˚C for 8h. Hydrogels were photo-polymerised (I2959 365nm or Ru/SPS 450nm, 5.4 J/cm2) with dithiotreitol (DTT) or thiolated 8arm-poly-ethylene-glycol (PEG8). Physico-chemical properties were characterized followed by 3D-bioplotting of porous, cell-laden constructs. Thiolated heparin (HepSH) was further incorporated into these hydrogels, encapsulated with human articular chondrocytes ($15x10^6$ cells/ml) and cultured for up to 5w in chondrogenic differentiation media to assess cell viability (live/dead), GAG (DMMB), DNA (CyQUANT), and matrix deposition (Saf-O, Coll I/II).

GelAGE was successfully synthesised and physico-chemical properties were highly tailorable (≈30-700Å mesh size, 7-176kPa compressive modulus). Bioplotted GelAGE (1A-C) yielded porous constructs with high cell viability (>75%). Shape fidelity was noticeably improved for PEG8 (<5% fibre diameter change post swelling) as compared to DTT (≈40%) and more commonly used gelatin methacryloyl (GelMA, ≈45%), possibly due to efficient crosslinking of PEG8 and resistance to oxygen inhibition in step-growth polymerisation. Similarly, addition of cells did not alter the compressive modulus post encapsulation in GelAGE hydrogels, while a significant reduction in modulus was observed in GelMA hydrogels, possibly due to cells quenching radicals and inhibiting chain-growth propagation. Finally, GelAGE allowed efficient conjugation of the bioactive molecule HepSH (>90%), yielding significantly greater differentiation compared to GelAGE alone (1D).

In conclusion, GelAGE can be applied as a cell-instructive bioink to enhance chondrogenesis, with tunable formulations and superior shape fidelity as compared to commonly used gelatin-based bioinks.

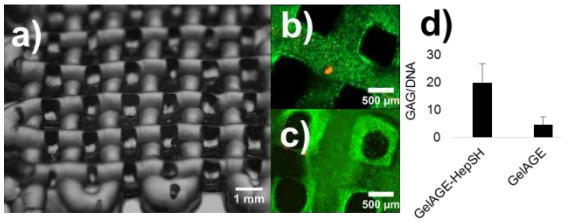

Fig 1. Images from a bioplotted GelAGE-PEG8 4 layered construct (A) and cell viability at d1 (B) and d7 (C). Addition of small amounts of HepSH significantly improved the cellular matrix deposition (D).

0079 New 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 employed in 3D bioprinting of cell-laden hydrogel constructs. However, most commonly used photo-initiators such as Irgacure®2959 (I2959) absorb in the UV region (300-400nm), which can potentially cause cellular DNA damage. In this study, we examine the feasibility of using an alternative set of photo-initiators, ruthenium (Ru) and sodium persulphate (SPS), which absorb in the visible light region (Vis, 400-450nm), for engineering cell-laden constructs using two kinds of bioprinting technology, 3D bioplotting and digital light processing (DLP). We showed that bioplotted MSC-laden gelatin-methacryloyl (gelMA) hydrogels photo-crosslinked using Vis+Ru/SPS have higher maintenance of shape fidelity, higher cell viability and metabolic activity over 3 weeks culture period compared to UV+I2959 polymerised constructs. The Ru/SPS system was also successfully combined with conventional digital light processing (DLP) machine, fabricating methacrylated-poly(vinyl alcohol) (PVA-MA) hydrogel constructs of complex but defined architecture at high resolution (25µm). Sophisticated designs such as the woven mat structure featuring intertwined struts that cannot be produced using other 3D bioprinting technology, can be achieved using DLP+Ru/SPS. MSCs were also printed into these constructs and showed long term survival (>90% viability after 21 days). We showed that this new Vis+Ru/SPS photo-initiating system is more advantageous than the conventional UV+I2959 system, as proven by the higher maintenance of shape fidelity, higher cell viability in 3D bioplotted constructs, as well as being compatible with conventional DLP machines to produce hydrogels with complex architecture at high resolution.

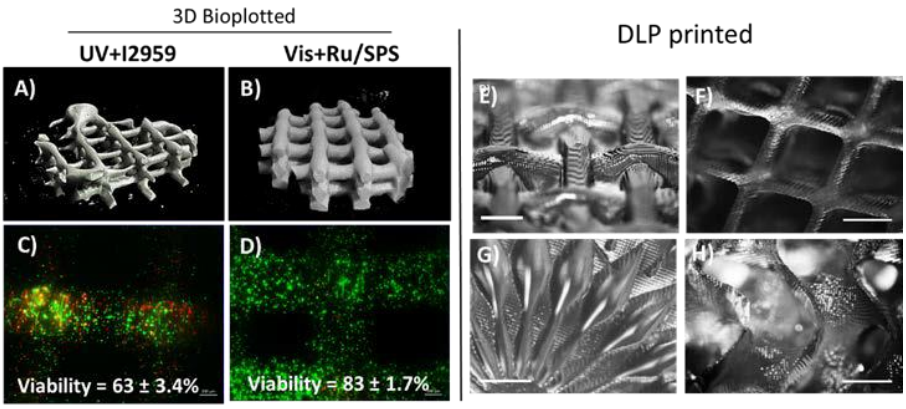

Figure 1: 3D bioplotted MSC laden GelMA hydrogels, photo-crosslinked using UV+I2959 (A & C); Vis+Ru/SPS (B & D); DLP printed PVA-MA hydrogels, E) Woven mat structure; F) Porous lattice; G) Flower; H) Gyroid. Scale bar = 500µm.

0080 Bioengineering of cortex-like constructs by bio-acoustic levitational assembly of primate neural stem cells

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We recently developed a strategy to bioengineer multilayered tissues with micrometer-scale control over biological and structural features of native layered tissues, relying on bulk acoustic levitation, and we applied the method to the assembly of neural progenitors in 3D fibrin hydrogels (1).

Here we combine this method with layer-by layer assembly to bioengineer multilayered single constructs containing heterogeneous layers of cells, using primate Neural Stem Cells (NSCs) at different stages of differentiation. NSCs were derived from monkey embryonic stem cells that stably express a tau-GFP fusion protein (2). By binding the GFP to microtubules, tau-GFP tagging enables detailed visualization of living cell morphology, including dendrites and axons.

The constructs were obtained by levitating NSCs within a mix of fibrinogen and thrombin, to create multilayered cell arrangements with an interlayer distance of 180µm. The process was repeated several times to superpose, in a single final construct, the NSCs at three stages of differentiation: (i) self-renewing NSCs corresponding to brain progenitor cells, (ii) NSC derivatives derived after one and (iii) two weeks of neuronal differentiation, corresponding to immature cerebral cortex neurons. The constructs were cultured in neuronal differentiating medium, fixed and immunostained for confocal microscopy analyses. Figure 1 shows examples of constructs after 1 and 7 days of culture post-assembly. The multilayered organization is clearly visible, and immunostaining analyses revealed cells exhibiting neuronal and glial phenotypes. Immunolabelling against Ki67 showed that progenitors cells undergo sustained proliferation in the substrate. Extending processes and inter- and intra-layers connections between layers of differentiated NSCs were monitored and immunostainings revealed complex intercellular connections formed by neurons and glial cells derived from tau-GFP- NSCs in the multilayered constructs.

The proposed method is potentially useful to create informative *in vitro* models of the developing primate brain, such as the multi-layered cerebral cortex, as it allows the assembly of heterogeneous cell populations, and supports proliferation and differentiation in 3D. Primate brain displays species-specific features, such as a massive expansion and diversification of neuronal output during development, which cannot be properly studied in model organisms like rodents. The limited access to primate biological material urges the need to develop *in vitro* models.

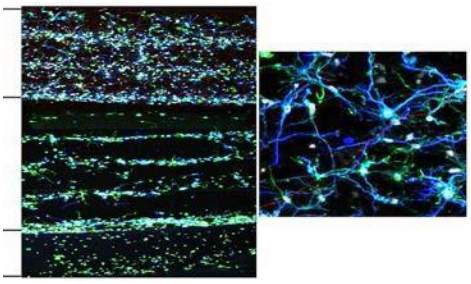

Figure 1. Left: Multilayered construct with NSCs (bottom), NSCs differentiated 7DIV (middle) and 14DIV (top). Right: Zoom on intralayer cellular connections in a gel after 7 days of culture. Immunostaining:βIIITubulin(Red)/GFP(Green)/GFAP(Blue)/DAPI(Grey)

0081 Human fibroblast-derived extracellular matrix induces human mesenchymal cells condensation and chondrogenesis in 3D mesh scaffold

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Extracellular matrix (ECM) is a complex but highly organized network of proteins and other macromolecules that play a critical role in cell adhesion, migration, and differentiation. To harness the benefits of ECM on regenerative medicine, morphogenetic signals that the biophysical ECM environments provide must be fully defined and decoded. While the effects of individual ECM macromolecules, i.e., collagen, fibronectin, or laminin on stem cells behavior have been well documented, the real face of a physiologically more relevant ECM is much less understood, due partly to the lack of proper tool. To address such issues, we have utilized cell-derived ECM (CDM) that is obtained from in vitro-cultured cells via decellularization. In this work, biodegradable PLA/PLGA microfiber mesh scaffolds were treated with human lung fibroblast-derived matrix (hFDM) and subsequently conjugated with heparin via EDC chemistry for transforming growth factor-β1 (TGF-b1) immobilization. To examine our hypothesis, four test groups were prepared: mesh scaffold (control), fibronectin (FN)-coated one, hFDM-treated one, and hFDM/TGF-b1 tethered one. Differentiation of human mesenchymal stem cells (hMSCs) into chondrogenic lineage was greatly improved in vitro via hFDM-treated mesh groups. Interestingly, MSCs condensation that led to cell aggregates was clearly observed with time in the two hFDM-coated groups and the quantitative difference was obvious as compared to the control and FN group. They proved significant increase in glycosaminoglycan synthesis and upregulation of chondrogenic marker expression. Animal models via rabbit knee articular cartilage also support a bioactive role of hFDM in cartilage regeneration. Histological findings demonstrated that hFDM/TGF-b1 mesh found much better newly formed neocartilage in the cartilage defect site. They exhibited specific chondrogenic extracellular phenotype as confirmed by Col-II immunohistochemistry and Safranin O staining, respectively. Taken together, bioactive hFDM-treated mesh scaffolds can provide a very effective microenvironment for chondrogenesis in vitro as well as for neo-cartilage formation in vivo.

0082 Enhancement of chondrogenic potential in mesenchymal stem cells by regulating expression of transforming growth factor β receptors

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Therapeutic cells including stem cells have been developing rapidly as a potential cure for repairing or regenerating the functions of diseased organs and tissues. For successful cartilage therapy, it is very important to facilitate efficient differentiation of stem cells toward chondrocyte. However, stem cells derived from individual donors can show wide variations in differentiation potential. In addition, the regulatory mechanisms underlying stem cells differentiation remain unclear. In present study, we hypothesized that chondrogenic potential and stability of stem cells would be enhanced by regulating TGFβ receptor signalling such as transforming growth factor β receptor Type I (TGFβRI) and Type II (TGFβRII). This strategy would be useful to prepare a standard protocol providing therapeutic stem cells for cartilage tissue regeneration.

0083 Umbilical cord blood derived MSCs and hydrogel composite for articular cartilage restoration

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Repair of articular cartilage defect is still a challenging problem. We developed a novel method to repair the articular cartilage defect of human knee joint using the composite of mesenchymal stem cells isolated from human umbilical cord blood and hyaluronate hydrogel. We evaluated the safety and efficacy of the composite for the repair of articular cartilage defects of knee joint in various animal models. Then we performed a phase I-II clinical trial followed by a phase III clinical trial.

Arthroscopically proven ICRS grade 4 lesions were included in the phase I-II clinical trial. MSC concentration of 0.5×10^7cells/ml were implanted to the lesion as 0.5ml/㎠. Safety parameters included physical examination (swelling, tenderness, range of motion, pain), vital signs, lab tests and any adverse events which were evaluated by the WHO common toxicity criteria. Efficacy was evaluated according to the ICRS Cartilage Repair Assessment, Pain VAS and histological assessment. Second look arthroscopy and biopsy was performed in 2 out of 7 cases in this clinical trial after informed consent was obtained. There was no significant adverse event of more than grade 3 according to the WHO toxicity criteria. The overall Repair assessment was improved in 67%. The biopsy result showed highly hyaline like regenerative tissue according to the H&E, Saf-O staining and Col II immunostaining. From the result of this study, the safety of the application of human umbilical cord derived mesenchymal stem cell composite in the regenerative treatment of articular cartilage defect of human knee joint was assured. The efficacy was assessed as reasonable. The promising result of this early phase clinical trial warranted further investigation in this strategy of articular cartilage restoration. Moreover, the long-term follow up study of more than 7 years revealed the maintenance of the restored cartilage without significant deterioration of the improved clinical outcomes.

Phase III clinical trial was performed as a multi-centre study with a randomized control group of microfracture. One hundred eight patients with ICRS grade IV chondral lesions in osteoarthritic knees were included in this study. The evaluation included clinical scoring, arthroscopic grading of the repair tissue, and histological assessment. The midterm results of the clinical trials will be presented in this talk.

0084 Enhancing cartilage regeneration of synovium-derived mesenchymal stem cells by the treatment of human transglutaminase-4

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Introduction: Although MSCs transplantation has been shown to be a promising strategy for the repair of damaged articular cartilage, MSCs-based cartilage tissue engineering has a number of limitations. These include poor implanted cell adhesion, phenotypic alteration of cells, and engraftment rates after implantation. To achieve effective transplantation of synovium-derived mesenchymal stem cells (SDSCs) for the repair of osteochondral defects, we aim to investigate SDSCs encapsulated in COL/HA/FG composite gel by supplement of recombinant human transglutaminase4 (rhTG-4).

Material and Methods: Isolation of rabbit SDSCs: Synovial tissues were harvested aseptically from the knees of New Zealand white rabbits. The synovial tissues were minced in PBS, and then digested with 0.02% collagenase. After filtering through 70-mm cell strainers, the released cells were washed, and resuspended in a culture medium

Preparation of COL/HA/FG gel: The mixture ratio of composite gel was COL/HA/FG = 0.5: 0.5: 2.0. The COL/HA/FG solution with or without rhTG-4 was loaded into syringe A. Thrombin containing 40 mM CaCl2 was loaded into syringe B. Upon mixing, the two solutions in formed a gel; for each sample, the total volume of the gel was about 200 mL.

Surgical procedure: osteochondral defect (4 mm x 3 mm) was made in the patellar groove of the distal femur. The osteochondral defects were filled with in COL/HA/FG composite gels loaded with autologous SDSCs

Results: Treatment of rhTG-4 can induce expression of integrinβ1 and dynamic actin fiber, enhancing the SDSCs adhesion to fibronectin (Fig.1). rhTG-4 significantly induced the proliferation of SDSCs encapsulated in COL/HA/FG composite gel. Supplement of rhTG-4 in COL/HA/FG composite gel also significantly increased the expressions of aggrecan and type II collagen mRNA (Fig.2). Moreover, transplantation of SDSC encapsulated in COL/HA/FG/rhTG-4 composite gel in vivo led to reconstructed tissue resembling native hyaline cartilage (Fig.3).

Conclusions: These data suggest that rhTG-4 enhances cartilage regeneration of SDSCs encapsulated in hydrogel. This approach could be used in engineering cartilage tissue, to improve the properties of engineered cartilage and ultimately to improve clinical outcomes.

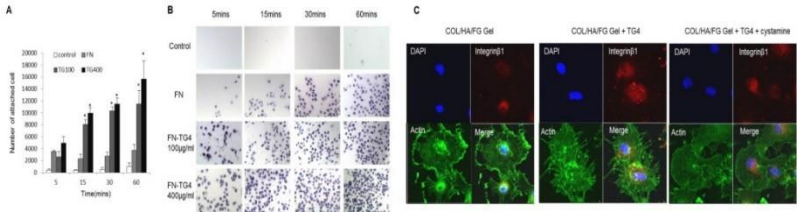

Figure. 1. rhTG-4 improves the adhesion of SDSCs. (A) MTS assay for the adhesion assay. (B) crystal violet assay. (C) Expression of integrin beta 1 and actin remodeling

Figure 2. The gene expressions of type I, II, and X collagen, sox 9, and aggrecan in the rabbit SDSCs encapsulated in supplement of rhTG-4

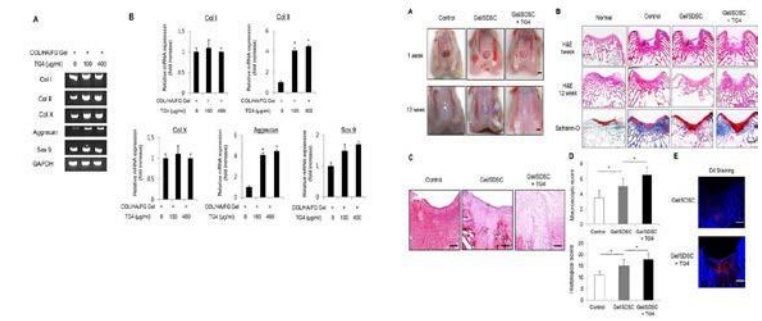

Figure 3. The in vivo potential of rhTG-4 supplement in the COL/HA/FG to regenerate and repair osteochondral defect.

0085 GMP-compliant bioreactors for scaleable decellularisation and recellularisation of scaffolds for tissue engineering

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UCL, London, UK

Tissue engineering offers a promising approach to tissue replacement and even organ engineering for which traditional techniques are unsuitable due to shortage of suitable donor tissues/organs and significant donor site morbidity. Synthetic scaffolds have proven largely unsuccessful in reproducing the complex structure and functionality of native tissues; notably in the cases of tracheal and oesophageal reconstruction. In contrast, donor derived acellular matrices appear promising; particularly following demonstration of their pre-clinical and clinical success in airways reconstruction by our group and others.

Groups from across the entire UCL Regenerative Medicine portfolio have developed tissue-specific decellularisation protocols for human and animal derived tissues to create scaffolds for re-seeding and surgical implantation. These techniques have been developed into semi-automated, closed systems to control the cost and complexity of delivery and ease the technology transfer to commercial manufacture when appropriate. In parallel we are developing scaffold seeding techniques into static and dynamic bioreactors using core materials and designs from the decellularisation bioreactors to minimise development costs.

Our procesess yielded acellular human and animal derived scaffolds that maintain putative integral biomolecular composition and structural architecture. In addition, biomechanical characterisation properties suggested the production of a viable constructs capable of extended patency during *ex vivo* repopulation. Repopulation of the acellular matrixes has been confirmed by the production of biocompatible constructs capable of supporting a variety of distinct cell types.

The successful production of a tissue engineered constructs within a GMP-compliant system realises not only the concept of complex organ regeneration, but also paves the way for scalable future clinical application.

0086 Surveying cellular and engineered tissue therapies in europe and associated countries in 2014 and 2015

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Cellular and engineered tissue therapies (CETT) are a growing sector for treating various diseases, malignances, and traumatic injuries. This field is not only thriving due to the increase of patients being treated, but also because the scope of clinical conditions, which could be responsive to CETT is broadening as well. As these advances take place it is important to maintain transparency of the treatments effectively carried out, as a basis to coordinate and further develop scientific, clinical and regulatory aspects. Due to this necessity our group has been conducting annual surveys since 2008 on the use of CETT (excluding hematopoietic stem cell treatments for the reconstitution of hematopoiesis) to establish a comprehensive, quantitative map of patients being treated in Europe and associated countries with such therapies. These surveys were supported by several international organizations such as EBMT, ISCT, TERMIS, ISCT, ICRS and IFATS. The reports include the number of treated patients without any reference to clinical outcomes. Data are sorted by indication, cell sources, processes and delivery modes as well as an appendix of active teams. Our presented work displays the results from the surveys for 2014 and 2015 separately and in comparison to one another, as our last published report reviewed the treatments of 2013.

More than 400 groups working in the field were contacted for both years. In 2014 277 teams reported performing CETT on 2054 (778 allogenic and 1276 autologous) patients, where as in 2015 210 teams reported therapies on 3338 (867 allogenic and 2471 autologous) patients.

The reported cell types for the years 2014 / 2015 were respectively mesenchymal stromal cells (MSCs) (56% / 36%), hematopoietic stem cells (26% / 13%), chondrocytes(7% / 14%), dendritic cells (3% / 2%), keratinocytes (3% / 1%), dermal fibroblasts (< 1% / 9%) and others (5% / 25%). These assessments clearly show that even though MSCs are still the predominant cell source used in CETT, there is trend towards an increasing use of differentiated cells, with the two dominant sources being chondrocytes and dermal fibroblasts. This comparison, as well as further evaluations of the collected data, identifies trends and the importance of their investigation in a still rapidly evolving field.

0087 A combined therapy of stem cells-derived 3d microtissue with angiopoietin-1 in peripheral arterial disease

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Human mesenchymal stem cells (hMSCs) are an attractive cell source in regenerative medicine and in the forefront of the cure for untreatable diseases. However, many limitations, including poor cell survival and low treatment efficiency, of using the stem cells for clinical applications remain. Recently, numerous studies have discussed the advantage of 3D cell aggregates, demonstrating that 3D aggregate or spheroid cultures can improve the differentiation, anti-inflammatory properties and therapeutic potential of various stem cell populations. Most cells are present three-dimensionally while in contact with the neighboring cells surrounded by extracellular matrices (ECMs) in a tissue. Mesenchymal cells are directly involved in the production of ECMs in the natural tissue. In the present study, we describe an innovative 3D cells spheroid, namely Angiocluster^{TM} (AC), culture method and assess the therapeutic effect of AC combined with Angiopoietin-1 (Ang-1) in mouse ischemia model. Human adipose-derived stem cells (hASCs) formed an AC on a maltose-binding protein (MBP)-linked basic fibroblast growth factor (bFGF)-immobilized polystyrene surface (MF) (as shown in Fig. 1), released various angiogenic factors, such as VEGF, bFGF, and IL-8, and differentiated into endothelial lineage cells. However, ACs rarely expressed Ang-1, an essential factor for vascular homeostasis via promoting vascular maturation and integrity. In this study, we proved that ACs formed well-organized vascular networks and incorporated significantly into host vessels by being combined with Ang-1 in a mouse hind limb ischemic model. Tissue regeneration and fibrosis was enhanced and reduced, respectively, in ACs+Ang-1 group as compared to other groups. Our results highlight that the combination of AC and Ang-1 contributes for therapeutic angiogenesis and has a promising method for wide applications in regeneration medicine.

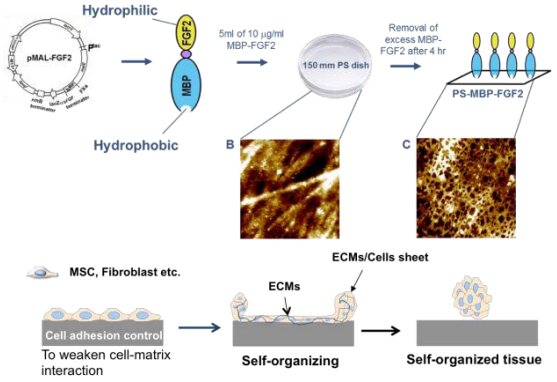

Figure 1. Scheme for Self-organizing tissue of hASCs

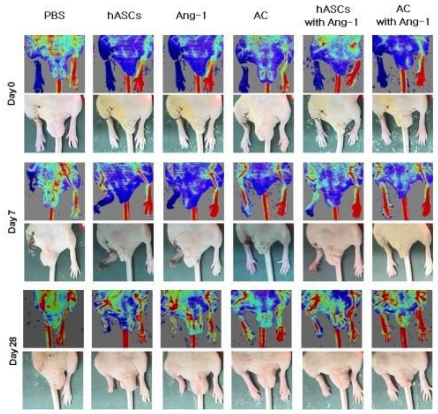

Figure 2. Angiogenic effect of AC with Ang-1

0088 Immunomodulatory characteristics of adipose-derived stem cells in the cell sheet format

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In this study we aimed to explore the immunomodulatory characteristics of different three-dimensional culture models of adipose-derived stem cells (ASCs), including cell sheets and cell spheroids. We examined the expression level of immunomodulatory genes, such as indoleamine-2,3-dioxygenase (*IDO*), Cyclooxygenase 2 (*COX-2*), TNF-α-induced protein 6 (*TSG-6*) and C1q / TNF-related protein-3 (*CTRP-3*). Relative to the monolayer culture condition, only *TSG-6* exhibited significant higher expression in the ASC spheroids. As for the cell sheets, both *TSG-6* and *CTRP-3* were found to be significantly upregulated. ELISA results also showed that ASCs within cell sheets produced significantly higher amounts of CTRP-3 than monolayer-cultured cells. Moreover, we found decreased secretion of inflammatory cytokines, such as TNF-α and CCL2, from activated macrophages treated with condition medium from ASC sheet. Previous studies have shown that CTRP-3 can inhibit macrophage aggregation and thus contribute to immunomodulation. Therefore, we designed an experiment of *in vitro* macrophage chemotactic assay in a transwell model with condition medium from monolayer ASCs or ASC sheet. Macrophage migration through Fluoroblok inserts toward activated macrophages in the bottom wells could be evaluated by staining the migrated cells at the undersurface of the inserts with crystal violet. Significantly fewer macrophages were noted in the group treated with condition medium from ASC sheet, and a similar effect could be observed when anti-CCL2 antibody was supplemented in the control medium (Fig 1). Therefore, ASCs may promote CTRP-3 production and improve their immunomodulatory properties by cell sheet formation. The finding may be important for further application of ASCs in treating diseases involving immune reactions.

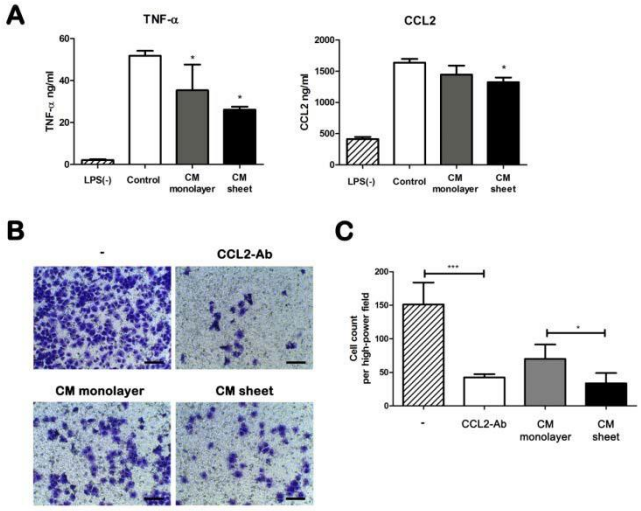

Fig 1. Responses of mouse macrophages toward different ASC conditioned medium (CM) under lipopolysaccharide (LPS) stimulation. (A) In the group treated with CM-sheet, decreased secretion of TNF-α and CCL2 was found. (B) *In vitro* macrophage chemotactic assay in a transwell model. (C) Significantly fewer migrated

0089 Anti-fibrotic effects of cardiac-derived progenitor cells in a 3D-model of human cardiac fibrosis

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Purpose: Excessive matrix deposition upon cardiac injury results in perpetuation of pro-fibrotic signalling, which contributes to progressive adverse cardiac remodelling during heart failure. It has been recently recognized that cardiac stem cell therapy for chronic ischemic heart failure might target fibroblast behaviour. However, the underlying mechanisms are poorly defined. Therefore, we developed a three dimensional (3D), tuneable model of cardiac fibrosis to allow for physiologically relevant *in vitro* testing of fibroblast behaviour upon stem cell therapy.

Methods: A photocrosslinkable hydrogel, composed of gelatin methacrylate (GelMA) combined with human fetal cardiac fibroblast (hfCF), was used to study fibroblast characteristics. hfCF-laden gels were cultured for 7 days in normal or pro-fibrotic medium (2 ng/ml TGF-β_1). To determine possible paracrine effects of cardiac-derived progenitor cells (CPC), hfCF were co-cultured with 1) CPC, 2) CPC conditioned medium (CM) or 3) CPC derived extracellular vesicles (EV). As a measure of hfCF activation, α-SMA and Col1a1 levels were analysed by qPCR and immunohistochemistry.

Results: 3D culture of hfCF resulted in a quiescent cell behaviour as demonstrated by a low α-SMA expression. In addition, reproducible cell activation and accumulation of extracellular matrix was observed in a pro-fibrotic environment and this fibrogenic response was strongly attenuated upon co-culture with CPC. We demonstrated that the anti-fibrotic effect was transferable via co-cultured CPC-CM and, despite the fact that pure CPC-CM caused the opposite effect, reproduced by isolated CPC-EV.

Conclusions: We showed the suitability of hfCF-laden GelMA as a 3D culture model to study cardiac fibrosis and the possibility to modulate cellular fibrotic responses. Moreover, our approach demonstrated inhibitory effects of CPC and CPC-EV on matrix remodelling *in vitro*.

0090 Dental stem cells potential benefits on neuroinflammation and remyelination after spinal cord injury

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Background: The cascade of secondary neurodegenerative events following spinal cord injury includes molecular events such as necrotic or apoptotic processes. Necrotic cell death initiates secondary damages and enlargement of the lesion area (Bianco et al., 2016). Secondary damage is largely mediated by inflammatory processes that involve macrophage and microglia activation (Beattie 2004). The presence of pro-inflammatory cytokines such as TNFα can exacerbate these events. Spinal cord injury also leads to oligodendrocyte cell death followed by progressive demyelination, axonal dysfunction and degeneration (Alizadeh et al., 2015). To improve recovery after spinal cord injury it is essential to interrupt the secondary damage process and provide neuroprotection.

Objective: The goal of this work was to assess the effect of dental stem cells from human apical papilla (SCAP) on neuroinflammation modulation and remyelination promotion, in order to highlight their neuroprotective potential.

Materials and Methods: SCAP were co-cultured 1) with LPS-activated BV-2 cells (a microglia cell line), 2) with rat spinal cord organotypic slices and 3) tri-cultured with spinal cord slices and oligodendrocyte progenitor cells (OPCs). Gene expression was assessed by RT-qPCR and protein quantification by ELISA. Immunohistochemistry was used for OPC identification and differentiation.

Results: When co-cultured with LPS-activated BV-2 cells, we observed a significant decrease of TNFα expression (gene and protein) and a significant increase of arginase gene expression suggesting a decreased microglia cell activation. TNFα expression (gene and protein) was also decreased in LPS-activated spinal cord organotypic slices when co-cultured with SCAP. Interestingly, we also detected a higher production of activin A (10 ng/ml) in SCAP-spinal cord sections co-culture compared to the SCAP or sections alone. Activin A significantly impacts OPC differentiation (Miron et al., 2013) so secretion of activin A observed when SCAP were in contact with spinal cord sections could induce a higher OPC differentiation.

Conclusion: In addition to demonstrating that SCAP can reduce inflammation, we showed for the first time that they induce the production of activin A, a protein that stimulates OPC differentiation. Thus, SCAP have interesting properties and could be an asset for spinal cord repair.

0091 Osteogenesis by adipose-derived cells and clinical use thereof

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This lecture will review how biomaterials and components of the extracellular matrix, i.e. structural proteins and growth factors, affect the osteogenic potential of adipose-derived mesenchymal stromal cells (ASC). Examples of bone formation by various human adipose derived cells-based engineered matrix/tissue, via either intramembranous or endochondral ossification will be presented.

This lecture will also present the development of an advanced therapy medicinal product (ATMP) based on an intraoperative use of the stromal vascular fraction (SVF) of human adipose, containing mesenchymal and endothelial cells, to support bone repair with tissue harvest, cell isolation, seeding onto scaffolding material and implantation within 3-4 hours. A translation of this concept into a first-in-man clinical trial (http://clinicaltrials.gov/show/NCT01532076), demonstrating safety, feasibility and providing proof-of-principle of the biological functionality (i.e., bone formation) of the implanted graft will be presented.

0092 Bone marrow mononuclear cell (BMC) supported therapy of large bone defects: Significant role of BMC's monocyte fraction for the bone healing response

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Introduction: Previous work demonstrated that BMCs seeded on a scaffold of □-tricalcium phosphate (□-TCP) promoted bone healing in an experimental critical-size femur defect. When applied for prevention of secondary dislocation of proximal humeral fractures in the framework of a phase-I clinical study, 10/10 patients showed primary fracture union. BMC being a variably mixed population of predominantly mature hematopoietic cells, it remained elusive, which cell(s) was instrumental. Its identification will be a significant step towards elucidating the cellular and molecular mechanisms of BMC-mediated osteo-induction, will enable more sophisticated prediction of “potency” of clinical BMC-products, and will also enable generation of clinical BMC-products enriched for the therapeutic agent(s).

Materials and Methods: Male athymic nude rats were used. A 5 mm femoral bone defect was created and filled with differentially pretreated BMC seeded on granular □-TCP scaffold (*ChronOs*,1.33x10^6 human BMC/mL □-TCP, granule size 0.7 -1.4 mm) applying the following group setup. Group 1: complete BMC, n=5; group 2: depletion of monocytes (-CD14), n=6; group 3: depletion of HSC (-CD34), n=6; group 4: depletion of MSC precursor (-CD271), n=6. Cell populations were depleted using magnetic bead separation. Healing time was eight weeks. Ultimate load of the defect zone was measured by means of three-point bending test. New bone formation was evaluated histomorphometrically on the basis of HE-stained histologic slides and presented as % of defect size corrected for the space covered by the □-TCP granules. Statistics: Results were presented as median values. Kruskal-Wallis-test with Bonferroni-Holm adjustment for multiple testing was applied. Non parametric Spearman-rank test was used for correlation analysis. A p-value <0.05 indicates statistical significance.

Results: BMC contained 19.4% monocytes, 6.3% HSC and 0.11% putative MSC before the depletion procedure. Efficacy of cell depletion was 84% for monocytes, 92% for HSC, 68% for MSC precursors. Compared to complete BMC, a significant drop of ultimate load was seen only in group 2 (-CD14, 10 N), but not in groups 3 and 4. Bone formation was highest in group 1 (BMC, 40%), and a trend towards lower values was seen in group 2 (-CD14, 12%, p=0.07) whereas no differences were seen compared to group 3 (-CD34, 38%) and group 4 (-CD271, 29%). Furthermore, a significant correlation between new bone formation and biomechanical stability was seen (rho=0.60, p<0.0045).

Discussion: Our data identify CD14+ monocytes as the major osteoinductive cell population within BMC when used for critical-size bone fracture augmentation. Future clinical studies should enumerate CD14+ cells within the BMC product and analyse clinical effects relative to CD14+ cell numbers in the products. As the potency of CD14+ cells may vary between donors, additionally differential proteomics of BMC and CD14-depleted BMC should be performed with the aim of characterizing osteoinductive mediators. Such data could either be used as potency assays for clinical BMC-derived products, or cocktails of osteoinductive mediators could potentially be used to functionalize □-TCP matrices, making cell aspiration and processing dispensable.

0093 Superior osteogenic potential of adipogenic predifferenciated human bone marrow derived multipotent stromal cells for bone tissue engineering

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In the present study, we evaluated the benefits of an adipogenic predifferentiation, the pathway most closely related to osteoblastogenesis, on the pro-osteogenic potential of human adult multipotent bone marrow stromal cells (hBMSCs), both *in vitro* and *in vivo*. Adipogenic differentiation of hBMSCs for 14 days resulted in a heterogeneous cell population from which the most adipogenic-committed cells were eliminated by their lack of re-adhesion ability. Our results provided evidence that the select adherent adipogenic differentiated hBMSCs (sAD+ cells) express a gene profile characteristic of both adipogenic and osteogenic lineages. *In vitro*, when cultured in osteogenic medium, sAD+ differentiated along the osteogenic lineage faster than undifferentiated hBMSCs. *In vivo*, in an ectopic mouse model, sAD+ exhibited a significantly higher bone formation capability compared to undifferentiated hBMSCs. We sought, then, to investigate the underlying mechanisms responsible for such beneficial effects of adipogenic pre-differentiation on bone formation and found that this outcome was not linked to a better cell survival post-implantation. The secretome of sAD+ was both pro-angiogenic and chemo-attractant but its potential did not supersede the one of undifferentiated hBMSCs. However, using co-culture systems, we observed that the sAD+ paracrine factors were pro-osteogenic on undifferentiated hBMSCs. In conclusion, adipogenic priming endows hBMSCs with high osteogenic potential as well as pro-osteogenic paracrine-mediated activity. This preconditioning appears as a promising strategy for bone tissue engineering technology in order to improve the hBMSC osteogenic potency *in vivo*.

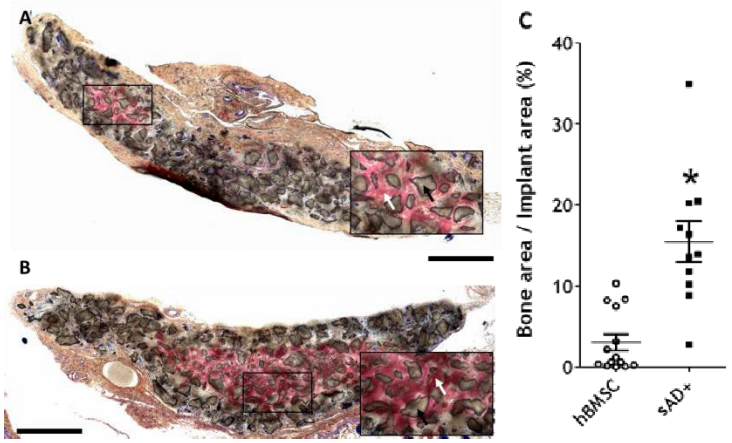

Figure: sAD+ cells associated with coral particles enhance de novo bone formation in vivo.

(A and B) Representative histology sections of cell-containing constructs stained with Picrofuchsin.

The constructs analyzed were seeded with either hBMSCs (A) or sAD+ cells (B) on coral particles and implanted in nude mice for 8 weeks.
Scale bar = 500 µm.

(C) Quantification of the total bone area as a percentage of the

0094 Translational research in the treatment of femoral head osteonecrosis with advanced cell therapy: bench-to-bedside translation

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Background

Osteonecrosis of the femoral head (ONFH) is a painful disorder that follows a common route to ischemia and cell death that can ultimately lead to structural collapse, articular incongruence and hip osteoarthritis. Preclinical studies in large animal models are developed to verify the practicability of tissue engineering and regenerative medicine approaches closer to real clinic situations. Here we present a femoral head osteonecrosis animal model in sheep to evaluate an advanced therapy medicinal product (ATMP) consisting of Bone Marrow-derived Mesenchymal Stromal Cells (BM-MSC) loaded in allogeneic bone tissue (XCEL-MT-OSTEO-ALPHA). Once the preclinical results demonstrated their feasibility and safety, they were translated into a clinical setting where they can demonstrate their safety and clinical benefit.

Materials and methods

Preclinical study was done in 15 mature sheep. Early stage osteonecrosis of the right hip was induced cryogenically. At 6 weeks, the sheep were divided into three groups: core decompression only group; core decompression followed by implantation of an acellular bone matrix scaffold; and core decompression followed by implantation of a cultured autologous BM-MSC loaded bone matrix scaffold. At 12 weeks, magnetic resonance imaging (MRI) hip studies were performed and the proximal femur was harvested for histological analysis. Clinical grade autologous BM-MSC loaded bone matrix scaffold (XCEL-MT-OSTEO-ALPHA) associated with a core decompression of the femoral head was implanted in 10 patients affected with early-stage ONFH. Follow-up was 12 months and clinical and radiological (MRI and positron emission tomography (PET)) reviews were performed.

Results and conclusions

Our findings in the preclinical study indicate that a cultured autologous BM-MSC loaded in a bone matrix scaffold (XCEL-MT-OSTEO-ALPHA) is capable of stimulating bone regeneration more effectively than isolated core decompression or in association with an acellular scaffold in a preclinical femoral head osteonecrosis model in sheep. MRI findings were not conclusive due to constant bone edema artifact in all cases but histology demonstrated a greater capacity of osteoid production in the cell therapy group. Neither tumorogenesis nor ectopic tissue formation were observed thus confirming the safety of the cell-based treatment. Preclinical data permitted the translation of the XCEL-MT-OSTEO-ALPHA use into a clinical setting. Patients who received the cellular treatment showed revascularization of the necrotic areas in MRI imaging and signs of osteoblastic activity in PET imaging, although one hip evolved toward collapse at one year. No serious adverse effects were reported in any patient at 12 months of follow-up. In conclusion, here we show the potential of a translational research in experimental cellular therapies from a clinically relevant animal model in sheep to a clinical context in patients affected with early-stage ONCF.

0095 Fractionated human adipose as a native construct for the generation of a bone organ by endochondral ossification.

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Adipose stem cells (ASC) generate bone organs through endochondral ossification if suitably primed *in vitro* (Osinga et al., 2016). However, many steps are required to generate such osteogenic grafts, from cell isolation to *in vitro* monolayer expansion of ASC, seeding into scaffolds and chondrocytic/hypertrophic differentiation of the grafts. Monolayer expansion and passaging of ASC, in particular, have been shown to strongly decrease their differentiation potential. We hypothesize that human adipose tissue is not only a source of regenerative cells (ASC and endothelial cells) but could also serve as an ideal scaffolding material, thanks to its native extracellular matrix and microenvironment, thus supporting chondrogenic differentiation of ASC. The goal of this study was thus i) to develop protocols allowing cell expansion, new matrix formation and chondrogenic commitment of ASC directly inside the native adipose tissue, and ii) to evaluate the ectopic bone formation capacity of such constructs *in vivo* by endochondral ossification.

Human liposuctions were fractionated by inter-syringe processing and cultured on agarose-coated dishes, with various growth factors, to allow cell expansion. After 3 weeks, 4-mm cylinders were punched out of the resulting constructs (named Adiscaff) and cultured for 4 weeks with chondrogenic induction medium and 2 more weeks with hypertrophic induction medium. Minimally expanded ASC from the same donors, seeded in 4-mm cylinders of collagen (Ultrafoam^{TM}, Osinga et al., 2016) and cultured with the same differentiation media were used as control. Both constructs types were then implanted subcutaneously in nude mice for 8 weeks, followed by tomography and histological/immunohistochemical analyses.

Cells in Adiscaff proliferated *in vitro* and generated a collagen and fibronectin-rich stromal matrix. Adiscaff contained clusters of ASC at the periphery of the adipose fragments as well as endothelial cells (positive for CD34 and von Willebrand factor). After the chondrogenic phase, Adiscaff produced cartilage, containing high levels of glycosaminoglycans, type II collagen and higher expression of Sox9 gene expression. Upon hypertrophic culture, gene and protein expression analyses showed upregulation of markers of terminal chondrogenic differentiation (type X collagen, osteocalcin and Indian hedgehog). *In vivo*, Adiscaff constructs generated bone tissue, with higher bone volumes than in control constructs. This bone, both of cortical and trabecular types, was vascularized and included a bone marrow compartment. In situ hybridization for human-specific sequences revealed a direct contribution of human implanted cells to bone formation in Adiscaff.

These data demonstrate that native human adipose tissue can be used as a construct for the generation of bone by endochondral ossification. This novel paradigm bypasses the steps of cell isolation and monolayer expansion, both of which are detrimental to the differentiation capacity of ASC and has translational relevance in the field of bone regeneration therapies.

0096 Assessment of a scalable 3D bioreactor system for the expansion of bone-marrow derived MSCs in a novel xeno-free medium

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The progression of MSC-based therapies towards the clinic increases pressure to address the current limitations of MSCs and their large-scale production for therapeutic use. Simply put, it is not practical or cost effective to manually culture large batches of MSCs for widespread clinical use. Scalable systems with the ability to produce large batches of MSCs reproducibly at a relatively low cost will be essential for the advancement of cell therapies. In this study, MSCs previously isolated and cultured in serum-free (SF) or 10% fetal bovine serum-containing (SC) medium were seeded into the GE Xuri Bioreactor system in 100mL spinner units using GE proprietary macrocarrier "waffles" and cultured for 7 days for two passages. MSCs were characterised and compared to their counterparts which were maintained entirely in standard 2D culture. Detailed analysis of these cells was performed including growth kinetics, tri-lineage differentiation potential and surface marker characterisation. Comparable growth kinetics of MSCs were observed in the 3D system and 2D culture indicating the suitability of the bioreactor for the proliferation of MSCs that have previously been isolated using conventional 2D plastic adherence. Tri-lineage differentiation assays were performed using standard 2D osteogenic and adipogenic assays and pellet chondrogenesis in addition to *in situ* differentiation of MSCs directly on GE bioreactor waffles. MSCs cultured on Xuri bioreactor waffles had significantly increased osteogenic potential compared to cells maintained in 2D culture. Similarly, the adipogenic potential of MSCs was increased with cultured and differentiated directly on the GE bioreactor "waffles". All cells passed ISCT guidelines for surface marker expression. These data indicate the potential suitability of the Xuri bioreactor system for the expansion of bone marrow MSCs. Current work is focussed on optimisation of the bioreactor system for the isolation of MSCs.

0097 Maximising plasticity for functional recovery after spinal cord injury

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Studies in recent years have shown that enhancing plasticity improves both anatomical and functional recoveries after various insults in the central nervous system (CNS), including spinal cord injury. We have previously demonstrated that an extracellular structure called perineuronal net (PNN) is important in controlling plasticity in the CNS. PNN is a supramolecular assembly of brain extracellular matrix molecules, forming hierarchical aggregates, on the surface of neurons. The key molecular composition of PNNs is chondroitin sulphate proteoglycans, hyaluronan, link proteins and tenascins. Enzymatic removal of chondroitin sulphate chains or disruption of the molecular aggregation increases axonal sprouting and the formation of functional synapses, and enhances functional recovery after spinal cord injury. I shall discuss the current technologies in maximising plasticity through PNN manipulation.

0098 Extracellular matrix hydrogels as scaffolds for spinal cord injury repair

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Restoring the neural functions lost after spinal cord injury (SCI) still remains a great challenge for current medicine. To find the optimal biomaterial for neural tissue repair, we developed several types of natural degradable and synthetic non-degradable hydrogel scaffolds and compared their neuroregenerative properties in vivo in the acute or chronic model of SCI either with or without seeded stem cells.

Synthetic non-degradable hydrogels were based on the superporous poly[N-(2-hydroxypropyl)-methacrylamide] (PHPMA) or poly(2-hydroxyethyl methacrylate) (PHEMA) with parallel or randomly oriented channels and modified with the integrin binding sequences IKVAV or RGD to promote cell adhesion and growth. Hyaluronic acid (HA) or extracellular matrix (ECM) hydrogels, prepared by decellularizing porcine urinary bladder or spinal cord tissue, were used as natural hydrogels. The hydrogels were implanted or injected into the SCI lesion alone or seeded with mesenchymal stem cells MSCs.

Both synthetic and natural HA or ECM hydrogels were well incorporated into the surrounding spinal cord tissue within the lesion site and promoted infiltration of connective tissue and the ingrowth of axons and blood vessels into the lesion. Axonal sprouting was more intense in HA or ECM hydrogels when compared to synthetic hydrogels. Transplanted cells further improved tissue regeneration. On the other hand, rapid ECM hydrogel degradation did not prevent cyst formation within the lesion and might prevent full tissue replacement.

When implanted into the SCI, the natural HA or ECM hydrogels were superior to the non-degradable synthetic ones in terms of promoting axonal sprouting, vascularisation as well as tissue bridging. Nevertheless, optimizing the scaffolds degradation rate is crucial for successful tissue remodelling. In conclusion, natural HA as well as ECM hydrogels in combination with stem cells are good candidates for bridging SCI lesions.

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0099 Stem cell therapy of amyotrophic lateral sclerosis: from bench to bedside

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Amyotrophic lateral sclerosis (ALS) is featured by the progressive degeneration of the cortical and spinal motoneurons (MNs), muscle fasciculation and dystrophy. ALS still but still lacks effective therapy, and current approach to treat patients includes riluzole and palliative therapy at the later stages. Stem cells brought new hopes for the field of neurodegenerative diseases. We have studied properties of human bone marrow derived mesenchymal stromal cells (BM-MSC) in vitro and in vivo and found that intrathecal and combined application of BM-MSC into symptomatic SOD1-transgenic rats slows disease progression, prolongs animals' survival, provides neurotrophic support to host MNs, preserves extracellular matrix structures, have antiapoptotic and immunomodulatory effects.

Based on our preclinical data a prospective, non-randomized, open-label clinical trial (phase I/IIa, EudraCT No. 2011-000362-35) has been designed. The trial aimed to assess the safety and efficacy of autologous multipotent BM-MSC in treatment of ALS patients. Autologous BM-MSC were isolated and expanded under GMP conditions. Patients were monitored for 6 months before treatment, and then for an 18 month follow-up period. Patients were injected intrathecally (via lumbar puncture) with 15±4.5 x 106 of autologous BM-MSC. Potential adverse reactions and clinical outcomes were evaluated by the ALS functional rating scale (ALSFRS), forced vital capacity (FVC), and weakness scales (WS). In total, 26 patients were enrolled in the study and were assessed for safety; 23 patients were suitable for efficacy evaluation. About 30% of the BM-MSC-treated patients experienced a mild-to-moderate headache, resembling those after a standard lumbar puncture. No suspected serious adverse reactions were observed. We found a reduction in ALSFRS decline at 3 months after application ($p<0.02$) that, in some cases, persisted for 6 months ($p<0.05$). In about 80% of the patients, FVC values remained stable or above 70% for a time period of 9 months. Values of WS were stable in 75% of patients at 3 months after application. These results demonstrate that the intrathecal application of BM-MSC in ALS patients is a safe procedure, and that it can slow down progression of the disease.

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0100 Neural progenitors derived from iPS cells can serve as a tool for CNS repair

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Neural progenitor cells derived from induced pluripotent cells (iPS-NPs) are hot candidates for treatment of various neurological diseases. We used iPS-NPs in the treatment of stroke, spinal cord injury (SCI) and Amyotrophic Lateral Sclerosis (ALS). In vitro the cells expressed neuronal (MAP2, bIII Tubulin), astrocytic (GFAP) and oligodendrocytic (Olig2) markers, differentiated into GABA-ergic, dopaminergic neurons and motor neurons. Female Sprague-Dawley rats were subjected to focal cerebral ischemia by reversible right MCAO for 90 min, while male Wistar rats were used for SCI (balloon-induced compression lesion). $SOD1^{93A}$ transgenic rats were used as a model of ALS. A suspension of iPS-NPs (300 000 cells in 3µl of CM) was transplanted into the lesions 7 days after MCAO or SCI; the control groups were injected with saline. Asymptomatic and symptomatic rats (7 and 25 weeks old, respectively) were intraspinally grafted with iPS-NPs. The animals' motor functions were tested throughout the course of the disease. Functional recovery in rats with MCAO lesion was regularly assessed after transplantation by the apomorphine-induced rotation test and tape-removal test. Rats with SCI were tested using the BBB test, flat beam test and rotarod. The grafted animals in the stroke model displayed a decreased number of clockwise rotations in the apomorphine-induced rotation test and performed better in the tape removal test. Animals with SCI significantly improved their locomotor activity when compared to control animals. iPS-NPs robustly survived in both models of injury, maintained their neural phenotype and migrated toward the lesioned area and survived there for 2-4 months after transplantation. In addition, some of the cells differentiated into more mature and tissue-specific neurons. The transplantation of iPS-NPs into symptomatic and presymptomatic ALS animals resulted in significantly prolonged survival (by 13 and 20 days, respectively) and slowed disease progression compared with vehicle-treated littermates. Presymptomatic grafting of NP-iPS also postponed disease onset. Both groups of cell-treated rats presented significantly better motor activity. Grafting of NP-iPS up-regulated the expression of growth factors' (NGF, IGF-1 and BDNF) and stabilized the expression of apoptosis-related genes' (BAX, BCL-2 and Casp-3) compared to vehicle-treated rats. Some of the grafted cells expressed neuronal marker bIII-tubulin or neurofilament NF200, but no differentiation into motoneurons was observed in this study. In conclusion, these results suggest that iPS-NPs can improve functional outcome in stroke and SCI and prolong lifespan in ALS animals. They survive and integrate into the host tissue, and under certain conditions undergo further differentiation after transplantation. Our findings therefore demonstrate great regenerative potential of iPS-NPs in the treatment of neurodegenerative diseases and CNS injury.

The study was supported by MEYS LO1309, GACR 15-06958S, GACR P304/12/G069 and Project Biocev and Center of Reconstructive Neuroscience CZ.02.1.01/0.0/0.0/15_003/0000419.

0101 Impact of the secretome of human mesenchymal stem cells on Parkinson's disease regenerative medicine: Neuronal differentiation, brain structure and animal behavior

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Parkinson's disease (PD) is clinically characterized by the progressive degeneration of dopaminergic neurons (DAergic neurons), leading to the appearance of characteristic motor and non-motor symptoms. The use Mesenchymal Stem Cells (MSCs) has emerged as a potential therapeutic route for PD regenerative medicine. In recent years several studies have indicated that the therapeutical effects of MSCs are mediated trough their secretome which withholds a wide panel of neuroregulatory molecules. Having this in mind the objective of the present work was to assess the feasibility and possible advantages of using a secretome based therapy for PD regenerative medicine, when compared to traditional cell transplantation approaches. To do so, human MSCs derived from bone marrow (hBMCs) were expanded in tissue culture flasks, and their secretome collected and characterized as previously described [1]. Subsequently embryonic ventral mesencephalic and human progenitor cell (hNPCs) cultures were incubated with MSCs secretome. Additionally, MSCs and its secretome were also injected intracranially in the substantia nigra (SNc) and striatum (STR) in a 6-OHDA induced rat model of PD [2]. Effects on functional motor outcomes were assessed through the staircase and rotarod tests [2].

In vitro experiments revealed that the secretome of hMSCs robustly increased neuronal differentiation of hNPCs and induced an overall survival of mature DAergic neurons. Additionally, it was also possible to observe that the injection of the secretome of hMSCs in a 6-OHDA rat model of PD potentiated an increase of DAergic neurons (estimated by neuronal densities in SNc and STR). Similar outcomes were observed in the motor performance of these animals as assessed by the rotarod and staircase tests. Interestingly animals injected with hMSCs secretome disclosed better outcomes then those transplanted with hMSCs (Figure 1).

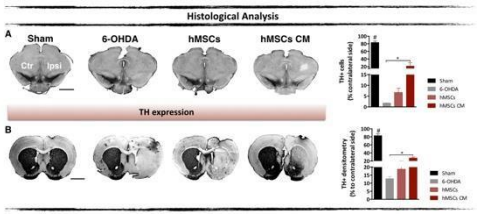

Figure 1. Representative micrographs of SNc and striatum slices stained for TH. Compared to the Sham group, all animals submitted to the 6-OHDA injections presented a reduction on the TH-staining both in the (A) SNc and (B) STR. However, animals injected with the hMSC CM (i.e. secretome) presented a significant TH-positive staining in SNc and STR when compared to the untreated group 6-OHDA. Sham: n=9, 6-OHDA: n=5, hMSCs=4, hMSCs CM: n= 6. Data presented as mean±SEM.*p<0.05; Sham animals statistically different from all the other groups, #p<0.001. (Scale bar: 2000 µm). CM: conditioned medium. TH: Tyrosine hydroxylase.

Finally, proteomic characterization of MSC secretome revealed the presence of important neuroregulatory molecules, namely Cys C, GDN, Galectin-1, PEDF, VEGF, BDNF, IL-6 and GDNF. Overall, we concluded that the use of hMSC secretome alone was able to revert the motor phenotype and the neuronal structure of 6-OHDA PD animals. This indicates that hMSC secretome could represent a novel therapeutic tool for the treatment of PD.

0102 Remote control of cell signalling using frizzled tagged magnetic particles for neuronal cell differentiation

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Introduction

Cell signalling pathways such as the Wnt pathway and ion channel signalling are instrumental in controlling cell fate. These pathways have been previously shown to be important regulators of neuronal development. There is therefore considerable interest in developing modulators of cell signalling pathways for neuronal repair and the treatment of neurodegenerative diseases. In our lab, we are developing a remote control system for tagging therapeutic cells with magnetic particles for activation of differentiation pathways.

The aims of this research was to investigate the effects of remote activation of Wnt signalling and potassium flux through Trek1 channels on neuronal differentiation of SH-SY5Y cells.

Methods

Expression of receptor targets was first assessed using rt-PCR. 250nm Magnetic nanoparticles (MNP) were then functionalised with peptides or antibodies thereby allowing remote targeting of the MNP to cell surface Frizzled receptors, coupled to the Wnt pathway, or the Trek1 ion channel. Remote stimulation of the MNP tagged receptors was then performed in 1h-3h sessions by exposing the receptor-bound MNP to external alternating magnetic field gradients provided by a magnetic force bioreactor (MICA Biosystems). Downstream signalling pathway activation was then assessed using a TCF/LEF luciferase reporter system and by monitoring end-point gene expression using real time PCR. Finally, the expression of neuronal differentiation markers was determined after 10 days treatment with functionalised MNP and magnetic field stimulation to assess the effects of remote signalling activation on neuronal differentiation of SH-SY5Y cells.

Results

Basal expression of target receptors Frizzled1, Frizzled2 and Trek1 was assessed and confirmed. Frizzled receptor expression but not Trek1 expression increased when cells were cultured in neurogenic induction media. TCF/LEF luciferase reporter activity was shown to fluctuate in response to MNP and magnetic field stimulation up to 24h after treatment. The expression of stress-response genes, NF-KB and COX2 was observed with time in response to Fz or Trek activation and found to vary during neurogenic induction. Finally, the expression of mature Neuronal markers expressed by differentiated SH-SY5Y cells in response to MNP and magnetic field treatment was determined. After a total of 10 days in culture (3x 1h stimulation), MNP and magnetic fields were found to alter the expression of neuronal markers BIII-tubulin, MAP2 and NeuN.

Conclusion

Our results suggest that remote activation of cell signalling pathways using tagged magnetic particles can be used for the control of neuronal precursor cell differentiation. This approach may offer a novel therapeutic strategy for treating neuro-degenerative diseases such as Parkinsons.

0103 Collagen scaffolds with stem cells for spinal cord injury repair: from animal models to clinical study

Jianwu Dai

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Spinal cord injury (SCI) is a devastating injury resulting in changes in the spinal cord's motor, sensory, or autonomic functions. Following SCI, an inhibitory environment develops at the injury site to inhibit neural regeneration. We have developed a functional biomaterial consisting of collagen scaffolds (NeuroRegen scaffolds) and biologically active molecules (neurotrophic factor or the antagonists to myelin-associated inhibitor), and stem cells to build a nerve regeneration microenvironment. Specifically, (1) the linear ordered collagen based NeuroRegen scaffold was developed to guide the neural regeneration along its fibers and decrease the formation of glial scars, (2) collagen binding neurotrophic factors were incorporated into the scaffolds to promote neuronal survival and neural fiber regeneration, (3) antagonists to myelin-associated inhibitors were added to the scaffold to direct the neuronal differentiation of neural stem cells at the injury site, (4) Mesenchymal stem cells (MSCs) were also added to the scaffold to reduce the acute inflammatory response due to SCI. These strategies were found to promote neural regeneration and functional recovery in SCI animals. NeuroRegen scaffolds with stem cells are in the clinical study of spinal cord injury repair.

0104 A controllable biodegradable injectable hydrogel in combination with ADSCs as a cellular skin substitute promotes humanized diabetic wound healing

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INTRODUCTION: Poly(β-amino ester)s (PBAEs) and their networks have attracted great attention as biodegradable polymers in the field of gene delivery and tissue scaffolds. However, the mechanical properties and biological effects are limited because of the linear structure of PBAE. Therefore, a series of novel hyperbranched PBAEs (HP-PBAEs) with three-dimensional dendritic architecture was developed as an improved construct for the generation of injectable hydrogel.

METHODS: HP-PBAE hyperbranched polymers incorporating multiple vinyl groups were synthesized via 'A2 + B4' Michael addition methodology. The injectable HP-PBAE/HA-SH hydrogel loaded with adipose-derived stem cells (ADSCs) was applied on humanized wound bed and the degree of healing was evaluated. (Fig. 1)

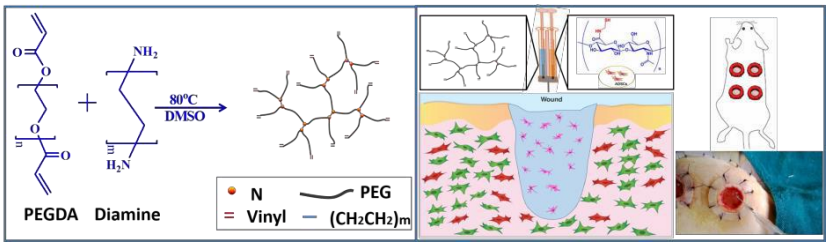

Fig. 1: Generation of injectable HP-PBAE/HA-SH based hydrogel and application in vivo.

RESULTS: A series of HP-PBAEs polymers were synthesized and thiolated hyaluronic acid (HA-SH) was used to crosslink with HP-PBAEs to form injectable hydrogel. The hydrogels showed negligible cytotoxicity and adjustable degradation properties. After application, the efficacy of the hydrogel in combination with ADSCs resulted in a significant improvement of wound healing in a diabetic wound model by inhibiting inflammation, enhancing angiogenesis and accelerating re-epithelialization.

DISCUSSION & CONCLUSIONS: The efficacy of skin substitutes in treating diabetic wounds is limited by the hostile microenvironment. To meet this challenge, we manufactured a well-designed hydrogel system for efficient delivery of stem cells into wounds and thereby promoting the microenvironment of ulcerated beds. Given that PBAEs and their networks have excellent biocompatibility and biological effects[1], we have developed hyperbranched PBAE via 'A2 + B4' Michael addition methodology and formed injectable HP-PBAE/HA-SH hydrogels with tunable properties and negligible cytotoxicity. This hydrogel system can provide a functional delivery niche for ADSCs and has potential as a novel medical device for tissue regeneration and wound healing.

ACKNOWLEDGEMENTS: This work was funded by Science Foundation Ireland (SFI), grant number 13/IA/1962, 10/IN.1/B2981(T), 15/IFA/3037, Health Research Board of Ireland (HRA-2013-412), University College Dublin and DEBRA Ireland.

0105 Injectable platelet lysate/cellulose nanocrystals hydrogels: a novel combined approach for regenerative medicine strategies

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Platelet lysate (PL) has shown outstanding properties as an autologous and inexpensive source of growth factors, cytokines and extracellular matrix precursors (e.g. fibrinogen) currently being explored in several tissue engineering (TE) strategies. However, the proposed PL-based formulations (gels, sponges and hydrogels) show limited ability to retain/protect biological active biomolecules from degradation, exhibit extremely low mechanical properties and structural stability, as well as fast *in vitro/in vivo* degradation rates. In order to overcome these limitations and further enabling the use PL not only as a source of growth factors but also as an injectable scaffolding biomaterial, we propose here reinforcing its protein content with cellulose nanocrystals (CNC). The superior strength of CNC, along with their tunable rheological properties and low cytotoxicity, makes it ideal nanofillers to reinforce low strength hydrogels without compromising the biological performance of the biomaterial. Our approach relies on the use of CNC baring surface aldehyde groups that can reversibly react with terminal amines of PL proteins and thus covalently crosslink its fibril structure.

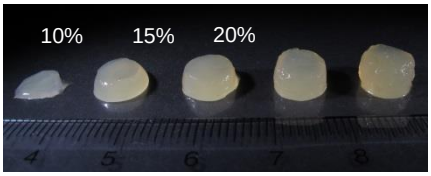

Fig.1 Freshly-prepared hydrogels with varying CNC content (equal initial volume).

PL/CNC hydrogels were produced using standard double barrel syringes promoting the *in situ* PL-clotting via thrombin and calcium activation along with the CNC/protein covalent crosslinking. The gelation, microstructural, mechanical, swelling, degradation and protein release profiles of the hydrogels were fully characterized.

In optimized conditions for PL gelation (1 $U.mL^{-1}$ thrombin and 5 mM $CaCl_2$), incorporation of up to 20% CNC (relative to PL organic content) considerably improved the microstructural organization, stability (Fig. 1) and degradation rate of the hydrogels. Moreover, the proposed strategy did not hinder a fast gelation process while markedly increased the hydrogels mechanical properties up to an impressive 2 orders of magnitude higher storage modulus compared to control (maximum *G'* of 1.2 kPa) and improved their ability to sequester native PL bioactive factors.

The proposed platform allows therefore using PL as stable injectable formulations for either the delivery of biological factors as well as a cell carrier matrix. Overall, this platform open new avenues to explore PL based hydrogels in TE applications, enabling a controlled modulation of the physical and chemical cellular microenvironments in *in vitro* settings, as well as upon *in vivo* injection. Their 3D *in vitro* biological performance was assessed using encapsulated human adipose tissue derived stem cells. Hydrogels formulations showed cell supportive properties, such as viability, metabolic activity, and proliferation rate.

Acknowledgements: Authors acknowledge FCT/MCTE, FSE/POCH, PD/59/2013 for PD/BD/113807/2015**,** SFRH/BD/96593/2013, SFRH/BPD/112459/2015, IF/00685/2012 and project Recognize (UTAP-ICDT/CTM-BIO/0023/2014).

0106 Clinical benefit of plant derived rhCollagen scaffold combined with PRP for treatment of lateral epicondylitis

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Introduction: A novel medical device composed of cross-linked recombinant human Type I Collagen. (rhCollagen) combined with autologous Platelets Rich Plasma (PRP) was developed. The product, rhCollagen/PRP, addresses the limitation of PRP treatments by providing a degradable matrix that retains the platelets at the vicinity of the injured tendon and extends the effect of growth factors.

Methods: The product was assessed in preclinical models followed by a multicenter, prospective, open label, single arm trial to demonstrate the safety and performance of a single dose of the product in patients suffering from lateral epicondylitis (tennis elbow). Product performance was assessed by reduction in pain and recovery of motion using standard evaluation methodology (PRTEE score).

Results: Preclinical evaluation showed that the product extended significantly the release of platelets related growth factors, PDGF and VEGF. In an Achilles tendon injury model in rats rhCollagen/PRP had better clinical markers than PRP alone. In a clinical trial, the product provided clear clinical benefit to the patients with 80% and 90% of the patients showing at least 25% reduction in PRTEE score after 3 and 6 months respectively. These results are significantly better than results reported previously in this indication with either PRP alone or corticosteroids.

Conclusions: The new product combined with PRP showed superior performance compared with PRP alone in preclinical models and clinical study. The results provide clear evidence supporting the use of this product combined with PRP for treatment of tendinopathy

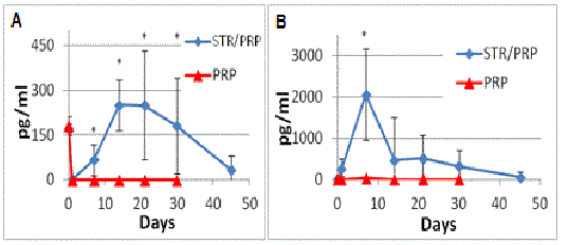

Figure 1: PDGF (A) and VEGF (B) content in subcutaneous pockets in rat model. *significance diffrerence ($p<0.05$) between STR-PRP and PRP.

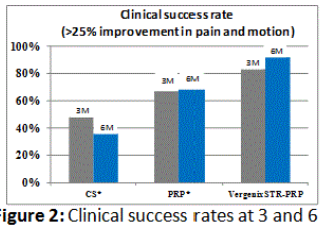

Figure 2: Clinical success rates at 3 and 6 months in patients treated for lateral epicondylitis with Corticosteroids (CS)*, PRP* or Vergenix™STR-PRP
*Peerbooms et al. Am J Sport Med 38(2):255-62, 2010

0107 Nitric oxide releasing injectable hydrogels for anti-infective and tissue regenerative matrices

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Nitric oxide (NO) is the endogenous molecular with many critical biological functions, such as neurotransmission, platelet function, immune response, suppression of tumor growth, vasodilation and wound healing. For wound healing applications, NO has been demonstrated to accelerate healing process. Furthermore, NO provides antibacterial effects on the broad spectrum through both its pathogen inhibition and killing ability.[1] Thus NO has become a potential candidate in preventing and/or treating infection to regulate the balance of inflammatory phase in wound healing cascade that leads to enhance the healing rate. However, the short half-life of NO has given great challenges for a clinical achievement of pharmaceutical dosage forms. Herein, we incorporated the NO donor of S-nitrosothiolated gelatin (GSNO) in the injectable gelatin-based hydrogels (GH) to release NO controllably in physiological conditions. Under the catalyst of HRP, H_2O_2 oxidizes phenol moieties functionalized on gelatin to form phenol-phenol crosslinks fast (few seconds to several minutes) and encapsulate GSNO (Fig. 1). Through thermal, visible light and oxidizing agent driven mechanisms, NO was released from the GH/GSNO hydrogels. By varying the GSNO concentration, the released NO was controlled in wide range of 0.054 ±0.011 – 2.050± 0.140 μmol/mL for up to 14 days (Fig.2A). In addition, the NO amount was fine-tunable control as a function of HRP and H_2O_2 concentration. The Kirby-Bauer method indicated that there was an inhibition zone against both E.Coli (EC) and S.Aureus (SA) contacted with GH/GSNO hydrogels releasing NO of 0.52 μmol/mL to higher amount (Fig.2B). The alarma blue was used to measure bacteria viability. The results shown that all EC and SA were completely killed at NO of 0.52 and 0.64 μmol/mL. SEM technique indicated that the bacteria was dead due to membrane damage caused by NO. Cytotoxicity test of GH/GSNO hydrogels on human dermal fibroblasts at the released NO amount for killing bacteria shown that no cell toxicity was induced. In summary, GH/GSNO hydrogels may offer the new platform of topical delivery of NO in treating wound infection as well as skin regeneration.

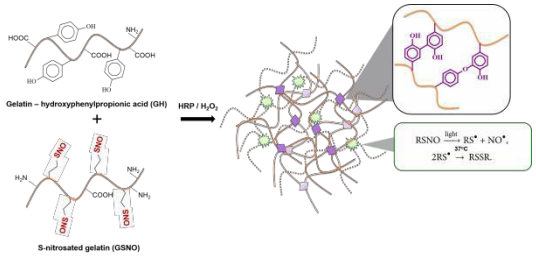

Figure 1 The formation of GH/GSNO hydrogels releasing NO

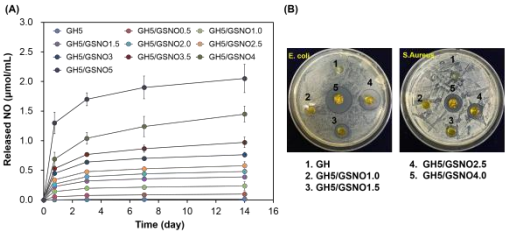

Figure 2 The released NO profile (A) and the Kirby-Bauer test of GH/GSNO hydrogels as a function of GSNO concentration.

Acknowledgement: This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIP) (NRF-2015R1A2A1A14027221)

0108 An in-situ crosslinkable, adhesive scaffold for cartilage repair: an in vivo characterization

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In this study we present a novel biomimetic hydrogel for cartilage repair. It is based on a modified hyaluronic acid (HA) that can be crosslinked with the activated transglutaminase (TG) factor XIII. This HA-TG hydrogel is injectable with a crosslinking time of less than 2 minutes and strongly adheres to cartilage tissue. When seeded with human chondroprogenitor cells (hCCs), HA-TG is able to promote cell proliferation and cartilaginous matrix deposition. HA-TG is stable in vivo where it supports tissue maturation in a subcutaneous mouse model.

HA-TG was synthesized according to a newly developed protocol (Broguiere & Cavalli, 2016) and mixed with 15 million human chondroprogenitor cells (hCCs) per ml of gel. A bovine explant model was established to better mimic a cartilage defect and to monitor the adhesion overtime (Figure 1A). After 3 weeks of culturing in chondrogenic media, the cells in the hydrogels expressed up to 100'000 fold increase in collagen type 2 and up to 10 fold aggrecan expression compared to cells in 2D. This resulted in a dramatic increase in mechanical properties in the softer gels: from the initial 6 kPa to up to 250 at 3 weeks. Adhesive properties of the gels were investigated by push-out tests and showed an initial bond strength 4 times higher than fibrin glue. After 3 weeks of culture of the hydrogel/hCCs within bovine cartilage explants, the bond strength increased to 60 kPa, likely due to matrix production and remodelling.

The gels and the gels in the explants were implanted subcutaneously in nude mice. HA-TG gels were stable for 6 weeks, resisted vascular ingrowth and supported integrative repair between the construct and the native tissue, reaching a bond strength of 160 kPa (Figure 1B-E).

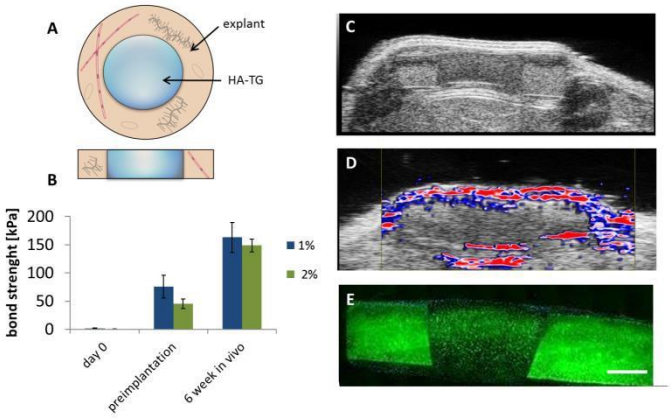

Figure 1 A: Schematics of the ex-vivo explant model. B: Bond strength of HA-TG to cartilage at d0, after 3 weeks of in vitro culture and after 6 additional weeks subcutaneous in a mouse model. Ultrasound image (C), Photoacoustic (D) and collagen 2 staining (E) of a gel-explant construct. Scale bar 2 mm.

HA-TG was transplanted in chondral defects of sheep. After 2 weeks, HA-TG was still present in the joint, suggesting that the adhesion was maintained.

In conclusion, due to its adhesive properties, injectable potential and in vivo stability, this novel scaffold represents a promising alternative to the current cartilage repair techniques.

0109 Integrating 3D bioprinting and bioreactor systems to engineer functional tissues and organs

Daniel Kelly

Trinity College Dublin, Dublin, Ireland

Our musculoskeletal system has a limited capacity for repair. This has led to increased interest in the development of tissue engineering strategies for the regeneration of musculoskeletal tissues such as bone, ligament, tendon, meniscus and articular cartilage. This talk will review our attempts to use biomaterials and mesenchymal stem cells (MSCs) to tissue engineer functional articular cartilage and bone grafts for use in bone and joint regeneration. In particular, it will describe how we can integrate hydrogel bioinks and MSCs into 3D bioprinting systems to engineer anatomically accurate constructs that could potentially be used regenerate large bone defects or damaged and diseased joints. In addition, the talk will describe the development of bioreactor systems that provide adequate nutrient availability and controlled oxygen conditions to enable the engineering of scaled-up tissues. Such dynamic culture regimes will be required to engineer large cartilage tissues for putative biological joint resurfacing strategies and for endochondral bone tissue engineering therapies.

0110 Patient-specific stem-cell based organ-on-chip

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University of Padova, Padova, Italy

The development of human organs-on-chips, in which microscale engineering technologies enable the recapitulation of the organ microenvironment, offers a unique opportunity to study human physiology and pathophysiology. We aim at developing a platform for drug screening applications and toxicological assays by using microfluidic technology and functional differentiated cells derived from human pluripotent stem cells. Induced-pluripotent stem cell derived tissue will allow patient specific study in the view of personalized medicine. The microfluidic cell culture device is specifically designed for simulating tissue- and organ-level physiology. We aim at creating a library of patient-specific hiPSC-derived hepatocytes to perform high-throughput screening of drugs. We developed a cost-effective high-efficiency somatic reprogramming for the development of hiPSCs in high-throughput manner.

In particular, we developed a robust and efficient microfluidic-based protocol for the differentiation of patient-derived hiPSCs into functional hepatocyte-like cells. The protocol allows long-term hepatocytes culture (over 1 month) and high expression of hepatic markers such as ALB, CYP3A4 and AAT together with enhanced secretory activity and response to temporally defined drug stimulation.

0111 Fabrication and assessment of a microfluidic chip for 3D bone cell culture

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Introduction: This study presents a novel bone tissue engineering system that combines a microfluidic bioreactor with microscale porous polymerised High Internal Phase Emulsion (polyHIPE) based bone scaffold. Bone regeneration is a well-organized but complex physiological process that involves synergistic chemical and mechanical stimulation [1]. However, the exact sequence, timing, and intensity of the stimuli remain unknown. A systematic study of these stimuli and their effect on cell function can only be achieved through precise control of the design, fabrication and culture of those scaffolds. A microfluidic bioreactor was designed to provide control over the fluidic stimulation in the scaffold, as well as enhance nutrient distribution. In summary, our system combines 1) high precision manufacturing of a porous, biocompatible PolyHIPE scaffolds via laser and soft lithography, and 2) a microfluidic bioreactor, for controlled mechanical and chemical stimulation which permits monitoring of cell viability via microscopy and enables CFD studies.

Materials and Methods: The mold for scaffold and microfluidic chamber were created via laser photocuring of polyethylene glycol diacrylate (PEG-DA). Scaffolds were produced by casting of 80% porous 2-Ethylhexyl acrylate and Isobornyl acrylate polyHIPE under UV light. They were properly sealed inside microfluidic chambers via air plasma sealing. Human embryonic stem cell-derived mesenchymal progenitors (hES-MPs) were cultured on scaffolds for one week both in static and dynamic conditions. MTT and resazurin reduction assay were implemented for cell viability assessment. Prostaglandin E2, alkaline phosphatase activity (ALP) along with DNA content were measured to investigate cell differentiation.

Results and Discussion: Fluorescent particle imaging in the microfluidic bioreactor showed that the scaffold was tightly encased in the microfluidic chamber. MTT and resazurin reduction assay indicated the cells were viable over one week culture. Additionally, MTT results indicated that the growth of cells was confined between PolyHIPE channels in the dynamic condition. Prostaglandin E2 and normalized ALP activity based on DNA content indicated that flow affected osteogenesis. Computational results showed that the fluid flowed in the free channels as well as porous structures and gave a detailed overview of shear stress in the Micro-Chip. These results aid understanding of the flow behavior and effects of different inlet and outlet conditions on flow during the culture.

Conclusion: We successfully created a bone scaffold/ microfluidic bioreactor platform for bone tissue engineering. The precise geometry of the chamber and scaffold enables the computational study of fluid flow in the chamber. Therefore, long-term studies of hES-MP cell lines in the bioreactor will enable us to explore the effect of high shear stress on bone deposition, and investigate in-vitro potential therapeutic reagents.

0112 Tissue engineering an in vitro model of osteoporosis

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Introduction

The most common method of studying osteoporosis and testing new therapeutics is through the use of in vivo models due to their ability to provide a uniform approach to research with a level of experimental control that is not possible in humans. Although animal models are viewed as the gold standard for testing the safety and efficacy of new therapies and are an essential step in preclinical development, their use should align with the principles of the '3Rs' – replacing, reducing and refining. Bone tissue engineering has shown that osteoblastic cells can be cultured on scaffolds to induce the formation of an immature mineralised bone-like matrix. Osteoclast cells are capable of resorbing this matrix and both cell types can respond to oestrogen. This work looks to combine these principles to see whether an applicable model of post-menopausal osteoporosis can be developed in vitro.

Methods

Three scaffolds (PolyHIPE, polyurethane, Biotek 3D Insert) were compared to select the most appropriate for the model by assessing reproducibility and bone formation over 28 days. Osteogenesis of different bone cell lines (MC3T3-E1, MLO-A5, IDG-SW3) and their responsiveness to oestrogen withdrawal was measured via alkaline phosphatase (ALP) activity and mineralisation to determine the most suitable. RAW 264.7 pre-osteoclasts were matured using RANKL and the effect of oestrogen on their maturation and activity was assed via cell viability and tartrate-resistant acid phosphatase (TRAP) activity.

Results

PolyHIPE scaffolds produced via microstereolithography with their multiscale porosity and high reproducibility were found to be the most suitable substrate for the model. Oestrogen exposure and withdrawal had no effect on MLO-A5 over a 28 day period but significantly upregulated ALP activity (exposure induced a 220% increase over untreated) and mineralisation (withdrawal caused a 142% increase over untreated) in MC3T3-E1. Oestrogen was found to reduce osteoclast number and TRAP activity by 28% in comparison to untreated controls. Initial co-cultures show it is possible to grow these cell types together in 2D and 3D and measure markers of bone turnover

Conclusion

An applicable *in vitro* model of osteoporosis would not only reduce the number of animals used each year in research, but would also provide a cheaper and faster alternative to *in vivo* models. This work has so far shown that all the constituent elements of a model can be created *in vitro* and that they can be united in 2D and 3D culture. Further work is being undertaken to simulate post-menopausal osteoporosis in the model by withdrawing oestrogen and comparing the results to established animal models.

0113 A new era of virtual histology: contrast-enhanced microCT to simultaneously visualize and quantify in 3D soft and mineralized biological tissues

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Introduction: Advanced 3D imaging is one of the enabling technologies that is of increasing importance in the field of tissue engineering (TE) to assess and guarantee high product quality, and to provide better knowledge on the mechanisms behind tissue formation and regeneration. Indeed, engineered tissues are 3D structures with complex spatial heterogeneity, for which traditional 2D imaging techniques such as standard histomorphometry are insufficient to comprehensively characterize them or assess their quality. In this overview, the potential and added value compared to standard histomorphometry of contrast-enhanced microfocus computed tomography (CE-CT) is presented. This recent development in the microCT imaging field allows virtual 3D quantitative histology of both soft and mineralized tissues.

Methods and results: For cartilage, both an anionic (Hexabrix 320) and cationic (CA4+) contrast agent have been validated on murine knee joints (as blueprint) and explants of cell-based TE constructs. CE-CT using these contrast agents not only allowed to visualize the cartilage (both mineralized and non-mineralized) along with the bone in a single, 3D dataset, but it also enabled to quantify the 3D structure of the different cartilage types and the bone, as well as the glycosaminoglycan content of the non-mineralized cartilage. For bioreactor-driven TE construct development, we showed that CE-CT using Hexabrix staining can be used as a 'whole-construct' imaging technique allowing to quantify *in vitro* formed, neo-tissue (cells and extracellular matrix) in large 3D TE constructs in a perfusion bioreactor. Staining with polyoxometalates (POMs – both commercially available as well as in-house developed) enabled to visualize and quantify in 3D the blood vessel network and the adipocytes in the bone marrow compartment along with the bone in explants of TE constructs (Fig. 1),

Conclusion: CE-CT is an important and innovative enabling technology to get a better understanding of complex mechanisms behind tissue formation and regeneration, both *in vitro* and *in vivo*, and it sets the stage for a new era of virtual histology.

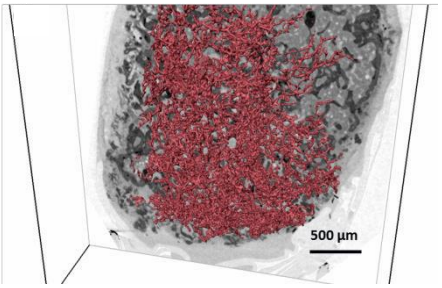

Fig. 1: A 3D representation of the blood vessel network (in red) in the bone marrow compartment of an explanted TE construct (using POM staining). In grey-scale, a cross-section through the explants is shown.

0114 Allogeneic mesenchymal stem cells are safe and stimulate cartilage repair upon co-implantation with chondrons

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Introduction: The purpose of this study was to demonstrate safety, feasibility and initial clinical outcome of an investigator-driven regenerative therapy that uses allogeneic multipotent mesenchymal stromal cells (MSCs) co-implanted with recycled autologous cartilage-derived cells (chondrons) for treatment of cartilage defects in the knee.

Methods: A total of 35 patients were treated in this first in man phase I/II prospective monocenter study. All patients had a focal defect on the femoral condyle or trochlea (mean size 3.2 ± 0.7 cm^2). Cartilage defects were treated with a combination of freshly autologous chondrons, rapidly isolated from cartilage taken from the rim of the defect, and allogeneic cryopreserved bone marrow-derived MSCs (ratio 1:9), which were implanted in fibrin glue within two hours. Clinical outcome was measured by the Visual Analogue Scale (VAS) for pain and the Knee injury and Osteoarthritis Outcome score (KOOS) at 3, 6, 12 and 18 months. Magnetic Resonance Imaging (MRI) and a second-look arthroscopy measured structural outcome at 12 months. Full-thickness biopsies taken from the centre of the repair tissue were used for histological analysis and DNA tracking with short tandem repeat (STR) analysis.

Results: No serious adverse events were found. All patients showed statistically significant and relevant clinical improvement in the VAS and KOOS scores from baseline to 18 months with the largest effect at three months follow-up ($p<0.001$). MRI scans and second-look arthroscopies indicated good defect fill and integration with native tissue. Histological analysis of the biopsies showed hyaline cartilage-like repair (Fig. 1). No DNA of the allogeneic MSCs could be detected in the repair tissue.

Conclusion and discussion: This is the first study to show preliminary safety of allogeneic MSCs, which together with autologous chondrons stimulate cartilage regeneration. All MSCs were disappeared 12 months after surgery, while the defects were restored with functional hyaline cartilage-like tissue. These findings are promising, as a new road has been opened in terms of safety of single-stage cartilage repair using allogeneic stem cells.

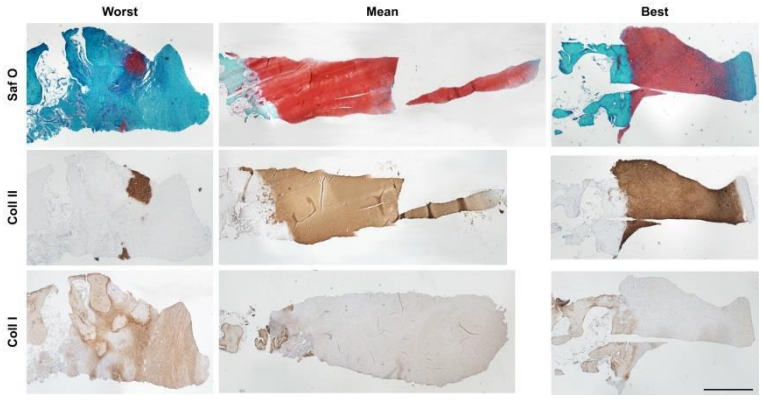

Figure 1: Safranin-O proteoglycan staining (Saf O) and type II and I collagen immunostaining (Coll II and I) on worst (N=2), mean (N=25) and best (N=6) scoring biopsies from the centre of the repair tissue twelve months after surgery. Scale bar indicates 1 mm.

0115 Regulating chondroprogenitor cell fate using mechanical stimulation

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The unique properties of mesenchymal stem cells (MSCs) and their natural presence within the bone marrow make them an attractive source of cells for novel cartilage repair strategies. Within the musculoskeletal system, one of the major drivers of repair is the mechanical load applied to the cells within the defect.

When developing new therapies in vitro, static culture is the most commonly used method. However, it is clear that due to the critical role mechanics plays in vivo, a more physiological loading regime in vitro would be most appropriate and this can be achieved by the use of bioreactors. Using a multiaxial load bioreactor system, we have been investigating the effect of mechanical stimulation on human stem cell differentiation. Performing studies in the absence of growth factors, specifically transforming growth factor β (TGFβ), allows the direct effect of the mechanical strain applied to be elucidated. Our bioreactor system allows for the application of shear, compression or a combination of both stimuli to establish the phenotypic changes induced within MSCs. In particular, the effect of the various mechanical stimuli on chondrogenic differentiation has been investigated.

As a model system, human bone marrow derived MSCs are embedded in a fibrin gel, which is then retained in a macroporous biodegradable polyurethane (PU) scaffold. This system provides a naturally occurring support matrix (fibrin), while allowing for cyclical load to be applied due to the resilience of the PU scaffold. Neither compression alone, nor shear alone can induce a change in MSC phenotype within this system. However, we have demonstrated that a combination of compression and shear is able to induce chondrogenic differentiation and this is due to increased endogenous expression of TGFβ from the loaded cells.

Using this multiaxial load bioreactor system we are able to investigate novel treatments and therapies in vitro, under physiologically relevant kinematic load. We are also able to search for novel markers and potential therapeutic targets that only occur under physiological loads. In addition, potential rehabilitation protocols to be used after cell therapy in cartilage repair can also be investigated.

0116 Cartilage mimics by 3D bioprinting of ipsc

Stina Simonsson[1], Dong Nguyen[1,2], Alma Forsman[1], Josefine Ekholm[1], Puwapong Nimkingratana[1], Daniel Hägg[3], Camilla Brantsing[1], Sebastian Concaro[4], Mats Brittberg[4], Paul Gatenholm[3], Annika Enejder[2], Anders Lindahl[1]

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Cartilage lesions that can develop into degenerated osteoarthritis (OA) cartilage are a worldwide burden. As a prospective treatment for such lesions, we show that human-derived induced pluripotent stem cells (iPSCs) can be 3D bioprinted into cartilage-mimics. The advantages by using an established iPSC line [1] are unlimited, immortal characterized cell source. The objective of this study is to develop a cartilage prototype by 3D bioprinting of human chondrocyte derived iPSCs with an OA protective genotype.

Methods: Designing protocols that generates hyaline cartilage from pluripotent cells *in vitro* is still a challenge, due to that joint formation are late in development and far from the pluripotent state. There are recent protocols for hyaline-like cartilage generation from iPSCs using combinations of growth factors (GF), or GF plus co-culture with irradiated chondrocytes [1].

Results: Increased cell density (500 million cells per ml) were detected by 2-photon-fluorescence-microscopy within the cartilaginous-like tissue, indicating the importance for good cell survival after printing. Collagen type 2 could be detected (Figure 1) and the pluripotencymarker Oct4 was lost in the cartilage mimics.

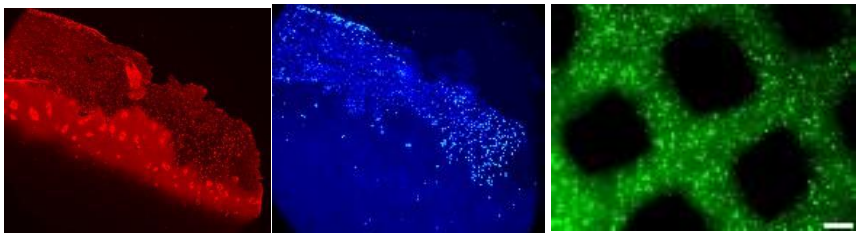

Figure 1 Collagen type 2 (red) in cartilage prototypes. Nuclei was counter stained with DAPI (blue). Right 3 D bioprinted iPSCs, live cells (green).

ACKNOWLEDGEMENTS:

This research was supported by VR Grant 2015-02560, Stina Simonsson

0117 Local biodistribution of hyaluronan-primed progenitor cells in a rabbit model of osteoarthritis: therapeutic implications for tissue repair

Giovanna Desando, Isabella Bartolotti, Carola Cavallo, Antonella Schiavinato, Cynthia Secchieri, Giuseppe Filardo, Elizaveta Kon, Maurizio Paro, Brunella Grigolo

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Introduction: The evaluation of key factors modulating cell homing after transplantation could provide important insights in the comprehension of biological mechanisms underlying tissue repair for osteoarthritis (OA) treatment. The purpose of this study was to investigate the local biodistribution of intra-articularly (IA) injected Mesenchymal Stromal Cells (MSCs) and Bone Marrow Concentrate (BMC) in a rabbit OA model, and whether the additional use of sodium hyaluronate (HA) could have effects on cell migration and wound-healing.

Methods: The study was performed in a rabbit model of OA through bilateral anterior cruciate ligament transection. After OA onset, both cells were intra-articularly delivered into the knee joint to assess: i) the biodistribution through cell labelling with a fluorescent dye at short-term follow-up; and ii) the healing potential of cells at long-term follow-up. Histology and immunohistochemistry aimed at studying markers involved in catabolic processes, or macrophage subset population, were done. Kruskall Wallis test, followed by post-hoc Dunns test and Spearman's rank-order correlation method, were used.

Results: The combination of both MSCs and BMC with HA promoted cell migration towards cartilage (Figure 1). The presence of cell clusters and OA changes in the extracellular matrix were proved to be important factors in driving cell homing in cartilage. In general, labelled cells combined with HA were detected in adjacent sites showing inflammatory and anti-inflammatory macrophages in the synovium. A promotion of joint repair was observed in all different tissues, mainly after BMC-HA treatment. This also provided proper stimuli to favor the expression of anti-inflammatory macrophages.

Conclusions: Studies tracking cell biodistribution indicate that priming progenitor cells with HA modulated cell homing favoring their attachment to articular cartilage. The treatment with BMC-HA, a "one-step" procedure, resulted the best therapeutic strategy supporting joint repair in our experimental model.

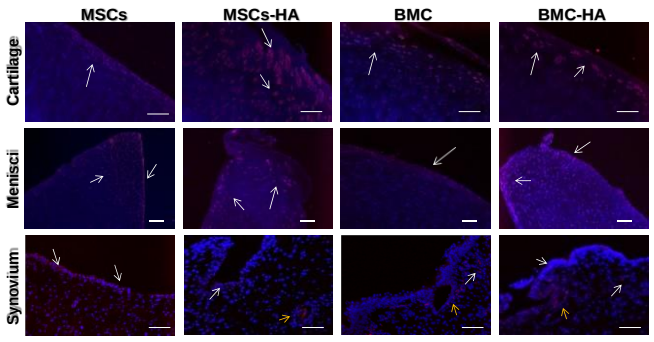

Figure 1: Images from epifluorescence microscope for labelled cells from MSCs, MSCs-HA, BMC and BMC-HA groups in cartilage, menisci and synovia. Arrows: labelled cells. Red staining: labelled cells (TRITC channel); blue staining: nuclear component (DAPI channel).

0118 Whole organ bioengineering

Shay Soker

Wake Forest School of Medicine, Winston-Salem, NC, USA

Irreversible end-stage organ failure process represents one of the leading causes of death and organ transplantation is currently the only curative solution. Donor organ shortage and adverse effects of immunosuppressive regimens are the major limiting factors for this definitive practice. Recent developments in bioengineering and regenerative medicine provide the scientific basis for creating implantable, personalized bioengineered organs. Whole organs detergent-perfusion protocols permit to gently remove all foreign cells, while preserving the original three-dimensional framework of the native organ. Decellularized organs including liver, kidney and pancreas, have been created using this technology to serve as a scaffolding platform for successful seeding of compatible cells. These scaffolds are composed of organ-specific extracellular matrix that contains growth factors important for cellular growth and function. The Macro- and microvascular tree anatomy is maintained and can be integrated with the recipients' vascular system after the implant. This presentation will focus on recent achievements in the whole-organ bioengineering and discuss the major barriers for success in order to achieve the clinical goal of functional bioengineered organ ready for transplantation.

0119 Engineering human liver: from 3D micro-scaffolds to whole organ engineer

Giuseppe Mazza, Massimo Pinzani

UCL, London, UK

Neural progenitor cells derived from induced pluripotent cells (iPS-NPs) are hot candidates for treatment of various neurological diseases. We used iPS-NPs in the treatment of stroke, spinal cord injury (SCI) and Amyotrophic Lateral Sclerosis (ALS). In vitro the cells expressed neuronal (MAP2, bIII Tubulin), astrocytic (GFAP) and oligodendrocytic (Olig2) markers, differentiated into GABA-ergic, dopaminergic neurons and motor neurons. Female Sprague-Dawley rats were subjected to focal cerebral ischemia by reversible right MCAO for 90 min, while male Wistar rats were used for SCI (balloon-induced compression lesion). $SOD1^{93A}$ transgenic rats were used as a model of ALS. A suspension of iPS-NPs (300 000 cells in 3µl of CM) was transplanted into the lesions 7 days after MCAO or SCI; the control groups were injected with saline. Asymptomatic and symptomatic rats (7 and 25 weeks old, respectively) were intraspinally grafted with iPS-NPs. The animals' motor functions were tested throughout the course of the disease. Functional recovery in rats with MCAO lesion was regularly assessed after transplantation by the apomorphine-induced rotation test and tape-removal test. Rats with SCI were tested using the BBB test, flat beam test and rotarod. The grafted animals in the stroke model displayed a decreased number of clockwise rotations in the apomorphine-induced rotation test and performed better in the tape removal test. Animals with SCI significantly improved their locomotor activity when compared to control animals. iPS-NPs robustly survived in both models of injury, maintained their neural phenotype and migrated toward the lesioned area and survived there for 2-4 months after transplantation. In addition, some of the cells differentiated into more mature and tissue-specific neurons. The transplantation of iPS-NPs into symptomatic and presymptomatic ALS animals resulted in significantly prolonged survival (by 13 and 20 days, respectively) and slowed disease progression compared with vehicle-treated littermates. Presymptomatic grafting of NP-iPS also postponed disease onset. Both groups of cell-treated rats presented significantly better motor activity. Grafting of NP-iPS up-regulated the expression of growth factors' (NGF, IGF-1 and BDNF) and stabilized the expression of apoptosis-related genes' (BAX, BCL-2 and Casp-3) compared to vehicle-treated rats. Some of the grafted cells expressed neuronal marker bIII-tubulin or neurofilament NF200, but no differentiation into motoneurons was observed in this study. In conclusion, these results suggest that iPS-NPs can improve functional outcome in stroke and SCI and prolong lifespan in ALS animals. They survive and integrate into the host tissue, and under certain conditions undergo further differentiation after transplantation. Our findings therefore demonstrate great regenerative potential of iPS-NPs in the treatment of neurodegenerative diseases and CNS injury.

The study was supported by MEYS LO1309, GACR 15-06958S, GACR P304/12/G069 and Project Biocev and Center of Reconstructive Neuroscience CZ.02.1.01/0.0/0.0/15_003/0000419.

0120 Advances in human iPSC-Derived tissues enabling microphysiological systems and regenerative medicine approaches

Sabine Lange, David Mann

Cellular Dynamics International - A FUJIFILM Company, Madison, USA

Stem cell technology affords unprecedented, limitless access to a consistent supply of human tissues. Current differentiation protocols yield robust sources of cardiac, hepatic, pancreatic, neuronal, endothelial and vascular cells. Bioengineered and microphysiological culture systems (MPS) add culture complexity via aspects of dimensionality, scaffolding, flow, and co-culture to elicit a more *in vivo* relevant model for study. Coupling iPSC-derived tissues with organotypic culture platforms synergizes to advance predictive *in vitro* biology for disease modeling and safety pharmacology and also enables regenerative medicine approaches to supply engraftable tissue as a therapeutic strategy. In this presentation, we review data highlighting the functional relevance obtained via deployment of iPSC-derived tissues in MPS ranging from simple 3D cultures with iPSC-derived hepatocytes to more complex MPS deployment. In addition, advances toward clinical application of iPSC enabled tissue generation will be presented.

0121 Tooth development and regeneration in Swine model

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Capital Medical University, Beijing, China

Objectives: The miniature pig provides an excellent experimental model for tooth development and regeneration because its diphyodont and heterodont dentition resemble that of humans. However, little information is available on the process of tooth development or the exact molecular mechanisms controlling tooth development in miniature pigs or humans. The purpose of the present study is to establish swine tooth development research model as first step, then to investigate the tooth regeneration using cell reassociation in this model.

Methods: The specific transcriptome in developing mandibular deciduous molars of the miniature pig was identified. cDNA profile, the specific microRNAome and expression profile were investigated. Swine tooth regeneration was performed using reassociation of swine tooth germ cells. We separated swine molar germ as epithelium and mesenchymal cells, made single cell pellet, reaggregate the cells, made subrenal transplantation in nude mice and jawbone of swine. Swine tooth germ served as positive control, only mesenchymal cells as negative control.

Results and Conclusions: cDNA profile, the specific microRNAome, expression profile and molecular expression pattern in developing mandibular deciduous molars of the miniature pig were obtained, which provided useful information for investigating the molecular mechanism of tooth development in the miniature pig. Our findings also identified the characteristic patterns about spatio-temporal morphogenesis of successional teeth in context of their predecessor and cascade initiation of additional molars in miniature pigs. The gene expression profiles indicated that spatio-temporal down-regulation patterns of gene expression were predominant; while, both dynamic activation and inhibition of pathways occurred during the diphyodont morphogenesis. We found that swine tooth germ cells were isolated to be single cells, reassiociated, and then continued to develop normal tooth by subrenal transplantation and swine jawbone in vivo. The sources, immunology and developmental biology of reassociated dental epithelium and mesenchymal cells are three key issues for whole tooth regeneration.

0122 Mesenchymal stem cell mediated repair of ischemically damaged human renal allografts

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Introduction: The chronic kidney shortage remains a huge obstacle in transplantation today. In our study we evaluated if a Mesenchymal Stem Cell (MSC) therapy could accelerate the repair of ischemically damaged human kidneys during an ex vivo warm perfusion. We hypothesized that by infusing MSC directly to the renal tissue, there would be an improved opportunity for repair mediated by the increased intrarenal paracrine effects.

Methods: Studies were performed using the Exsanguinous Metabolic Support (EMS) platform. Kidneys from DCD donors (n=5) with a mean ischemic insult of 40 minutes were studied. A paired human kidney model was used; kidneys were EMS perfused for 24 hours with or without the addition of MSC. Human MSC ($1x10^8$) were slowly infused into the renal artery during the perfusion. Kidneys were evaluated by histological grading, cytokine/chemokine synthesis and a panel of biomarkers indicative of DNA synthesis, cytoskeletal regeneration and injury markers.

Results: Treatment with MSC resulted in a reduction of inflammatory cytokines synthesized by the kidneys. MSC treatment led to a significant increase in the synthesis of ATP (Figure 1) and growth factors resulting in normalization of the cytoskeleton during 24 hours of perfusion. Toluidine Blue staining of MSC treated kidneys showed a significant increase in mitotic figures (23%) compared to EMS alone (Figure 2). PCNA and Clusterin staining also detected the same positive MSC mediated effect.

Discussion: To our knowledge, our work is the first to have achieved actual cellular regeneration while ischemically damaged human kidneys are perfused ex vivo for 24 hours. The observed regeneration entails: increased synthesis of ATP, a reduced inflammatory response, increased synthesis of growth factors, normalization of the cytoskeleton and mitosis. The ability to regenerate renal tissue ex vivo sufficiently enough to result in immediate function could revolutionize transplantation by solving the chronic organ shortage.

Figure 1: Renal Metabolism – ATP Synthesis

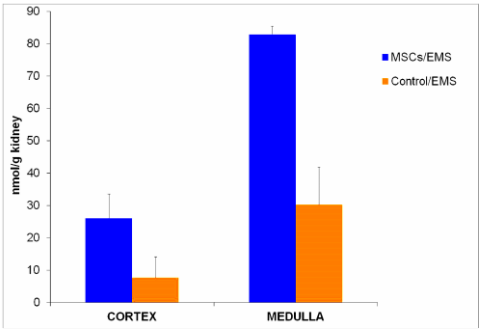
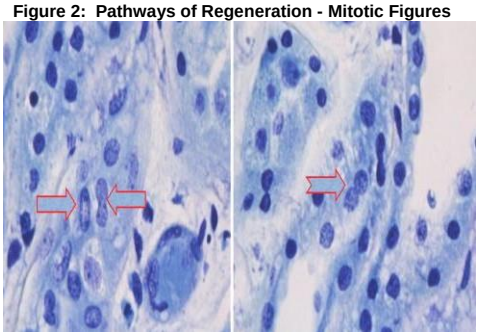


Figure 2: Pathways of Regeneration - Mitotic Figures



MSC Treated (23% Increase) **EMS Perfused**

0123 Development of an artificial native-like layered oesophagus engineered with primary mesoangioblasts, neural and epithelial cells

Carlotta Camilli[1], Luca Urbani[1], Claire Crowley[1], Demetra-Ellie Phylactopoulos[1], Dipa Natarajan[1], Conor McCann[1], Federico Scottoni[1], Anna Urciuolo[1], Marc-Olivier Baradez[2], Ed Hannon[1], Koichi Deguchi[1], Asllan Gjnovci[1], Giulio Cossu[3], Simon Eaton[1], Paola Bonfanti[1], Paolo De Coppi[1]

[1]UCL Great Ormond Street Institute of Child Health, London, UK, [2]The Cell and Gene Therapy Catapult, London, UK, [3]University of Manchester, Manchester, UK

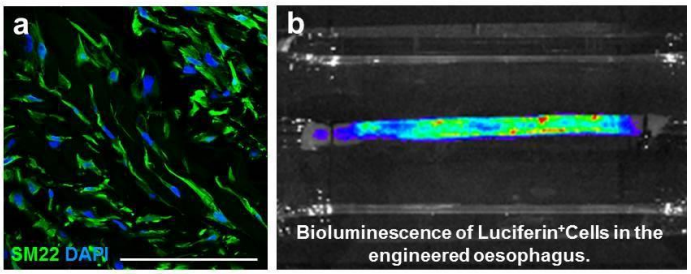
A suitable oesophageal replacement is needed in several genetic and acquired conditions, such as *long-gap* oesophageal atresia or tumours and lesions that lead to oesophagectomy. Oesophageal tissue engineering is emerging as a concrete therapeutic option aiming at developing functional tissue substitutes. Engineering the oesophageal layers is a key factor to restore mechanical and functional properties of the tissue and to foster complete regeneration *in vivo*.

The aim of this work is to develop a fully mature tubular oesophageal construct for *in vivo* replacement. In a customised bioreactor, a decellularized scaffold was combined initially with smooth muscle and enteric neural crest precursors to engineer the *muscularis externa*, followed by epithelial cells to reconstitute the oesophageal luminal barrier.

Decellularization of rat oesophagi was achieved using an established detergent-enzymatic protocol. Human mesoangioblasts, mouse fibroblasts and neural crest cells and rat epithelial cells were isolated from different sources and/or labelled in order to facilitate their identification within the engineered construct and throughout the culture. Cells were seeded into the acellular scaffold in 2 stages and cultured for 14 days in the bioreactor.

Mesoangioblast precursors engrafted and migrated within the muscle layer of the acellular scaffold, obtaining a cell distribution comparable with a native oesophageal smooth muscle layer. Smooth muscle cell spread was significantly improved by the co-injection of fibroblasts favouring an overall homogeneous distribution with no fibrotic effects. Our fine-tuned combination of cell delivery, media sequence and 3D culture allowed marked cell proliferation and differentiation, confirmed by expression of Calponin and SM22 and morphological cell orientation with pre-existing ECM structures (Fig1a). Notably, a pool of proliferating cells ($Ki67^{+}$) was identified at the end of the culture, resembling the native tissue homeostasis. Enteric neural crest cells-derived mature neurons (identified by TuJ1 expression) integrated within the scaffold with axonal projections which made cell-cell contacts with mesoangioblast-derived smooth muscle cells. Functional integration was confirmed by electrical point stimulation of the repopulated scaffold resulting in cellular calcium transients using Ca^{++} imaging. Finally, a second cell seeding step allowed successful delivery of epithelial cells into the lumen of the oesophageal scaffold, without affecting the 3D culture in the bioreactor (Fig1b). Epithelial cells showed proliferation and self-organized in a continuous monolayer after 3 days of culture, expressing the basal cytokeratin marker CK14.

Decellularized scaffolds, preserving tissue-specific ECM information, acted as ideal templates driving cell behaviour within the 3D structure. Using a 2-stage seeding technique, we developed for the first time a fully mature tubular construct, in which different cell types show specialized location and function. Moreover, our finely regulated 3D culture promoted crosstalk between distinct cell populations and final maximising cell re-population maturation. This tissue engineering approach shows robust validation and potential for the development of artificial constructs for pre-clinical and clinical application in oesophageal replacement.



0124 Tools for Accelerated Innovation: Stories from Bioinspiration to Innovation

Jeffrey Karp

Harvard-MIT, Boston, USA

When developing technologies to solve medical problems, often one encounters significant hurdles, that at times seem insurmountable. Overcoming these hurdles requires new ways of thinking. One approach is to turn to nature for inspiration. Millions and millions of years of research and development at our fingertips, and all we need to do is look outside to the amazing creatures that inhabit our planet. This talk will explore medical technologies being developed that harness lessons from nature for inspiration, from creatures such as geckos, spider webs, jellyfish, porcupine quills, snails, to spiny headed worms. Some of the technologies that will be described are rapidly advancing to the clinic and some are already on the market helping patients.

0125

Paul Kemp

0126

Marie Paule Richard

0127

Alain Vertres

0128 Circulating stem cells as a source for endogenous stem cells mobilization and their clinical implications

Gang Li[1,2]

[1]The Chinese University of Hong Kong, Shatin, Hong Kong, [2]Shenzhen Research Institute of Chinese University of Hong Kong, Shenzhen, China

Mesenchymal stem cells (MSCs) have been found in cord blood and peripheral blood (PB) of mammalian species including human, guinea pig, mice, rat, dog, horse and rabbit. The number of MSCs in PB (PB-MSCs) is rare and their biological role was not fully defined. We have found increased numbers of circulating MSCs in peripheral blood in patients with long bone fracture, non-union and in patients with cancers. The number of PB-MSCs was approximately 9 times higher in the cancer patients, suggesting there is systemic recruitment of MSCs during cancer development. We have compared the difference between the circulating MSCs and bone marrow derived MSCs and found that they share similar phenotype in vitro, but the gene expression profile between the two cell populations was significantly different. cDNA microarray analysis and quantitative RT-PCR confirmed some genes that are differentially expressed with more than 10 folds difference, such as cellular retinol-binding protein 1 (CRBP1), N-cadherin, SRY-box containing gene 11 (Sox11), the aquaporin 1 (AQP1), et al. These genes are now being further investigated for their role in MSCs migration, homing and multiple-differentiation potential. In terms of potential clinical implications of PB-MSCs, we have demonstrated that allogenic PB-MSCs enhanced bone regeneration in rabbit ulna critical-sized bone defect model. We also demonstrated that BM-MSCs can be recruited via circulation toward the sites of bone fracture and participate fracture healing in rabbits. We have demonstrated that systemically administrated MSCs could home to tumor sites and participated tumor growth. We are now working on using MSCs as a systemic gene delivery vehicle for management of wound healing and cancer therapy, and the ways of enhancing the homing and recruitment of MSCs toward specific sites after their systemic delivery. In conclusion, PB-MSCs are new cell source of cells that may play very important roles in development, repair and disease progression. PB-MSCs may be used for disease monitoring, diagnosis, cell and gene therapy applications.

0129 Targeting AMPK signal pathway to regulate the stem cells differentiation and bone regeneration

Yugang Wang, Qiming Fan, Tingting Tang

Shanghai Key Laboratory of Orthopedic Implants, Department of Orthopedic Surgery, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai, China

The cell senescence and related imbalance between osteogenesis and adipogenesis of bone marrow mesenchymal stem cells (MSCs) are the major pathological factors in age-related osteoporosis. Low-grade age-related systemic inflammation have also been reported to accelerate the senescence of stem cells. It is reported that AMP-activated protein kinase (AMPK) as an integrator and mediator of several pathways and processes linking energetics to inflammation and ageing. We established MC3T3-E1 and 3T3-L1 cell models of AMPK α subunit overexpression through lentivirus vector, in which AMPK was over-activated. Further study indicated that the α 2 and α 1 subunits of AMPK had several functional differences, with α 2 conferring stronger osteogenic potential (Fig 1) and a weaker ability to induce osteoblasts-associated osteoclastogenesis in MC3T3-E1 cells as well as conferring a lower adipogenic potential to 3T3-L1 cells. The functional differences of α 2 and α 1 in primary MSCs have also been demonstrated. These findings provide a basis for developing drugs that can differentially target the α 1 or α 2 subunits of AMPK to treat ageing related diseases such as obesity and osteoporosis.

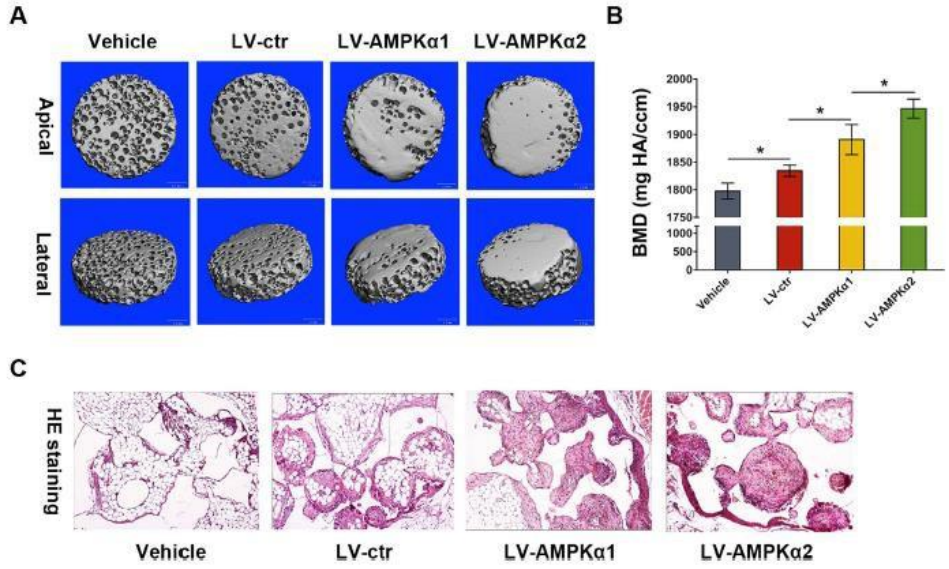


Figure 1. In vivo ectopic bone formation in MC3T3-E1 cells over-expressing AMPK α1 and α2 at 8 weeks. β -TCP scaffolds loading with or without MC3T3-E1 cells over-expressing AMPK α 1 and α 2 were implanted into the intramuscular pocket of the femur of nude mice. (A) Micro-CT images. (B) Measurement of BMD. (C) Histological micrograph, H&E staining. (Scientific Reports. 2016, 6:32771. DOI: 10.1038/srep32771)

0130 Tendon stem cell fate and treatment of tendinopathy by autologous tendon cell injection in human

Minghao Zheng

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The pathogenesis of tendinopathy has traditionally been viewed as a continuum from reactive change through to partial- and full-thickness tendon tears. Intrinsic degeneration within the tendon(s) and repetitive microtrauma are thought to contribute to the process. Despite its clinical significance, tendinopathy has been poorly studied from both a cellular and molecular perspective. However, tendon degeneration has been shown to involve disruption of the collagen matrix, calcification, vascularisation and adipogenesis. Programmed tendon cell death, an important physiological process that maintains homeostasis in many tissues, is believed to play a major role in the progression of tendinopathy. Elevated apoptotic and autophagic cell death rates in common extensor and rotator cuff tendinopathy are often observed. This has led to the suggestion that the healing process is suppressed by a lack of cellular components. The depletion of cell population and reduced collagen synthesis results in accelerated collagen deterioration during tendinopathy, which compromises the ability of the tendon to maintain its structural integrity, eventually leading to tendon tear. Based on these studies, we proposed that restoration of functional cells capable of synthesising extracellular matrix and repairing the damaged tissue within the tendon may be an effective therapeutic strategy for tendon repair.

Tendon-derived cells, containing a population of tendon-derived progenitor cells (TPCs), possess the potential for tendon regeneration as they have the capacity for collagen synthesis, proliferate rapidly and are self-renewable. The homologous application of tendon-derived cells provides strong indication for use in tendon tissue regeneration. The efficacy of tendon cell introduction for tendon repair has been verified in a number of in vitro and animal studies. Our animal studies have revealed that implantation of in vitro expanded autologous tenocytes improved the tendon structure and facilitated the healing process in both an acute tendon tear model (36) and a chronic degenerative tendon disease model (35).

In an effort to develop an autologous and homologous cellular engineering approach for tendon repair our laboratory has established the first autologous tenocyte therapy technique (ATI). The technique involves obtaining a patellar tendon biopsy from the patient, isolation of cells from the tendon tissue by enzymatic digestion, in vitro expansion of the cell population to a sufficient number in a GMP certified laboratory, and lastly, delivery of cells to the defect site by an ultrasound guided needle injection.

Phase I/IIa studies of ATI for common extensor tendinopathy show that ATI significantly improves disability and function for up to 5 years post-treatment. However, randomised studies of autologous tenocyte injection as compared with other treatments are needed to further prove the efficacy of the technology.

0131 The effect of three-dimensional bionic collagen/silk scaffold on rotator cuff tendon regeneration

Weiliang[2], Ouyang[1]

[1]Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cell and Regenerative Medicine,Zhejiang University,, Hangzhou, China, [2]Department of Orthopedic Surgery, 2nd Affiliated Hospital, School of Medicine, Zhejiang University,, Hangzhou, China

Objectives: To develop an advanced scaffold (3D bionic collagen/silk scaffold, B scaffold) for rotator cuff tendon (RC) repair by modifying the arrangement of collagen fibers of our basic sponge collagen/kintted silk scaffold (sponge collagen/silk scaffold, S scaffold).

Methodology: The structure, biological compatibility and cell seeding effect of B scaffold were investigated in vitro, while the optional collagen structure and efficacy of B scaffold in promoting RC regeneration was evaluated in vivo within a rabbit model.

Results: With in vitro studies, Scanning electron micrographs showed that tendon stem/progenitor cells (TSPCs) were spindle-shaped and well orientated on the B scaffold. The expression of tendon-specific genes was significantly higher in TSPCs growing on B scaffold than those on S scaffold in both normal and osteogenic media. In addition, alkaline phosphatase activity and alizarin red staining showed that the S scaffold induced osteogenesis, while the B scaffold hindered the process. With in ectopic study, the B scaffold elicited little inflammatory reaction and 10 mg/ml was the optimal collagen concentration for 3D bionic collagen structure with more regularly aligned cells and larger collagen fibers. With in vivo studies, RC treated with B scaffold showed more native microstructure with larger diameter collagen fibrils, deposited more collagen, had better mechanical properties, and stronger scaffold-tendon interface healing than those treated with S scaffolds.

Significance: The findings are the first to highlight the important roles of 3D bionic substrate in tendong regeneration biology. This 3D bionic collagen/silk scaffold can therefore be a practical application for tendon tissue engineering.

0132 Nature’s cues towards ECM engineering - Learning from human development

Katja Schenke-Layland

[1]Fraunhofer IGB, Stuttgart, Germany, [2]Dept. of Women's Health, Research Institute for Women's Health, Tübingen, Germany, [3]UCLA, Los Angeles, USA

In addition to cells, extracellular matrix (ECM) is one of the most important components of all tissue types in the human body. It consists of fibers and networks composed of structural proteins, such as collagens or elastin. The ECM directs cell orientation in the three-dimensional (3D) space, is essential for cell migration and affects cell communication and differentiation. In my presentation, I will focus the impact of ECM on the cardiovascular cell fate and its impact on stem cell biology and regenerative medicine.

0133 Making elastic arteries from synthetic polymers without exogenous cells

Yadong Wang

University of Pittsburgh, Pittsburgh, USA

Introduction The last decades of arterial tissue engineering research have largely focused on cell-based approaches. This study focuses on materials, we set out to investigate the potential of mammalian host to remodel synthetic polymeric grafts into living artery-like tissues. This bypasses cell harvesting, seeding and culturing completely, further reducing donor site morbidity and production cost.

Materials and Methods We designed the graft to have two layers, the inner tubular core is made of the elastomeric poly(glycerol sebacate),1 the outer sheath is made of polycaprolactone fibers. The sterilized grafts are coated with heparin and implanted as interposition grafts in rat abdominal aorta or carotid artery.

Results and Discussion Fast polymer degradation led to rapid host remodeling. The regenerated artery mimicked native artery mechanically, biochemically, and anatomically. The neo-arteries were well integrated with the host, remained patent and pulsed synchronously with host arteries.2 The elastin and collagen content of the regenerated arteries matches that of native arteries. The regenerated arteries are free from aneurysm, stenosis and calcification 1-year post-implantation. Preliminary data indicate that the same regeneration also occurs in the carotid arteries, a more challenging and clinically relevant model for small arteries. It remains to be seen if this is translatable to small arteries in large animal models and humans.

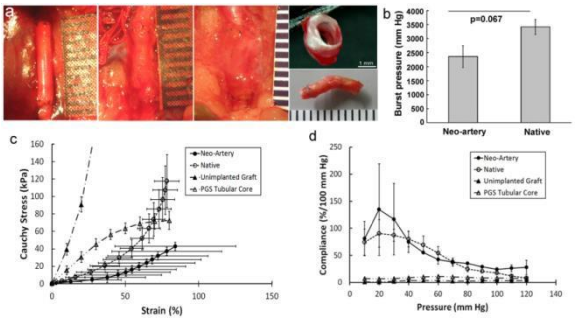

Fig 1. Remodeling of the synthetic graft. (a) Gross appearance of the graft at day 0, 14 and 90. (b) The burst pressure, (c) Stress-strain curve, and (d) Compliance of the neo-artery, approximate that of the native aorta.

Disclosures Nothing to disclose.

0134 Engineering a highly elastic protein-based surgical sealant

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Approximately 114 million surgical and procedure-based wounds occur annually worldwide, including 36 million from surgeries in the US. Post-operative reconnection of tissues is crucial for restoring adequate function and structure. Sutures, wires, and staples are widely used for this purpose. Despite their common use in the clinic, these methods exhibit limitations when being applied to fragile and soft tissues, especially if the sealing is intended to prevent liquid or air leakage against high pressure, as e.g. in vascular and lung surgeries.Various types of surgical materials have been used for sealing, and reconnecting tissues, or attaching devices to tissues. These surgical sealants/adhesives can be used in adjunction with sutures for better closure or even can potentially replace sutures and staples to close the wound rapidly and improve clinical outcomes. However, existing surgical sealant materials often display limited adhesive strength, toxicity, lack of appropriate mechanical compliance and importantly, do not function well in wet and dynamic environments in the body. To address these limitations, we designed and developed a novel human protein-based tissue sealant that combines several critical characteristics and can outperform the currently available sealant products in the market.

An ideal surgical sealant is required to be highly elastic to be able to adapt with dynamic movement of native tissues, have excellent biocompatibility and controlled biodegradability, and provide high adhesive strength and burst pressure particularly in the presence of body fluids. Therefore it is very important to tailor the physical and biological properties of the biomaterials that are used to engineer surgical sealants in accordance with the indicated tissue. These biomaterials should also rapidly polymerize in situ to seal the wound areas without inducing toxicity. We have showed that UV crosslinkable MeTro can produce a class of highly elastic, human protein-based hydrogels with high biocompatibility. MeTro exhibited tunable mechanical properties depending on different MeTro concentrations and methacrylation degrees. Adhesion strength of MeTro sealant, measured by wound closure and lap shear tests, showed properties superior to the clinical standard glues such as Evicel and Coseal. Improved cell viability and proliferation were achieved using MeTro gels in vitro. Subcutaneous implantation also showed excellent biocompatibility and material integration into the host environment. In addition, burst pressure of MeTro sealant covering a rat lung leakage was measured 7 days after surgery. The value of bust pressure for the MeTro-sealed lung was similar to that of healthy lung tissue, confirming that MeTro sealed the incision and promoted lung tissue healing.

A highly elastic, biocompatible, and biodegradable hydrogel-based sealant was engineered through photocrosslinking of a modified human protein. Our in vitro and in vivo data suggest that this material is superior to the existing products in the market and may generate a paradigm-shifting surgical sealant that, due to its excellent mechanical and adhesive properties, may not require additionally supporting sutures.

protein-based elastomer.

0135 Pro-elastic matrix regenerative effects of doxycycline-release nanoparticles targeting inhibition of c-Jun N-Terminal Kinase

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Introduction: Abdominal aortic aneurysm growth is driven by chronic proteolysis of aortal wall elastic fibers by matrix metalloproteinases (MMPs) and their inability to naturally repair/regenerate. We previously showed that at low (< 10 g/ml) doses, doxycycline (DOX) continues to inhibit MMPs as it does at much higher oral doses, but also stimulates elastic matrix neoassembly and crosslinking. In this work, we have shown that both these effects of low dose DOX are linked to its upregulation of transforming growth factor beta (TGF-β1) upon targeted inhibition of a regulatory protein c-Jun-N-terminal kinase 2 (JNK2). Using JNK2 inhibition as a metric for pro-regenerative matrix effects of DOX, we also investigated if sustained and steady release of DOX from biodegradable polymer nanoparticles (NPs) we have developed that independently provide pro-elastogenic and anti-MMP effects, is able to synergistically improve quantity and quality (crosslinking, fiber formation and density, stability against proteolysis) in in vitro cultures of cytokine-activated rat aneurysmal smooth muscle cells (EaRASMCs).

Methods and Materials: EaRASMC cultures were stimulated with cytokines to augment an activated phenotype and treated with DOX as a function of dose (1-20 g/ml) or not (treatment controls) and compared with cultures of healthy rat aortic SMCs (RASMCs). Western Blots were performed on cell lysates to detect expression of JNK isoforms, pJNK, and TGF-β1 at 30 min of treatment and outcomes were correlated with elastic matrix amounts, desmosine crosslinks, elastic fiber counts, MMP protein amounts and enzyme activities in the cell layers at 21 days of culture. In a second study, polylactic-co-glycolic acid-polyethylene glycol (PLGA-PEG) nanoparticles encapsulating DOX were formulated by double emulsion solvent evaporation using DMAB as a surfactant. NP size and charge were measured and DOX release profiles determined as a function of DOX loading and NP dose to determine conditions necessary to ensure steady state DOX release at the levels of exogenous DOX deemed most useful for JNK inhibition. Cytokine-activated EaRASMCs were cultured with the DOX-NPs for 30 min or 21 days, with RASMCs and EaRASMCs cultured with blank NPs and no NPs serving as controls. Assessments were performed as with the previous set of experiments.

Results: DOX inhibited expression and phosphorylation of JNK. Levels of JNK and pJNK, especially JNK2, were significantly lower in treated cultures and were comparable to the healthy control. DOX augmented TGF-β1 activity upon inhibition of JNK. These outcomes correlated positively to elastic matrix amounts, crosslinking and fiber counts and negatively to proteolytic activity indicators. These effects were found to depend on DOX dose. DOX-delivery from the NPs was deemed to be as or more effective in stimulating elastogenesis and fiber formation and inhibiting elastolysis relative to exogenous DOX, likely due to synergy between the nanocarriers and released DOX.

Discussion and Conclusion: The study results suggest JNK inhibition as a useful metric to assess pro-elastic matrix regenerative effects and point to the synergistic regenerative benefits provided by DOX and DMAB-functionalized DOX-NPs.

0136 Functional tissue formation in in situ engineered heart valves based on a resorbable elastomeric scaffold

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In situ heart valve tissue engineering (TE) is a prospering new concept based on acellular, bioresorbable scaffolds to replace malfunctioning heart valves. Upon implantation, the scaffold stimulates host cell colonization and subsequent tissue formation, while the initial starter matrix is gradually being resorbed, leading to a living, adaptive replacement valve. This process hinges around the host inflammatory response, which can be modulated by the scaffold properties (e.g. microstructure, material properties, bioactivities) in order to achieve functional regeneration. Our goal is to develop a readily-available and fully tailorable synthetic scaffold for heart valve replacement that induces tissue regeneration directly *in situ*. One of the main challenges therein is the induction of *functional* extracellular matrix regeneration, and elastogenesis in particular, in the inflammatory and hemodynamic environment.

We developed a synthetic replacement valve for *in situ* TE, based on an electrospun polycarbonate bisurea scaffold. Upon implantation in the pulmonary position in sheep, the valves exhibited: 1) extensive cellularization and neotissue formation, 2) cell-driven scaffold resorption, and 3) preserved valve functionality at 6- and 12-months follow-up (n=4 per time point). Although promising, native-like tissue organization formation was not achieved. Therefore, we aimed to detailedly map the spatio-temporal expression of neo-matrix and the phenotype of co-localizing cells in these *in situ* TE valves. To achieve this, we developed a species-specific antibody panel for detailed analysis of the sheep explants.

These elaborate morphologic analyses revealed that tissue formation is region- and layer-specific. (Tropo)elastin expression strongly co-localized with collagen expression at both timepoints. However, only very locally tropoelastin expression colocalized with the presence of a microfibrillar network (fibrillin- 1 and -2). Mature elastic fibers were mostly detected on the pulmonary side of the valve leaflet, in contrast to native. However, after 12 months, mature elastic fibers were also detected on the ventricular side of the leaflet, near the hinge region, albeit marginal compared to the native valve. In addition, we identified a clear remodeling front in the hinge region, from which new tissue formation progressed. The remodeling front is characterized by extensive cellularization, scaffold degradation and expression of factors involved in valve development, such as periostin and TGF-β_1.

Current work is directed at expanding the morphologic analysis to elaborate on the role of specific immune cells, and investigating if the observed inhomogeneities in tissue formation are related to the local hemodynamic loads. This knowledge will be used to rationally design the next generation heart valve scaffolds, based on our supramolecular materials platform. In this respect, incorporation of bioactives (e.g. immunomodulatory cytokines) into the scaffold is being explored as a strategy to (locally) boost *in situ* elastogenesis.

0137 Stimulating the formation of an extracellular matrix microenvironment in vitro using macromolecular crowding – first glimpse on effects on microfibrillar networks

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Elastic fibres are composite materials that convey recoil properties to tissues. In essence, elastic fibres are a mixture of elastic microfibrils, mostly composed of fibrillin-1 and associated glycoproteins that are invested with amorphous elastin. While fibrillins are ancient molecules, already found in cnidarians (hydra, for example), elastin is found only in vertebrates. The combination of fibrilin-1 microfibrils that have intrinsic recoil with the rubber-like properties of elastin, allow numerous tissues to be reversibly deformed, such as arterial blood vessels, elastic ear cartilage, dermis, lung tissue, or ligaments. The formation of elastic fibres in tissue engineering organotypic constructs is still a rare observation, in particular in blood vessel tissue engineering where the formation of elastin-containing fibres has remained a major hurdle. The successful formation of elastic fibres must start with the successful formation of elastic microfibrillar aggregates. Therefore, we investigated the formation of fibrillin-1 microfibrils by monolayer cultures of mesenchymal stem cells (MSCs) in vitro. The classical assays established with dermal fibroblasts in the 1990s required seeding cells at immediate hyperconfluence (f.e. 100,000/sqcm) and culture for 72 hours. This system led to frequent losses of monolayers due to contraction and peeling off. However, seeding cells at lower densities would produce unsatisfactory microfibrillar deposits. Here, we developed a new system by implementing macromolecular crowding (MMC) in the culture medium at a fractional occupancy level Ψ of 18%v/v using Ficoll with human bone-marrow derived MSCs seeded at a density of only 2800 cells/sqcm. As MMC has been reported to be a powerful driver of the supramolecular assembly of extracellular matrix components such as fibronectin and collagen, we expected that a reduced cell density would be sufficient for fibrillin deposition in vitro under MMC. After 3 days of incubation with MMC, MSCs showed clear fibrillin-1 deposition in the form of fibres as compared to sparse deposition in standard cultures. After 5 days, 3 times more deposition occurred, and after 7 days at least 5 times more, with dense and substantial fibrillin-1 meshwork. Microfibril-associated glycoprotein 1 (MAGP-1) was associated with the microfibrillar fibres only under MMC, after 7 days of culture. This is the first demonstration of the efficacy of MMC in the assembly of elastic microfibrils in vitro, with 37 times less cells than hitherto known. In the presence of ascorbic acid more collagen IV was deposited. We also tested the efficacy of MMC in collagen I hydrogels and likewise, MMC enhanced elastic microfibril formation in vitro in 3D hydrogels. However, under ascorbic acid collagen IV prevailed. When we differentiated MSCs into adipocytes in hydrogels, we noted a remodelling of fibrillin-1 microfibrils and a reduction of the fibrillin-positive meshwork, while differentiated adipocytes were preferentially enclosed by collagen IV cocoons. Interestingly, under MMC these collagen IV cocoons were connecting with each other. As with collagens, macromolecular crowding appears to be a promising tool to drive the deposition of elastic microfibrils and thus might help to move tissue engineered constructs closer to the goal of achieving elastin fibre formation.

0138 Biological bases of therapeutic vascularization

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Therapeutic angiogenesis, i.e. the generation of new vessels by delivery of specific factors, is required both for rapid vascularization of tissue engineered constructs and to treat ischemic conditions. Vascular endothelial growth factor (VEGF) is the master regulator of angiogenesis. However, uncontrolled expression leads to vascular tumors (angiomas). Major challenges to fully exploit VEGF potency for therapy include the need to precisely control in vivo distribution of growth factor dose and duration of expression. We previously found that the therapeutic window of VEGF delivery depends on the VEGF amount in the microenvironment around each producing cell rather than on the total dose, since VEGF remains tightly bound to extracellular matrix and a few "hotspots" of high expression are sufficient to cause angioma growth even if the total dose is rather low [1]. On the other hand, short-term expression of less than about 4 weeks leads to unstable vessels, which promptly regress following cessation of the angiogenic stimulus [2].

Here I will present recent work aimed at: 1) translating fundamental principles of VEGF function into clinically applicable approaches to induce controlled angiogenesis, through the use of genetically modified progenitors or extracellular matrix engineering with recombinant factors [3], particularly for the regeneration of bone [4] and cartilage [5]; and 2) investigating the mechanisms that regulate the switch between normal and aberrant angiogenesis *in vivo*, to identify novel molecular targets that may prevent the toxic effects of VEGF.

0139 Synthetic high-efficient morphogens for guided angiogenesis

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Coronary heart disease and stroke are leading causes of mortality in Europe, resulting in a loss of function of the affected tissue. The challenge of this project is to restore the myocardial tissue functionality using linear peptides which possess angiogenic bioactivity, mimicking the growth-factors involved in the angiogenesis process, to stimulate the development of pre-existing blood vessels and enhance myocardial tissue regeneration.

The hypothesis behind the experimental process is in the use of hyperbranched molecules, called dendrons, which can be used as protein scaffolds for the spatial exposure of angiogenic bioactive peptides. Indeed, the overall objective of the project is to investigate the efficacy of the spatiotemporal delivery of dendron-tethered angiogenic linear bioactive peptides to induce angiogenesis *in vitro*. In this research, poly (ε-lysine) dendrons of branching generations G0 will be used as protein scaffold to present, at their uppermost branching generation, the Angiopoietin-1 (Ang-1) mimicking peptide, QHREDGS, which has previously been shown to promote blood vessel formation and stabilization. To evaluate the angiogenic potential of functionalised dendrons both as “free drug” and surface functionalization method, the novel molecule will be incorporated into hollow nanosphere. The modulation of angiogenic stimuli will be tested *in vitro*.

Functionalised dendrons were characterized successfully using MS, HPLC and FT-IR. Ang-1 peptide-functionalised G0 dendrons clearly induce the formation of a network of endothelial sprouting (Fig.1) where cells appeared to establish connections.

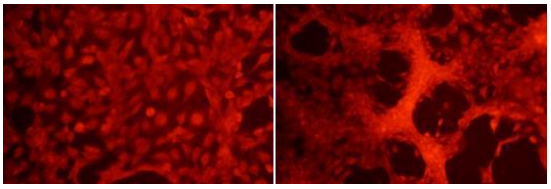

Figure 1: Induction of endothelial cell sprouting when HUVECs are spiked with Ang-1 peptide-functionalised G0 dendrons (right panel)

Consequently, hollow nanosphere coated with collagen has been produced and analysed by SEM and FT-IR (data not shown). Ang-1 peptide-functionalised G0 dendron has been loaded onto hollow collagen nanospheres resulting in a loading capacity of 25% and an encapsulation efficiency of 98% proving that the diffusion method used for the functionalization of the nanospheres has occurred successfully and that hollow collagen nanosphere coul be used as delivery carrier for dendritic angiogenic peptide.

The present study for the first time unveils a novel biomaterial approach to stimulate angiogenesis through nano-structured biomaterials and emphasises the need for a temporospatial presentation of the relevant peptide sequence to obtain endothelial sprouting.

0140 Therapeutic angiogenesis by a VEGF and PDGF-BB gene-activated matrix

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Therapeutic angiogenesis aims at promoting vascularization of ischemic tissues by delivery of exogenous growth factors. Vascular Endothelial Growth Factor (VEGF) is the master regulator of vascular growth. However, uncontrolled VEGF delivery can cause the growth of angioma-like tumors or edema. We previously found that co-delivery of PDGF-BB can prevent the toxic effects of VEGF and induce only normal angiogenesis despite uncontrolled VEGF levels. For clinical gene delivery, integrating viral vectors raise safety concerns, due to the risk of oncogenic transformation, while plasmid DNA vectors have an excellent safety profile, but suffer from limited transfection efficiency *in vivo*. In order to overcome these limitations and achieve safe and effective angiogenesis, here we sought to develop a gene-activated matrix (to ensure prolonged and efficient delivery of a plasmid vector) in combination with balanced co-expression of VEGF and PDGF-BB (to prevent the side-effects of uncontrolled VEGF expression *in vivo*).The gene-activated matrix was based on an Elastin-like polymer (ELP) biomaterial, because: 1) it is an injectable liquid that self-assembles into a hydrogel at body temperature; 2) it is fully biocompatible; 3) after functionalization with RGD peptides it sustains cell adhesion and provides a favorable environment for endothelial migration and vascular assembly. The plasmid, carrying the VEGF and PDGF-BB genes in a single bicistronic expression cassette (named VIP for **V**egf-**I**RES-**P**dgfb), can ensure co-expression of both factors in every transfected cell. In order to assess material biocompatibility *in vivo*, empty ELP gels were implanted in hind-limb skeletal muscle of mice. Seven days after implantation a robust infiltration of $CD11b^+$ monocytes as well as efficient ingrowth of microvascular structures was observed. One month after, ELP hydrogels were partially degraded with no signs of necrosis of muscle tissue. These data confirm the biocompatibility of the ELP biomaterial and its ability to sustain colonization by host cells and vascularization. The efficacy and duration of gene expression after VIP plasmid delivery from the hydrogel will be assessed *in vivo*.

Functionality and morphology of induced angiogenesis will be analysed in non-ischemic muscles and, lastly, therapeutic potential of the gene-activated matrix will be tested in a pre-clinical model of mouse hind-limb ischemia.

0141 Photo-grafting of VEGF protein gradients into elastin-like polymer hydrogels for improved vascularization

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Rapid and efficient *in vivo* vascularization of clinical-size tissue-engineered grafts is a key requirement to enable progenitor survival and tissue formation. Vascular endothelial growth factor (VEGF) is the master regulator of vascular growth and is widely investigated to induce therapeutic angiogenesis. However, when VEGF is produced, it binds to the surrounding extracellular matrix and forms concentration gradients, which are sensed by endothelial cells and guide the sprouting of new vessels towards the hypoxic areas. This is a key aspect of VEGF physiological function, but under-appreciated in the design of vascularization strategies for tissue engineering. Here we sought to investigate whether the functionalization of a biocompatible biomaterial with VEGF protein in the pattern of a gradient could be more effective than a homogenous distribution to drive rapid ingrowth of vascular networks. Elastin-like polymers (ELP) are an attractive material because they: 1) self-assemble into a hydrogel at body temperature; 2) mimic the elastic properties of natural tissues; and 3) provide a favorable environment for endothelial migration and vascular assembly. Since VEGF does not have any natural affinity for ELP, two-photon absorption was used to induce photo-grafting of the protein in the presence of multi-functional aromatic azides, allowing fine control over the spatial distribution of the grafted protein. Recombinant human VEGF-A_{165} was fluorescently labelled with the NT-647-NHS far-red dye. Labelled protein retained approx. 40% of its bioactivity tested by its ability to induce phosphorylation of VEGF-R2 in endothelial cells *in vitro.* VEGF was grafted on ELP hydrogel using 4,4′-Diazido-2,2′-stilbenedisulfonic acid (DAS, 2 mM) as a photosensitive cross-linker. After grafting, both DAS and VEGF were visualized by confocal microscopy. ELP hydrogels with photo-grafted VEGF sustained greater endothelial cell proliferation than empty ELP, showing that protein bioactivity was preserved after the grafting. In order to create 2D gradient of VEGF on ELP hydrogel, two-photon grafting method was employed. Laser parameters (speed and power) were optimized to enable dose-dependent grafting. VEGF gradient pattern was successfully created by increasing laser power from 20 to 80 mW, while homogenous distribution of the protein was achieved by using constant laser power (80 mW). Current experiments aim at testing ability of 2D grafted VEGF gradient to direct migration of endothelial cells *in vitro* in comparison to homogenously distributed VEGF. Finally, these results provide the basis for the generation of 3D constructs functionalized with VEGF protein gradients, enabling the testing of their potential for accelerated vascularization *in vivo*.

0142 Effect of ischemia on the glyco-envirnoment: a study on a murine model

Grazia Marsico Marsico[1], Dilip Thomas[1], Abbah Akogwu Sunny[1], Paolo Contessotto[1], Michelle Kilkoyne[2], Lokesh Joshi[2], Karl McCullagh[3], Abhay Pandit[1]

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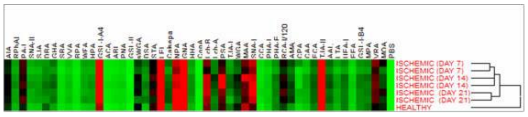
Introduction: Glycans play a central role in and muscle development and physiology [1] and in regulating angiogenesis by binding pro-angiogenic growth factors and clustering receptors [2]. Furthermore, a few studies have reported alterations in GAGs bioactivity and in N-linked glycoproteome in ischemic conditions [3, 4]. It was hypothesised that there are overall alterations in the glycocalyx of the muscle fibers in ischemic conditions. The objective of this study is to compare the glycocalyx in healthy and ischemic muscle using a mouse model of hindlimb ischemia (HLI).

Methods: HLI was induced in the left leg of C57/bl6 mice (n=12) by ligation of the femoral artery as previously described [5]. The right leg was used as control tissue. Samples were collected from both legs after 7, 14, and 21 days. A lectin microarray of 48 lectins spotted on a slide was performed on both healthy and ischemic muscles. Alexafluor®555-labelled glycoproteins extracted from cell membrane were incubated on the lectin microarray. Fixed frozen sections (7µm-thick) were incubated with FITC-labelled *Sambucus Nigra* agglutinin (SNA-I, 15 µg/ml) and Wheat Germ Agglutinin (WGA, 20 µg/ml). Quantification of the fluorescence intensity was carried out using the software ImageJ and statistical significance was determined using SPSS software. N-Glycans were released from both healthy and ischemic tissues via digestion with peptide N-glycosidase F (PNGase F) and quantified by mass spectrometry.



Results: Lectin microarray showed differential binding profile of the lectins associated to Galactosylation, Sialylation and GlcNac in the ischemic and the healthy tissue.N-linked glycans. (Figure 1). Lectin histochemistry of SNA-I revealed an increase in SNA-I binding sites at seven days after induction of ischemia. On the other hand, WGA binding decreased at seven days after induction of ischemia (Figure 2). Differences in of N-glycans levels between the healthy and the ischemic samples were observed in the mass spectrometry analysis.

a b

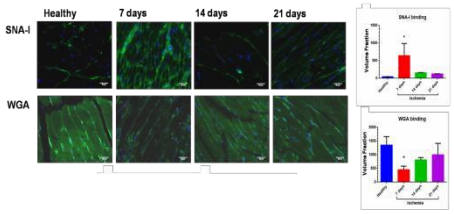


Figure 1. a) Lectin array output **b)** SNA-I and WGA binding pattern on healthy and ischemic muscle at day 7, 14, 21 after the induction of ischemia and relatives volum fraction binding quantification. The values are expressed as mean and SD,*=P value≤0.05 (n=4).

Discussion & Conclusions: Lectin microarray showed differential expression of Galactosylation, Sialylation and GlcNac in the ischemic and the healthy tissue. Furthermore, these differences have been confirmed with the lectin histochemistry suggesting that early ischemic conditions can affect both α(2-6) linked sialic acid and GlcNAc presence.

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0143 Elastin like recombinamers based hydrogel designed to support angiogenesis through a combination of cell adhesion cell induced biodegradation and remodeling

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INTRODUCTION: Angiogenesis plays a critical role in development processes, wound healing and pathological conditions. It is a complex sequential process tightly regulated which involves endothelial cell proliferation, migration and anastomosis along with the coordinated action of growth factors and

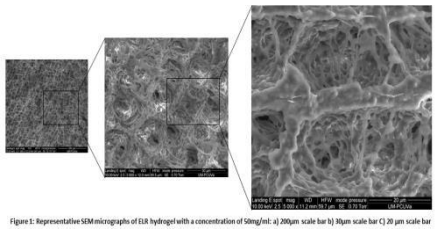

Figure 1: Representative SEM micrographs of ELR hydrogel with a concentration of 50mg/ml: a) 200μm scale bar b) 30μm scale bar C) 20 μm scale bar

their receptors extracellular proteins adhesion molecules and proteolytic enzymes.[1] This work presents four different ELRs which contain proteolytic target sites that degrade in response to tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA) with tunable degradation rates. Endothelial cells are able to degrade these engineered proteins specifically at the site of uPA and tPA creating in this way tunnels for new blood vessels. By varying the rate of the scaffold degradation it may be possible to influence the speed of vessels elongation promoting the regeneration of new blood vessels. METHODS: Elastin-like recombinamers were designed, synthetized and produced using recombinant protein technology. They were purified by inverse transition cycling and characterized by NMR, DSC, MALDI-TOF, FT-IR and HPLC. Moreover, comparative degradation rates were evaluated using tPA and uPA enzymes. Subsequently, they were chemically modified with azide and cyclootyne group for hydrogels formation. Their degradation properties were characterized by monitoring changes in the elastic modulus over time. *In vitro* cytocompatibility studies were performed using HUVEC cells. RESULTS: Chemical and physical characterization confirmed the purity and the structure of the recombinamers as well as their chemical modification. Correlation between SEM micrographs, porosity and rheological measurement have been carried out. SDS-page analysis confirmed that the full proteins degrade to smaller fragments throughout the course of the reaction. *In vitro* studies shown that these materials are biocompatible and biodegradable. *In vivo* studies are in progress using male mices. DISCUSSION&CONCLUSION. Recombinamers to fabricate regenerative scaffolds that can be systematically tailored to obtain the optimal stimuli required for the formation of new blood vessels were attained. tPA and uPA are primarily produced by endothelial cells and have been linked physiological roles in thrombolysis and ECM degradation.[2] Due to their excellent cytocompatibility, these are promising scaffolds for tissue engineering able to induce angiogenesis.

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0144 Engineering organoid development in 3D artificial microenvironments

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The earliest steps of development are characterized by cellular reorganization and

differentiation within a three-dimensional (3D) microenvironment. This 3D context allows for a complex spatial interplay between biochemical and mechanical signals, and governs important cellular rearrangements leading to morphogenesis. In vitro approaches have attempted to recapitulate key features of these processes, and it has now become possible to generate an increasing variety of self-organizing multicellular tissue constructs termed ‘organoids’. While important aspects of the 3D in vivo organization have been recreated in these organoid systems, such studies have been exclusively performed in animal-derived matrices whose properties cannot be readily modulated. As such, the uncharacterized interactions between cells and this extracellular matrix (ECM) have proven to be a major challenge to understanding the underlying regulatory mechanisms governing morphogenesis. In this talk, I will highlight recent efforts in my lab to employ tunable synthetic hydrogels in order to disentangle the contributions of biochemical and mechanical effectors of the microenvironment in the specification of stem cell fate and self-organization.

0145 Bi-directional interactions between stem cells and their pericellular matrix direct fate in 3D hydrogels

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Combining stem cells with smart, instructive materials has the potential to deliver tissue-engineered regenerative therapies, but effectively controlling differentiation remains challenging. In modifiable hydrogels, physical characteristics of a stem cell's 3D environment, including its stiffness and degradability are known to direct lineage specification. Yet, in their *in vivo* niche, stem cells do not passively respond to signals delivered to them. Rather, they actively modify their local environment by secreting a proteinaceous extracellular matrix (ECM) and degrade their surroundings to suit their needs. In 3D hydrogels, the role of such dynamic bi-directional interactions are not well studied, and indeed, how they develop over time and direct the differentiation of encapsulated stem cells remains relatively unexplored.

Here, we formed hydrogels based on a well-described Michael addition between thiol-modified hyaluronic acid (S-HA) and PEGDA, a system which limits integrin-mediated interactions to those with cells' own secreted ECM. We characterised the hydrogels physical properties over time using a combination of atomic force microscopy-based indentation and cryoSEM. We then encapsulated human marrow stromal cells (hMSC) and analysed their differentiation in the absence of chemical induction and assessed how they modified the hydrogels' physical properties at both the bulk and local levels. Our analyses show that when encapsulated within S-HA-PEGDA hydrogels, hMSC quickly modify (within 24 h) their pericellular environments by secreting a proteinaceous ECM, which they rely on for survival. Encapsulated hMSC also either secrete pericellular matrix to stiffen an initially soft local environment or degrade their surroundings to soften initially stiffer pericellular milieus. Indeed, within 72 h after encapsulation, hydrogels have similar pericellular stiffnesses (~2-5 kPa), regardless of their initial bulk properties. Gene expression analyses by qPCR show that cell-secreted pericellular matrices play a central role in regulating hMSC fate. Pericellular stiffening correlated with adipogenesis, and softening with osteogenesis. Blocking cell-ECM interactions with RGD sequencing-containing peptides for the first 72 h after encapsulation eliminated matrix-mediated differentiation, confirming that hMSC's interactions with their own secreted ECM drove lineage specification.

Our observations suggest that hMSC do not respond directly to the physical properties of their surrounding 3D matrix, but rather participate in a bi-directional interplay between the initial and time-dependent properties of the 3D space and their own secreted pericellular matrix and this combination of interactions governs their fate. In short, hydrogel properties may not direct stem cell fate *per se*, but rather impact cells' pericellular matrix, which in turn drives stem cell lineage specification. This insight may explain why 3D culture systems with outwardly similar physical characteristics can produce different biological responses.

0146 Collective cell behaviour in depth-sensing of extracellular matrix stiffness

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The stiffness of the extracellular matrix (ECM) has a profound effect on the behaviour of many cell types. Cells contract, exerting tensile and compressive forces on ECMs, and sense the resultant deformation that develops. This is dependent not only on the elastic modulus of the material of which the ECM is composed, but also on other factors including its thickness, with the corollary that single cells are able to sense underlying substrata through ECMs at low (<10 µm) thicknesses. Here, we hypothesised that groups of cells would be able to mechanosense underlying substrates at greater depths than individual cells.

To test this, we fabricated polyacrylamide hydrogels in the range of 1 - 1000 µm in thickness and of 0.5 – 40kPa elastic modulus adhered to glass substrates. Hydrogel surfaces were then covalently modified with ECM proteins, and MG63 and MDCK cells were plated on hydrogels either at low density or in compact colonies. We measured cell density, cell aspect, and cell perimeter by microscopy.

We found, as expected, that cells plated at low density responded in an elastic modulus-dependent fashion, spreading more on gels of a high compared to a low elastic modulus. In contrast, when plated as colonies no differences in the phenotype of cells were observed, regardless of measured gel elastic modulus. When gel thickness was increased, however, cells in colonies showed morphological differences dependent on gel elastic modulus. Cells were significantly denser at peripheral colony regions on thick, soft (0.5 - 2kPa) gels than on stiff (40 kPa) gels, and had higher aspect ratios and perimeters.

These results support the notion that groups of cells are able to mechanosense rigid materials beneath elastic hydrogels at greater depths than individual cells. This raises the possibility that the collective action of cells in tissues such as epithelia may allow contributing cells to sense structures of differing stiffness at comparatively large distances.

0147 Writing on the blank slate - Endogenous ECM deposition in synthetic PEG hydrogels and its role in microvascularization

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The physiological material surrounding cells in tissues is the extra cellular matrix (ECM). The ECM is a complex network of many structural and adhesive molecules that display a dense 3-dimensional layout and provide form and function to tissues. Synthetic materials such as poly(ethylene glycol) (PEG) hydrogels can mimic the natural ECM and enable the study of cells on a blank slate in 3D [1]. Moreover, such artificial ECM mimics have been applied to instruct (stem) cell behaviour by tailoring their properties in a user-directed manner. In this regard, some very recent and excellent publications have nicely shown the direct coupling/correlation of artificial ECM properties and cellular responses [2,3,4,5]. A key feature and advantage of artificial ECM mimics is the absence of exogenous ECM and thereby of confounding biological signals. However, cell cultures within synthetic materials do deposit and assemble ECM. Astonishingly, this endogenously assembled ECM and related biological implications have been overlooked in the field of artificial ECM mimics. Here, we report on the ECM formation by mesenchymal stem cells (MSCs) and its role in microvascular network formation in synthetic PEG hydrogels.

For this study we use FXIIIa cross-linked MMP-sensitive TG-PEG hydrogels functionalized with the cell adhesion peptide RGD [6]. Human MSCs alone or in combination with human endothelial cells (EC) are 3D encapsulated in TG-PEG hydrogels. By various microscopy techniques we visualize that MSC weave a 3-dimensional meshwork of several fibrillar proteins into the extra-cellular space. Furthermore, in co-cultures MSCs enable ECs to form stable and lumenized microvascular tubes that are surrounded by a dense ECM layer. Intriguingly, the ECs influence the ECM production by MSCs, altering protein expression levels and ECM assembly pattern of MSCs. We demonstrate that key ECM proteins of the vascular basement membrane are induced in MSCs only when exposed to ECs.

We show the endogenous ECM formation by MSCs and ECs in synthetic PEG hydrogels. This newly endogenously assembled ECM is an underestimated feature of artificial ECM mimics and bears the potential to interfere with engineered material properties, by altering the biochemical properties of the matrix. Nevertheless, due to the absence of exogenous ECM proteins we envision synthetic environments to be a powerful tool to investigate the role of the ECM in 3D cell biological processes such as the formation of the microvasculature.

0148 Near infrared light-mediated PEG hydrogel degradation: a new light-responsive system to study cell-material interactions

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'Smart' responsive materials such as enzymatically degradable[1] and photodegradable hydrogels are attractive platforms for directing cell-material interactions. Photodegradable hydrogels have gained a lot of attention in recent years due to the ability to achieve a high degree of spatiotemporal control over hydrogel structure. By incorporating UV-cleavable *o*-nitrobenzyl groups within hydrogel crosslinks, high resolution 3D photopatterning was achieved in the presence of living cells[2], allowing the study of real-time cellular responses to changes in the mechanical environment, or localized release of biochemical factors. Due to toxicity and mutagenic concerns with UV light, there is interest in developing similar techniques with more cytocompatible wavelengths of light. Near-infrared (NIR) is advantageous because it is a lower energy wavelength with much higher penetration depth than UV. Additionally, because of increased attention on the uses for nanoparticles in biological systems[3], incorporating light-reactive nanoparticles into photodegradable hydrogels is an attractive strategy. Jayakumar *et al*. showed that upconversion nanoparticles could be employed *in vitro* as transducers that locally convert NIR to UV, using NIR light (980 nm) to release photocaged siRNA for intracellular delivery[4]. To demonstrate that these nanoparticles can be used to mediate hydrogel photodegradation, a UV-cleavable *o*-nitrobenzyl crosslinker was synthesized and used to make hydrogels by reacting with a suspension of 8-arm PEG-maleimide and NIR to UV upconversion nanoparticles, entrapping nanoparticles within the hydrogel network (Fig. 1). To verify photodegradation and to simulate the release of tethered biomolecules, the fluorescent dye AlexaFlour® 546 C5 maleimide was added, covalently binding to the photodegradable crosslinker resulting in its release upon photocleavage. It was demonstrated that as little as 10 minutes of NIR light exposure at 980 nm was sufficient to result in photorelease of tethered AlexaFluor® 546. Future experiments will aim to fully characterize NIR-mediated hydrogel degradation, and also to encapsulate stem cells to demonstrate spatiotemporal delivery of biomolecules to cells in a 3D hydrogel environment. Overall, this platform has high potential for studying complex cell-material interactions without requiring direct UV light, and could be used for triggered delivery of biomolecules on demand to direct stem cell fate.

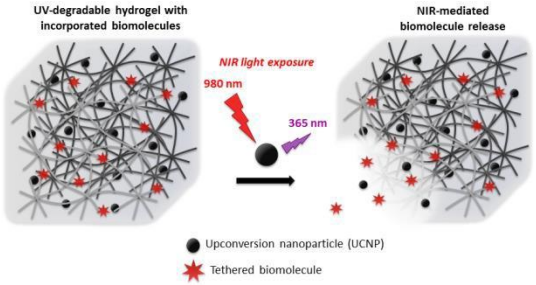

Figure 1. Schematic of near-infrared (NIR) light-mediated photodegradation and release of tethered or entrapped biomolecules from poly(ethylene glycol) hydrogels containing embedded NIR-to-UV upconversion nanoparticles.

0149 Injectable supramolecular double-network hyaluronic acid hydrogel towards stem cell chondrogenesis

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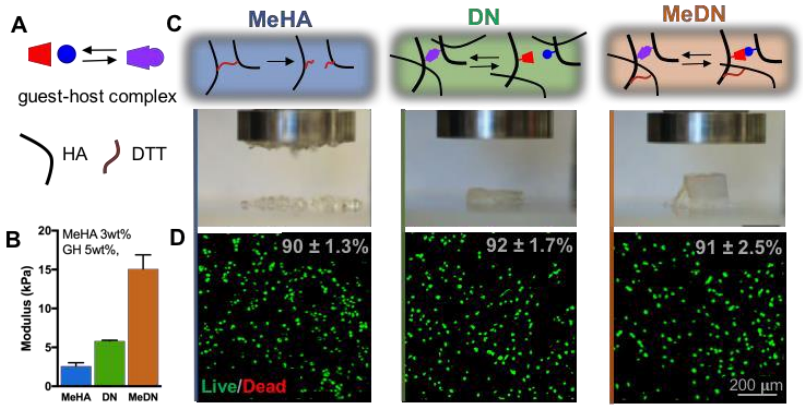
Introduction: Towards engineering of cartilaginous tissue, hyaluronic acid (HA) hydro-gels have emerged as supportive microenvironments for chondrocyte matrix deposition and chondrogenic differentiation of mesenchymal stem cells (MSCs)[1]. However, these typically covalently crosslinked hydrogels are often not capable of providing the resilience of cartilage tissue under repeated loading. Here, we present HA-based double-network hydrogels with resistance to mechanical failure through a combination of covalent networks and supramolecular guest-host networks, amenable to the encapsulation of viable MSCs.

Methods: HA hydrogels were prepared from methacrylated HA (MeHA, 100% modified) through addition of dithiothreitol (DTT, ratio thiol/methacrylate 0.2, Media 199). Secondary networks of guest-host hydrogels of cyclodextrin and adamantane HA (CD-HA and Ad-HA, 22% modified) were included by physical interpenetration (DN) or with additional covalent crosslinking between the two networks (MeDN; 35% methacrylated CD-HA and Ad-HA)[2]. Mechanical characterization was performed in compression (TA Q800). Viability of human MSCs (P2) was assessed with Live/Dead staining and confocal imaging (Zeiss LSM710).

Results and Discussion: Supramolecular double networks (DN) formed readily upon mixing due to high-affinity guest-host (GH) complexation (*Fig. A*). DN hydrogels demonstrated increased moduli (*Fig. B*) with highest values when both networks were tethered (MeDN). Loading to 90% strain (*Fig. C*) showed brittle (MeHA) and ductile (DN) failure modes, and recovery of MeDN constructs with limited cell deformation under load. High MSC viability (>90%, 24 h, *Fig. D*) illustrated cytocompatible crosslinking at physiological conditions (pH 8.5, 37°C), i.e. after *in vivo* injection.



Conclusion: We developed an injectable hydrogel platform based on supramolecular interactions and secondary formed covalent crosslinking, endowing macro- and microstructural self-healing. Ongoing work is investigating the effect of microenvironmental cues on the formation of pericellular matrix into the hydrogel. Due to the importance of micromechanics on stem cell chondrogenesis, this double-network hydrogel design presents a unique approach to study and direct chondrogenic matrix formation.

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Vincent Ronfard

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Daniela Marino

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Mustapha Najimi

0153 Enhancing tissue regeneration with electrospun fibers and scaffolds

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Musculoskeletal disorders comprise an important and increasing component of the global burden of disease. On average men will live 16 years and women 19 years with disability due to musculoskeletal disease. There is a need to improve this position, particularly with the demographic trends showing a universal increase in the aged population. Shoulder pain due to rotator cuff tendon tears is a very common manifestation of musculoskeletal disease. Tendon tears are found in around 15% of 60 year olds, 25% of 70 year olds and 30% of 80 year olds. Pain which significantly disrupts sleep is common. Unrepaired tears may grow in size and very large tears are often associated with significant functional disability. Initial management in primary care is conservative with rest, time off work and simple analgesia. Some patients will respond well to a structured exercise programme but in many patients pain persists. A common second line treatment strategy is locally injected steroids (glucocorticoids), but there is increasing evidence from randomised trials that steroid injections have long term harmful effects on tendon structure and may increase the number of tendon tears. In patients with persistent symptoms surgical repair is commonly performed. Currently around 16,000 rotator cuff repairs are carried out in England and 400,000 in the USA every year. Rates of surgery have increased by over 700% in the past decade in England, and the annual treatment cost to the NHS is around £68 million.

Evidence from international cohort studies and from the recent NIHR Health Technology Assessment programme funded randomised trial in of open versus arthroscopic repair in 275 patients (UKUFF) revealed that 40% of repairs fail within 12 months irrespective of the surgical technique used and that a failed repair adversely affected patient outcomes. There is a need to improve the success rate of surgery.

Given the concerns reported regarding the safety, efficacy and cost of the current repair solutions research has focused on developing a new generation of biomimetic materials, which provide molecular and morphological cues mimicking key aspects of the structure and function of extra cellular matrix (ECM). Recent advances in this field include the development of electrospun materials which, through their close structural resemblance to ECM, provide morphological cues encouraging endogenous cell growth and tissue healing. We have developed a novel technology that enables us to produce, as a composite material, a nanofibrous mesh which is bonded in a laminated design to a much stronger woven layer. In vitro tests have demonstrated excellent cellular response and improved healing potential. We have also evaluated these scaffolds in vivo in rodent and ovine model to ensure safety and absence of adverse tissue response. These implants are now entering first in man clinical trials.

0154 Lessons from knockout mouse models for design of tendon tissue boosters

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Tendon healing is a lengthy four-stage process, involving activation of exogenous blood-derived cells as well as endogenous tendon stem/progenitor cells (TSPC). Existing medical and surgical treatments mainly aim at controlling inflammation and regaining tissue integrity and stability, however they frequently fail in full recovery of tendon structure and function. Moreover, the tendon field is still lacking tendon-specific drugs that can boost the tendon repair process.

Development of novel therapeutics has been hampered due to limited understanding of basic tendon biology. Knockout mouse models are powerful tools for pinpointing single gene function in a tissue of interest and can aid in a better understanding of the molecular mechanisms governing tissue development, function and repair.

In the last decade several mouse models have been shown to exhibit intriguing tendon phenotypes, such as Scleraxis and tenomodulin. Scleraxis knockouts demonstrated severe tendon changes, while tenomodulin knockouts showed mild tendon alterations in vivo. This data suggested that Scleraxis expression is essential in the maintenance of healthy tendon tissue and identifying regulators of Scleraxis transcriptional activity could be one promising way to discover potent tendon biologics.

Despite the mild phenotype of tenomodulin knockout mice we performed a further investigation on tenomodulin effects on TSPC revealing that its loss results in dis-balanced self-renewal and senescence propensity, but sustained multipotential of TSPC. Additionally, in vitro scratch assays showed that tenomodulin-deficient TSPC were delayed in wound closure and had slower migration rates. Rescue experiments with tenomodulin C-terminal domain could restore the proliferation of knockout TSPC and even enhance the division rate of control TSPC. In a pilot tendon rupture model we could also clearly detect lower activation of stem cells in tenomodulin knockout mice. Hence, our data propose that in contrast to Scleraxis, tenomodulin is a fine-tuning gene and identifying agonist-like molecules could be attractive another strategy for designing novel tendon boosters.

0155 The role of SPARC for achilles tendon enthesis development and maturation

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Acute tendon injury and chronic tendinopathies remain clinically challenging and novel treatment modalities are urgently needed. However, despite significant advancements in tissue regeneration and engineering strategies, the clinical impact for the regeneration of tendons and tendon-to-bone insertions remains limited. By investigating the developmental programs driving tendon tissue formation and the mechanisms contributing to the senescence of tendons, potentially novel targets for clinical intervention can be revealed.

In a previous study we showed that the age-related decrease of Sparc in tendons modulates the cell-ECM interaction of tendon-resident stem and progenitor cells and together with a change in ECM properties potentially favours adipocyte differentiation.

We now provide evidence that Sparc is involved in the temporal and spatial fine tuning of Achilles tendon enthesis development and biomechanical stability.

Hind limbs from Sparc-/- and age-matched WT mice at various developmental stages (p7, p14, p21, 2 months) were prepared and analysed by histology. Cartilage tissue architecture and collagen deposition were investigated by conventional histology and stainings for Sparc, Col I, and Col III were performed. Finally, collagen fibre arrangement was studied using polarisation microscopy and the biomechanical properties of Sparc-/- tendon-to-bone insertions were compared to their WT counterparts.

The development of the secondary ossification centre with their ingrowing blood vessels as well as the development of the 4 characteristic zones of the Achilles tendon enthesis was significantly delayed in the Sparc-/- animals. In young adult animals, Sparc specifically localizes to the Achilles tendon enthesis, almost precisely demarcating the tidemark and the mineralized fibrocartilage region. Further, the matrix content (most likely Col I) in 2 month old WT mice was higher compared to Sparc-/- animals. However, overall no collagen fibre arrangement differences were observed. Finally, biomechanical testing revealed that the Achilles enthesis of Sparc-/- animals was less able to withstand force.

Taken together, our data demonstrate that Sparc is not only required for collagen fibril formation/maturation in tendons, but also for the proper development of the Achilles tendon enthesis.

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0156 Longitudinal analysis of human achilles tendon tears: Determination of changes in MMP and TIMP expression and tendon structure

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INTRODUCTION: A balance between the matrix degrading enzymes, Matrix Metalloproteases (MMPs), and their inhibitors (TIMPs) is required to maintain tendon homeostasis [1] and might have an impact on the tendon healing potential. Until now, however, the processes and timing of matrix remodeling and formation that occur after trauma are not known in human. It was the aim of the present study to analyze structural tendon changes and the expression profile of MMPs and TIMPs of acute human Achilles tendon tears at different time points after trauma.

METHODS: After local IRB approval, tendon tissue samples were collected from 40 patients (Age: 39±10; BMI: 25±3) with acute Achilles tendon tears directly from the tear site. The surgery was performed 2 to 11 days after trauma and the samples were grouped according to the time of surgery (early (2-4 days; n=15), middle (5-6 days; n=15) and late (>7 days; n=10)). Movat-Pentachrome, H&E and a-SMA staining was performed and a modified Movin score [2] was used to determine pathologic tendon changes by analyzing tendon architecture, amount of aligned collagen, GAG-content, amount of fat tissue, cellularity and vascularity. Each variable is scored between 0 and 3, with 0 being normal and 3 markedly abnormal resulting in a maximum score of 18 points. The expression of MMPs (MMP-1, -2, -3, -9, -10, -13) and TIMPs (TIMP-1 to -4) was analyzed by qRT-PCR and normalized to the expression of 18S-rRNA. Statistics**:** Mann-Whitney-U Test, $p<0.05$.

RESULTS: The age and BMI of the 40 patients was equally distributed over the 3 groups. The histological score significantly increased from the early time point post tear compared to the late time point (6.5±2.3 versus 8.8±2.0), with a lower amount of aligned collagen, a higher amount of fat tissue, as well as a higher cellularity and vascularity. The expression of MMP-2, MMP-9, and MMP-13 significantly increased from the early to the late time point, MMP-13 also from early to middle. In contrast, the expression of MMP-10 significantly decreased over time, while MMP-3 only tended to be decreased. Only the expression of TIMP-1 was significantly decreased from the early to the late time point.

DISCUSSION: The study shows for the first time the expression profile of important MMPs and TIMPs as well as structural tendon changes in a longitudinal human Achilles tendon analysis. MMP-2, -9 and -13 increased and MMP-10 and TIMP-1 decreased with the time after trauma. As shown previously the same MMP regulations were found in tenocytes of supraspinatus tendons of patients with higher age and degenerative status of the tendon [3]. This might imply that the later the Achilles tendon surgery takes place after trauma the more the tendon tissue is turning into a degenerative milieu, which could also be confirmed by the increasing histological score and might impede tendon healing processes. Therefore, the time point of surgical intervention after trauma might be critical and should be considered to obtain an optimal functional outcome.

0157 Optimization of mesenchymal stem cell cultured in PLCL-braided scaffold for ligament tissue engineering

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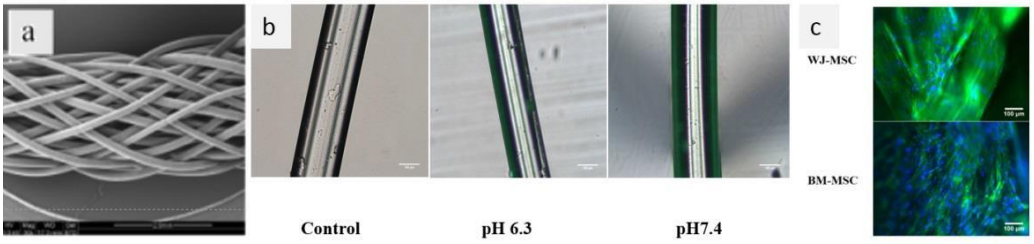
Ligament injuries are among the highest incidence of musculoskeletal disorders, especially for sportive or aged people. The main strategy for repairing injured ligament is an autograft-based surgical intervention. Even if there are some improvements for the patients with current therapies, they still involve drawbacks including donor site mobility, immune rejection, limited graft sources or graft rupture. With the rise of tissue engineering, ligament tissue engineering could be a novel, attractive and alternative approach to recover the function of injured ligament.

In our present study, Bone Marrow Mesenchymal Stem Cells (BM-MSC) and Wharton-Jelly Mesenchymal Stem Cells (WJ-MSC) have been seeded on a 6-layer braided scaffold (figure a) made of poly (L-lactide-co-caprolactone) (PLCL). Culture were performed with and without dynamic stimulation to induce MSC-scaffold substitutes to develop ligamentous tissue. In order to improve the cell attachment and uniform cell distribution on the scaffold, Layer-by-Layer technology (LBL) has been used to modify the surface of the scaffold (figure b). Poly-lysine (PLL) and Hyaluronic acid (HA) have been used as polycation and polyanion to develop PLL/HA polyelectrolyte film. Different pH7.4 and pH6.0-pH6.5 for film build-up have been compared, as well as different PLL/HA layer numbers (n=1, 3, 5) (SnL) with Scaffold Blank(SB), Scaffold-PLL(SP). The two cell sources (BM-MSC and WJ-MSC) have been compared with densities of 300 000cells/scaffold and 100 000cells/scaffold in order to optimize the seeding conditions. The growth of PLL/HA film was observed by fluorescence microscopy; the film morphology was observed by scanning electronic microscopy. The cell metabolic activity on the scaffold was detected by Almar Blue, and cell morphology and cell location on the scaffold were characterized by fluorescence microscopy (figure c) and scanning electronic microscopy (figure d). Specific extracellular matrix expression of ligamentous tissue as collagen I and collagen III have been verified by immunofluorescence staining and RT-PCR. The results indicated that BM-MSC showed increased cell metabolic activity on SB, SP and S1L in both pH6.3 and pH7.4, while WJ-MSC showed increased metabolic activity on SB, SP, and S1L only in Ph6.3. For both cell types, cell density of 300 000cells/scaffold encouraged cell metabolic activity. Collagen I and collagen III have been detected, indicating that cell-scaffold substitutes have potentials to develop a ligamentous tissue.



(a) PLCL braided scaffold (b) Layer-by-Layer coating of PLCL fibres (c) WJ-MSC and BM-MSC on the braided scaffold (phalloidin (green) and DAPI (blue))

0158 Effect of intermittent mechanical loading in a model of the early inflammatory phase of tendinopathy

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INTRODUCTION: The presence of immune cells in human tendinopathies points to inflammation as a player in the regeneration of diseased tendon. However, many questions on the crosstalk between immune-cells and tenocytes remain unanswered. In the present study we have analyzed the effect of different topographies and cyclic intermittent mechanical loading during the early macrophage induced inflammatory phase on tendon healing. We hypothesized that pro-inflammatory M1-like macrophages would stimulate markers of catabolic matrix activity in tenocytes, and that this adverse regulation would be suppressed by intermittent mechanical loading. **METHODS:** THP-1 monocytes, differentiated to macrophages (M0), were chemically polarized to the pro-inflammatory M1-like phenotype, and verified using surface markers CD197 (M1). Primary human tendon-derived cells (TDCs) were cultured on nanofiber polymer mats that structurally mimic either healthy (aligned nanofibers) or diseased (randomly distributed nanofibers) tendon environments. After 24h, TDCs were exposed to medium conditioned by macrophages and clamped in a custom-made bioreactor (Fig. 1). Static loading (0% dynamic strain) or cyclic intermittent loading (5% dynamic strain) was applied for 3 days. Morphological differences were quantified by fluorescent staining of nuclei and actin cytoskeleton. Cell alignment was calculated as deviation from the loading axis. TDC response was assessed using qPCR of lineage-specific markers, such as tenogenic gene expression markers and collagen I/III. Analysis of the expression of matrix metalloproteinases (MMP) was used to study matrix remodeling activity of TDC in both conditions. **RESULTS:** Fluorescent staining of the actin cytoskeleton indicates significant differences in the morphology of TDCs spreading on aligned and randomly distributed nanofibers (Fig. 1). While cells growing on aligned fibers display elongated cell bodies, and thus resemble healthy tenocytes, on the substrates with random topographies cells acquire polygonal shapes characteristic of diseased tendons. After mechanical loading cells growing on aligned substrates orientate along the loading axis (86 ± 19.2° for static and 87 ± 17.7° for intermittent loading). In turn, TDCs seeded on randomly aligned nanofibers are randomly oriented (33 ± 31.9°) but then partially align to the applied loading axis in case of cyclic loading (75 ± 39.2°). Equivalent tenogenic gene expression occurs on all tested substrates (not shown). Macrophage-conditioned medium on both substrates induces a significant up-regulation of genes related to matrix degrading enzymes and down regulation of genes involved in matrix synthesis (Fig. 2). Interestingly, application of mechanical loading results in a trend for a down-regulation of MMPs and an increased extracellular matrix production. **DISCUSSION:** We have previously described the impact of topology on the response of TDCs to immune cell-derived factors. We now expand this study to explore the combined effect of topographical cues and mechanical loading on TDCs exposed to soluble factors released by macrophages. Independently of the topography of the substrates where TDCs are cultured, medium conditioned by M1 macrophages causes a significant up-regulation of matrix degrading potential and a decrease in extracellular matrix synthesis. Our results suggest that mechanical loading can suppress response of TDCs to a shift in proinflammatory conditions. The increased expression of genes related to extracellular matrix synthesis in cells subject to mechanical load can be further interpreted as a recovery-related signal. These results shed new light on inflammation- and mechanically-driven healing processes in tendon tissue and may help in the development of better therapeutic strategies.

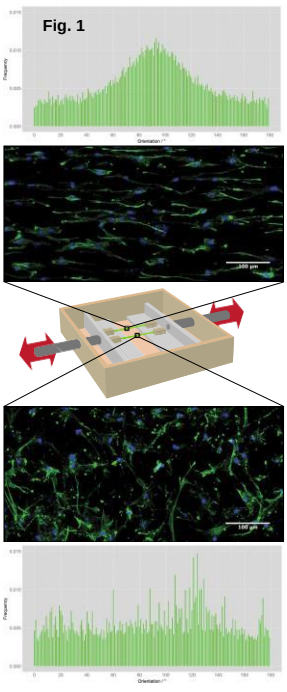
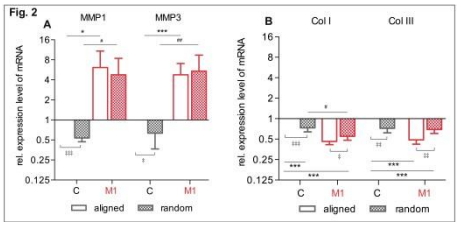


Fig. 1: *Bioreactor model system. Fluorescent staining of nuclei (blue) and actin (green) of TDCs. Cells were cultured on microstructured substrates that mimic healthy or disease geometries and mechanically stimulated using a custom-made bioreactor. The directionality analysis revealed an aligned orientation of the actin cytoskeleton along the loading axis for the cells seeded on the aligned substrates and a random orientation for the cells seeded on the random substrates. Conditioned M1 medium lead to a* ***Fig. 2:*** *significant re-regulation of matrix remodelling proteins. A) M1 conditioned medium lead to a significant up-regulation of matrix degrading proteases MMP1/3 and a significant down regulation of matrix production genes Col I/III in TDCs growing on differentially patterned substrates. Gene expression is normalized to control (C: medium only, aligned substrates).*



0159 Biocompatibility of antimicrobial polymeric materials for medical applications - How much in vitro is mandatory, how much is sufficient?

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Since the late 30s when E. B. Chain and H. W. Florey, as part of a comprehensive program of research on antibacterial substances, began work on penicillin at Oxford, antibiotic drugs performed a triumphal procession by saving countless patients life. But in the last decades a dramatically increasing antimicrobial resistance (AMR) poses a dangerous threat to the global public health that requires measures across all government sectors and society. Without effective antibiotics and alternative, complementary antimicrobial strategies the success of major surgery and interventional therapies, temporarily applied medical devices or implanted mid- and long-term devices would be compromised. In addition to ceramics and alloys polymers, both synthetic and natural, play an indispensable role in modern medicine. In this context antimicrobial polymeric materials (AMPMs) are becoming important for clinical applications as biomaterials applied in coatings or with intrinsic antimicrobial properties for implants, wound dressings and tissue engineering approaches to prevent particularly bacterial adhesion and biofilm formation. But to prevent microbial attachment to implant surfaces and to inhibit their proliferation and organization in biofilms on inner and outer implant surfaces will open the critical question regarding their biocompatibility. This means not only cyto-, but hemo- and immunocompatibilty. This sounds compared to most of the formerly and currently published scientific papers somehow exaggerated. What does it mean for academics, medical device industry, clinicians, patients and hospital operators? The awareness of requirements for testing biocompatibility correctly, comprehensively and carefully can prevent the industry from making common mistakes that could slow a product's time to market, the academics from giving clinicians and patients wrong hope of contemporary help, the hospital operators and clinicians from buying and applying products only tested by inadequate standards and finally the patients from harming by worse medical products and treatments. The key questions and issues the author would like to highlight in this presentation are the following ones:

What are the best standards for evaluation of biocompatibility of new AMPMs worldwide? A comparison between USP, ISO 10993 and the Japanese guidance document will be presented. - The difference between standard and advanced in vitro biocompatibility evaluation will critically be discussed and the difference between commercially available cell-based assays and individually developed ones will be compared. - The importance of analyzing the immunocompatibility in case of using more unspecific AMPMs or polymers modified with antimicrobial leachable ions, small molecules or antimicrobial nanoparticles. Discussing the necessity of adapting standardized biological assays to a real clinical problem. Can the application of in vitro 3D cell culture models and disease models help to reduce the number of in vivo animal trials? Future challenges for standardization and future perspectives: In vivo simulation and organ-on a-chip approaches.

0160 Antibacterial polymers derived from bacteria

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Device-related infections are one of the main causes of failure of medical implants. Biofilms are difficult to treat and once formed the only solution is to remove the infected medical device or perform a surgical excision of the infected tissue[1]. This affects the patient's well-being and simultaneously increases healthcare costs. To overcome this situation, development of antibacterial materials is becoming more pertinent. The design of such materials can be based on different approaches including loading biopolymers with antimicrobial molecules and chemical modification with antibacterial functional groups, as well as developing materials possessing intrinsic bactericidal properties[2].

Polyhydroxyalkanoates (PHAs) are bacteria derived polyesters which accumulate within the cell under nutrient limiting conditions[3]. Due to their highly biocompatibility and biodegradability, PHAs are excellent candidates for the development of tissue engineering scaffolds and other medical devices. Different types of PHAs with varied material properties and degradation rates can be obtained by changing the growth conditions and type of bacteria used, making them versatile for a variety of medical applications.

This study focused on the development of PHAs with antibacterial properties using two different approaches. The first approach involved loading PHAs with naturally derived small molecules which in turn conferred antibacterial properties to the polymer. Trans-cinnamaldehyde (TC) was selected as the natural agent to be added to the polymer. This compound has been shown to have antibacterial activity against many bacteria and it has also been known to inhibit biofilm formation[4]. In this work, MCL-PHAs were produced using *Pseudomonas mendocina*[5]. The polymers were characterized with respect to their mechanical and thermal properties. Solvent cast films were produced using the MCL-PHA in to which TC was incorporated in varied concentrations. Antibacterial tests confirmed the antibacterial activity of the polymer films against *Staphylococcus aureus* ATCC 6538.

The second approach involved the production of PHAs with intrinsic antibacterial properties. Recently, PHAs containing thioester linkages in the side chains have shown to inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) both in vitro and in vivo[6]. The mechanism behind its activity was hypothesized to be related to the cationic nature of the thioester group. In this work, we have investigated the production of thio-PHAs by *Pseudomonas putida* KT2440 using different thio-group containing carbon substrates in order to obtain a range of novel thio-PHAs with inherent antibacterial properties.

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0161 Biological response of silk-based fibers functionalized with antimicrobial peptides by mimicking bacterial infection *in vivo*

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Surgical site infections are often caused due to microbial contamination of surgical material, such as sutures, thus being necessary to explore new polymer with antimicrobial properties to impair such infections. Spider silk proteins present interesting properties, such as mechanical stability and biocompatibility, useful for tissue engineering. Furthermore, the accessibility of these proteins to bioengineering, allows the generation of spider silk proteins fused to other peptide domains not normally found in spider silks, thereby expanding their function. The *in vivo* functional behaviour of silk-based fibers that combine the mechanical properties of spider silk (6mer) with the antimicrobial properties of human neutrophil defensin 1 (HNP1) was addressed in this study. The local inflammatory response was assessed histologically and by gene expression, and compared with controls of spider silk alone (6mer), silk fibroin, commercial sutures (Perma-Hand® silk suture and VicrylPlus® suture) and empty defects. To mimic bacterial infections *in vivo*, silk-based fibers with 6mer-HNP1 and commercial suture controls were inoculated with methicillin resistant *Staphylococcus aureus* (MRSA) before implantation. Histological analyses of local inflammatory response indicated the presence of inflammatory infiltrates at the implant site after 1 day. Also, transcript levels of inflammatory mediators were upregulated in relation to the empty defects. No apparent differences were observed between the implanted materials after 7 days, suggesting that silk-based fibers with 6mer-HNP1 did not elicit a long-term immunological reaction. The materials inoculated with MRSA generated transcript levels of inflammatory mediators upregulated after 1 day, corroborated by histological analysis, suggesting a mild host response. After 7 days, the inflammatory mediators in the presence of silk-based fibers with 6mer-HNP1 and VicrylPlus® sutures were down regulated. In contrast, the inoculated Perma-Hand® sutures generated the formation of fibrous capsule in histological sections. It was still possible to identify inflammatory cells with phagocytised bacteria in the silk-based fibers with 6mer-HNP1, suggesting an ongoing immunologic response. The histological analysis of VicrylPlus® sutures showed a reduced presence of bacteria in the implanted sutures, probably due to the controlled released of the antibiotics. Overall, the silk-based fibers with antimicrobial peptides elicited no significant immunological reactions, and supported an active response to bacterial infection. These findings provide new insights to the *in vivo* functional response of bioengineered spider silk proteins with antimicrobial properties, highlighted by their immunological response to bacterial infection, representing a promising approach to reduce surgical site infections and improve medical care.

Acknowledgments:

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0162 Novel amphipathic antimicrobial polyurethanes: Design and development of new biomaterials with intrinsic antimicrobial properties

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Microbial colonization is the cause of local infections that eventually can evolve in severe sepsis, a life-threatening condition for patients that can finally end up in a multi-organ failure and death. Several bacteria and fungi can colonize biomaterials but most belong to endogenous bacteria or to the commensal community of the skin. Polyurethanes (PU) are a large family of synthetic polymers, which are widely used in biomedical applications. Their chemical versatility makes them ideal candidates to design and engineer drug-free antimicrobial biomaterials.

In this framework, the synthesis, characterization and antibacterial efficacy of the chosen monomers used to prepare two different novel amphipathic antimicrobial PU containing quaternary ammonium (QA) functionalities is described. The cationic charge stemming from the positively charged hydrophilic QA moieties should facilitate binding to the negatively charged head groups of the bacterial cytoplasmic membrane (BCM). Subsequently, the hydrophobic block should get inserted inside the lipid core of the BCM disrupting bacteria stability and integrity. In one of the synthesis process, an hydrophobic ionic liquid (IL) monomer (N-Butyldimethylmethacryloyloxyethylammoniumbis (trifluoro methanesulfonyl)imide) containing QA moieties was grafted to the amphiphilic PU backbone (PU-g-PIL) by ceric ammonium nitrate initiated redox reaction in aqueous phase. Further, it was possible to decorate the PU-g-PIL colloidal particles with the hydrophilic cationic Poly 2-(Acryloyloxy)ethyl] trimethylammonium Chloride (AETMAC) shell. In another approach, *N*-isopropylacrylamide monomer (NIPAM) was grafted from the amphiphilic PU backbone in order to impart a thermoresponsive property and subsequently it was decorated with cationic AETMAC shell.

The chemical structures were confirmed by FTIR, Raman, ^{1}H NMR and ^{13}C NMR spectroscopies. Moreover, characterization of the size and morphology was performed with cryo-TEM, cryo-SEM and dynamic light scattering measurements. Whereas, zeta potential measurements provided information regarding the stability of colloidal dispersions. The antibacterial properties of the monomers which were subsequently grafted from amphiphilic PU backbone were tested on Gram-positive (*S. aureus* and *S. epidermidis*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*) by the microdilution broth method to determine the minimum inhibitory concentration (MIC).

The chemical and structural analyses confirmed the successful synthesis of the amphiphilic PU and the subsequent grafting of either hydrophobic PIL block or thermoresponsive NIPAM block to the PU backbone and subsequent decoration of AETMAC shell. Preliminary indication of the antibacterial effect of the constituent monomers of the newly PUs was also evaluated. Interestingly, AETMAC showed higher bacterial inhibition against Gram-positive bacteria. On the other hand, NIPAM presented a lower MIC against Gram-negative bacteria. Antimicrobial studies of functionalized amphiphilic PU are required in order to understand the bactericidal properties of polymerized monomers forming constituent hydrophobic/thermoresponsive and hydrophilic blocks.

0163 Collagen - copper-doped bioactive glass composite scaffolds for the treatment of infection and regeneration of bone

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The bone infection osteomyelitis (OM) is most commonly caused by S*. aureus.* Following surgical debridement, long-term systemic high dose antibiotics and bone grafting are typically required. Antibiotic resistance is an increasingly concerning issue: 70% of bacteria are resistant to some/all antibiotics [1]. An alternative approach is to utilise proven non-antibiotic antibacterials, such as copper. In this project, we utilised bioactive glass (BG), an established osteoinductive and osteoconductive material with immense capacity for bone repair [2], as an antimicrobial copper ion delivery vector. The copper-doped BG was incorporated into collagen scaffolds previously developed within the TERG which have proven bone regenerative capacity [3] to produce a bifunctional antimicrobial and osteogenic scaffold.

Freeze-dried collagen scaffolds containing 0-15 milligrams/millilitre of 2 mol% copper-doped BG (sol-gel derived) were fabricated. Backscatter SEM was used to examine the distribution of BG within the scaffold. The effect of the addition of BG on the porosity and elastic modulus of the collagen scaffold was investigated. Antibacterial activity of the scaffolds was assessed against *S. aureus.* The scaffold with the best antibacterial response was carried forward for cellular viability and osteogenesis studies with a pre-osteoblast cell line (MC3T3) (PicoGreen® assay, calcium assay, and alizarin red staining).

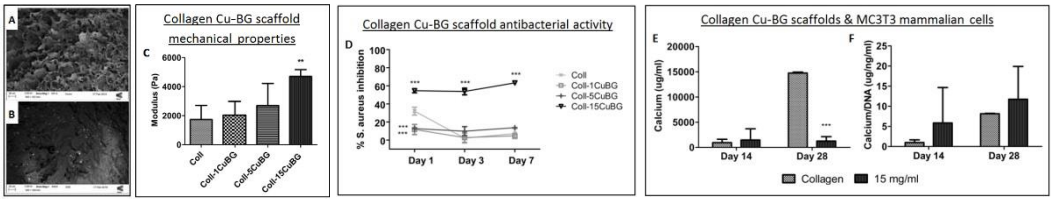

Figure 1 - Backscatter SEM images showing (A) highly porous collagen scaffold, (B) homogenously distributed BG. Note the bright copper against the dark collagen background. (C) Increasing BG concentration increases scaffold compressive modulus. (D) Coll-15CuBG significantly reduces bacterial viability. (E, F) Coll-15CuBG scaffolds show some toxicity towards MC3T3s (not shown) however, calcium values are maintained when normalised on a per-cell basis.

The collagen copper-doped BG scaffolds demonstrated both antibacterial and osteogenic effects. Although by increasing the concentration of BG within the scaffold a reduction in viability of osteoblasts was observed, calcium production was maintained on a per-cell basis and the antibacterial activity was increased on average by 57% compared to the collagen control. The collagen copper-doped BG scaffolds developed here show potential to elicit bone healing, whilst eliminating infection in the osteomyelitis defect site.

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0164 A platelet lysate antibacterial bioactive patch for tendon repair

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Platelet lysate (PL) is a class of platelet-rich hemoderivatives produced by cryogenic disruption of platelet concentrates, originating a pool of supra-physiological concentrations of growth factors (GFs) that is being widely explored in the medical field, namely in sports medicine and orthopaedics. In this concern, patch augmentation strategies have been receiving increased attention as the basis for the development of novel biomaterials aiming at tendon regeneration. In the present work, we assessed PL-membranes as prospective bioinstructive patches under the hypothesis that tendon cells positively respond to PL-derived biochemical signals.

For this purpose, PL membranes were fabricated as previously described by Babo *et al*[1] and characterized in terms of degradation, PL-derived proteins and GF release profiles. Cell behaviour was studied in terms of metabolic activity and proliferation, as well as extracellular matrix (ECM) production by culturing human tendon-derived cells (hTDCs) up to 21 days. In addition, the potential of PL membranes as antibacterial surfaces for biomedical implants was evaluated against *Staphylococcus aureus* ATCC 29213 by determining the number of viable counts, as well as biofilm formation and distribution up to 72h, using PDMS films as controls.

Overall, our results showed that PL membranes remained stable for up to 30 days in PBS. In addition, PL-derived proteins, as well as specific GFs like basic fibroblast growth factor (bFGF) and platelet derived growth factor (PDGF)-BB followed a typical controlled release profile, as described by Babo *et al*[1]. Regarding the biological performance, PL-membranes were able to control the proliferation of seeded hTDCs, as demonstrated by maintenance of DNA content over 21 days of culture, in comparison to the controls in standard culture plastic. This result strongly suggests that PL-membranes can avoid an extensive proliferative phase, which *in vivo* is responsible for the formation of scar tissue, a major concern during tendon healing. These cells were metabolically active over time in culture and deposited tendon-related ECM proteins, including collagen types 1 and 3 and tenascin-C. Additionally, PL-membranes exhibited a significantly reduced number of viable counts of *S. aureus*, together with diminished bacteria adherence after 24h of incubation. No biofilm formation was observed in comparison to PDMS controls.

Altogether, our results demonstrate that these PL-membranes can modulate cellular activity *in situ*, acting as a reservoir of bioactive molecules derived from PL, which supports their application as bioinstructive and protective patches for tendon regeneration. Finally, exploring the multitude of features of crosslinked PL proteins can potentially uncover uncharted prospective applications in regenerative medicine.

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0165 Macrophages as important players in tissue engineering and regenerative medicine

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Macrophages are present in almost any type of tissue, and depending on stimuli they receive, they will obtain a certain phenotype. Initially, these phenotypes were classified as classically activated or M1, and alternatively activated or M2, implying only two ends of this spectrum. The M2 macrophages can however be subclassified into several phenotypes such as repair or anti-inflammatory, and different subclassifications such as M2a, M2b etc are given to these phenotypes. In the end, macrophages seem very plastic and they can occur in a large variety of phenotypes. Stimuli from the environment can lead to these different phenotypes. Cytokines and growth factors are the usual suspects here, but extracellular matrix, immune complexes, bacterial fragments, and implanted biomaterials[1] can also alter the phenotype of macrophages.

The response of these macrophages to differentiation stimuli such as biomaterials depends on the health or disease status of the person. Obesity for instance leads to a predominant pro-inflammatory differentiation.[2] It is no surprise that different macrophage phenotypes differentially influence surrounding tissues. Macrophages influenced by different types of materials in for instance have differential effects on adipose tissue derived stem cells (ASCs) regarding fibrotic and immune modulatory parameters.[3] In addition, macrophages residing in the infrapatellar fat pad located in the joint and macrophages residing in the lining of the joint capsule (i.e. synovium) negatively influence chondrogenesis of bone marrow derived stem cells (MSCs)[4,5]. When comparing the reaction of these specific tissue macrophages on MSCs with the effect of M1 and M2 macrophages on MSCs, the effect of the tissue macrophages was mostly comparable to the effect of M1 macrophages.

Logically, it seems interesting to be able to modulate the macrophage phenotype in the tissue, aiming to create an environment that fosters tissue regeneration or to prevent adverse outcomes after the implantation of a tissue engineered construct that might include biomaterials. First attempts are made in our lab to modulate macrophages residing in the synovium[6], infrapatellar fat pad, or seeded on biomaterials. The results of these experiments seem really promising; depending on the phenotype that is desired, a certain compound can be chosen and the effect of the macrophages on surrounding tissues can be modulated.

Taken together, targeting macrophages seems a good strategy when aiming for regenerative therapies and possibilities seem to exist to improve this regeneration via the macrophage.

0166 Immunomodulation with hyaluronic acid derivative coatings

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Hyaluronic acid (HA) is a major extracellular component of connective tissues where it plays an important role in cell differentiation and growth.1 Although it has been widely used for tissue engineering applications, it does not support cell attachment and spreading and needs chemical modification to promote cellular adhesion. In this study, in order to develop immunomodulatory coatings for regenerative medicine application, two substrates with modified HA were developed by Layer-by-Layer method (polylysine/HA-aldehyde)2 and by spin coating (gelatin/HA-tyramine). HA conjugates present the advantage of being crosslinkable: i) HA-aldehyde can react with amino group of poly(lysine) (PLL) either via formation of ionic pair or via formation of covalent imine bond –CH=N- between aldehyde and amine groups; ii) HA-tyramine crosslinking is based on dimerization of tyramine to dityramine by horseradish peroxidase mediated reaction. Moreover, gelatin is also crosslinked with transglutaminase (TGA). The final aim of this work is to significantly decrease the implant and medical device failure due to adverse immune reactions by developing an innovative immunomodulatory system. Our current focus is to use the controlled release capacities for the delivery of immunomodulatory cytokines such as Interleukin 4 (IL-4) and quantification of its effect on primary macrophages (monocytes). IL-4 is a key cytokine in type 2 immunity, which is essential for wound healing and allergy.

Concerning the cytokine production, M1 macrophages produce larger amounts of the pro-inflammatory cytokines: TNF-α, IL-12, IL-1β; while M2 macrophages produce larger amounts of the anti-inflammatory cytokines: IL-1RA, CCL18, IL-10. Regarding the cytokine production from the monocytes seeded on PLL/HA-Aldehyde embedded with IL-4, it is observed that IL-4 released from the film had induced the release of anti-inflammatory cytokines while significantly reducing the release of pro-inflammatory cytokines. The second substrate, gelatin/HA-tyramine, presents a very good stability (75% of remaining film after 21 days), which is related to the fact that the film is double crosslinked. Furthermore, the metabolic activity of macrophages cultured on the top of the film (2D culture) and encapsulated in gelatin hydrogel deposited on the top of the film (3D culture) increases with IL-4 releasing from day 14. In summary, PLL/HA-Aldehyde derivative and Gelatin/HA-tyramine based films are good candidates as surface coatings of complex architectures with controlled physicochemical properties and release capabilities

0167 Mesenchymal stem cell-derived extracellular vesicles (EVs) as mediators of anti-inflammatory effects: endorsement of macrophage polarization

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Mesenchymal Stem Cells (MSCs) are effective therapeutic agents enhancing the repair of injured tissues, not only through direct engraftment, but also through their paracrine activity. Preliminary results indicate that in an inflammatory environment as the one generated during the early phases of the wound healing process, MSC paracrine activity is significantly modulated promoting a functional switch of macrophages from a pro- (M1) to an anti-inflammatory (M2) state, thus corroborating evidences showing that the mobilization of innate immune cells mediates the activation of regenerative processes. Among the factors responsible for the paracrine effects of MSCs, extracellular vesicles (EVs) have been recently described as new players in cell-to-cell communication by serving as vehicles for transfer between cells of membrane and cytosolic proteins, lipids, and genetic information. Since EVs represent physiologically relevant and powerful component of the MSC secretome, the aim of the present study was to carry out a detailed characterization of EVs released by human adipose derived-MSCs to investigate their involvement as modulators of MSC anti-inflammatory effects inducing macrophage polarization. The EV-isolation method was based on repeated ultracentrifugations of the medium conditioned by MSC exposed to either normoxic or hypoxic conditions (EVNormo and EVHypo). Both types of EVs were efficiently internalized by responding bone marrow-derived macrophages, eliciting their switch from a M1 to a M2 phenotype. Observations that different macrophage subsets are associated with different stages of muscle regeneration led us to investigate whether EV treatment could influence macrophage polarization from M1 to M2 phenotype in vivo. We opted for a cardiotoxin (CTX) injury in the mouse Tibialis Anterior (TA) muscle, a reproducible model that recapitulates all healing phases. Muscles, subjected to CTX-damage followed by injection of either EVNormo or EVHypo, were examined at different times. EVNormo and EVHypo interacted with macrophages recruited during the initial inflammatory response. In injured and EV-treated muscles, a down-regulation of IL6 and the early marker of innate and classical activation Nos2 was concurrent to a significant up-regulation of Arg1 and Ym1, late markers of alternative activation. These effects, accompanied by an accelerated expression of the myogenic markers Pax7, MyoD, and eMyhc, were even greater following EVHypo administration. Collectively, these data indicate that MSC-EVs possess effective anti-inflammatory properties, making them potential therapeutic agents more handy and safe than MSCs.

0168 Allogeneic chondrogenic human bone marrow mesenchymal stromal cells induce Th2 T cell differentiation and inhibit Th1 T cell differentiation *in vitro*

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Introduction: Allogeneic bone marrow stromal cell (BMSC)-mediated endochondral ossification may be a promising new approach to replace autologous bone transplantation as the current gold standard of bone regeneration. Implantation of autologous chondrogenically differentiated BMSCs leads to bone formation *in vivo* (1). However, the success of using allogeneic cells depends on the interaction between the implant and the host's endogenous immune cells. Our group has previously shown that chondrogenically differentiated BMSCs do not affect the proliferation of CD4 T cells (2). However, we hypothesise that chondrogenic BMSCs may effect a particular subset of these CD4 T cells. Th17 cells and their secretion of IL17 has been shown to be involved in osteoclastogenesis and bone remodelling (3). It is not clear which of the CD4 subtypes are modulated by chondrogenic BMSCs and how they use these cells to facilitate bone regeneration. The aim of the current work was to assess the effect of chondrogenic hBMSC pellets on the differentiation of Th1, Th2 and Th17 T cells. Materials and Methods: Human BMSCs were differentiated in chondrogenic medium with and without TGFβ3 for 7 days. CD4+ T cells were isolated from peripheral blood mononuclear cells (PBMCs). Naïve and memory T cells were sorted from this population using antibodies against CD4 and CD45RO. Naive and memory T cells, as well as unsorted PBMCs co-cultured with allogeneic chondrogenic hBMSC pellets for 3 days, were harvested and analysed by flow cytometry to assess the effect on the differentiation of Th1, Th2 and Th17 cells. Secretion of IFNγ, IL4, IL10, IL6, IL17A, IL17F and IL22 in supernatants was measured by ELISA. Data was analysed using a repeated measures ANOVA with Bonferroni post-test. $P<0.05$ was considered statistically significant. Results: Co-culture of allogeneic chondrogenic hBMSC pellets and memory T cells resulted in a significant increase in Th2 T cells and a decrease in Th1 T cells. Th17 T cells were not affected. Allogeneic chondrogenic hBMSC pellets did not induce Th1, Th2 or Th17 T cell differentiation in co-culture with naïve T cells. Following 3 days co-culture with unsorted PBMCs, allogeneic chondrogenic hBMSC pellets induced the differentiation of Th2 T cells. Th1 and Th17 T cell differentiation was not affected.

Discussion and conclusion: Chondrogenically differentiated BMSCs were hypothesised to induce/inhibit specific CD4 T cell subsets to facilitate bone formation. It was initially expected that Th17 cells would have been involved in MSC-mediated endochondral bone formation, however, in this study Th17 T cell differentiation was not affected by chondrogenic or non-chondrogenic BMSCs. Interestingly, there appeared to be an induction of Th2 differentiation and inhibition of Th1 T cell differentiation in memory T cell co-cultures. This effect was the same in both BMSC pellet types, despite the obvious differences in these cells ability to form bone *in vivo*. More research is required to unravel the effects of this skewing of the balance of Th1/Th2 T cells in MSC-mediated endochondral bone formation.

0169 Generating an M2 pro-regenerative macrophage phenotype using nanoHA particles for bone tissue engineering

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Introduction: The ultimate functional success of materials used for implantable medical devices relies heavily on the host tissue response. Cells of the innate immune system such as macrophages are one of the first to participate in the immune response after implantation of a biomaterial and are therefore crucial determinants for the success or failure of the implant (1). These are a heterogeneous population of mononuclear cells which can be broadly divided into M1, pro-inflammatory and M2, anti-inflammatory macrophages. M1 macrophages secrete potent pro-inflammatory cytokines which can drive chronic inflammation and scar tissue formation. However, M2 macrophages, which secrete anti-inflammatory and pro-healing components, have been associated with a more constructive remodelling outcome in vivo (2). Although much research has been carried out examining the osteoinductive activity of HA particles, little research has been performed to determine the macrophage phenotype induced by these particulates.

Aims: The objective of our research is to characterise the phenotypic profile induced by different sized HA particles in primary human macrophages.

Methods: Primary human macrophages were treated with commercially available Hydroxyapatite (HA) particles, (1-2 µm), or nano sized HA (100nm), prepared as previously described, (3) for 24 hours. Supernatants were harvested and IL-1α/β, TNFα and IL-10 cytokine concentrations were quantified by ELISA. Real-time PCR was carried for the detection of CCL19, CXCL11, CCL13 and MRC1 mRNA. Flow cytometry was used to assess the surface markers CD86, CD163 and CD206.

Results: Larger HA particles, ranging in size from 1-2µm, are highly pro-inflammatory in primary human macrophages and drive the expression of IL-1α/β and TNFα cytokines in addition to the M1 markers, CD86, CXCL11 and CCL19 (not shown). In contrast, nanoHA (100nm) particles do induce the production of the M2 anti-inflammatory cytokine IL-10. Furthermore, these particulates drive robust expression of the M2 markers, CCL13, CD206 (MRC1-mannose receptor) and CD163.

Conclusions: This study has demonstrated that nanoHA particles exert a pro-regenerative M2 phenotype as evidenced by increased expression of the M2 markers IL-10, CCL13, CD206 and CD163, in comparison to larger HA particles which are potent drivers of pro-inflammatory mediators. The mechanistic basis for these differences is not yet known and while further in vivo study is required, this data suggests that nanoHA particles may support a more constructive tissue remodeling outcome in comparison to their larger counterparts.

0170 Macrophage polarization and their cross-talk with mesenchymal stem cells can be modulated by substrate stiffness

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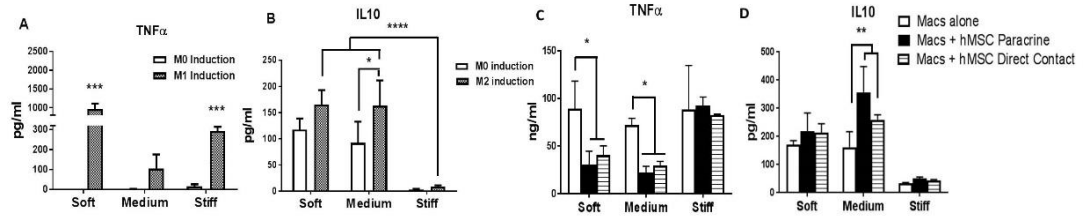
Following implantation of regenerative materials into the body, immune cells such as macrophages are the first to respond where they direct the tissue healing process through pro-inflammatory (M1) and anti-inflammatory (M2) polarization states. Mesenchymal Stem Cells (MSC) are another important component of this process, and together with macrophages drive tissue repair. It is known that MSC differentiation into different lineages can be controlled by stiffness properties (1); and recent evidence suggests that MSC adapt an immunomodulatory role in the presence of macrophages (2). However, little is understood about the cross-talk between the two cell types in the context of diverse biophysical and biochemical cues, a situation analogous to the implant environment. The overall aim of this study was thus to elucidate the role of substrate stiffness in macrophage polarization, MSC immunomodulation and the MSC/macrophage cross-talk. Towards this, macrophages (derived from THP1 monocytes) were cultured on polyacrylamide gels of three different stiffness values (Soft: 11kPa, Medium: 88kPa and Stiff: 323kPa). Macrophages on stiff gels assumed a pro-inflammatory phenotype (high secretion of pro-inflammatory TNFα) whereas cells on soft and medium gels assumed an anti-inflammatory (high secretion of IL10), highly motile, highly phagocytic phenotype (A, B). Using actin inhibitors, we confirmed that this stiffness-mediated polarization was dependent on the actin cytoskeleton. Moreover, when MSC were cultured in the presence of macrophages either in direct contact or in transwell inserts (to assess paracrine signalling), we observed in both cases that macrophages secreted lower levels of TNFα and higher levels of IL10 (C, D). Interestingly, the cross-talk between the two cell types was dependent on the stiffness cues presented to them – the immunomodulatory effect of MSCs was most profound on soft and medium gels and was severely diminished on stiff gels. Taken together, these results underline the importance of biophysical cues presented to the cells arriving at the site of implantation. Biomaterials that are designed to incorporate favourable biophysical and biochemical cues will promote positive immune and stem cell responses after implantation, resulting in successful remodelling outcomes that ensure complete integration into the host.



0171 Relevance of NP progenitor cell niche in the endogenous regeneration potentials of the intervertebral disc

Daisuke Sakai

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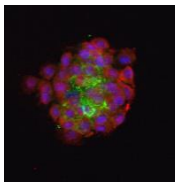
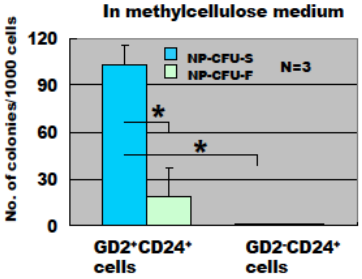
Stem/progenitor cells have been isolated from various musculoskeletal tissues. Recently, cells with MSC-like properties have been isolated from degenerate human intervertebral discs, providing support for the presence of mesenchymal progenitors (Blanco et al., 2010; Risbud et al., 2007). We have isolated progenitor cells from human and mouse nucleus pulposus(NP) of the intervertebral discs that are different to adhesive MSCs, able to form spheres similar to neural progenitors when cultured in a methycellulose-containing medium (Sakai et al., 2012).





These cells have self-renewal (*in vitro* and *in vivo*) and multipotential characteristics consistent with progenitor cells. These progenitors express Tie2 as a marker that progress toward Tie2+/GD2+ cells, with the subsequent expression of CD24 and CD44 as markers for nucleus pulposus (NP) cells in culture, and the down-regulation of Tie2 and GD2 expression with maturation towards NP cells, and self-renewal property is then lost.

In this symposium, NP progenitor niche and its correlation with intervertebral disc homeostasis and various disc diseases in clinics are discussed.

0172 Intervertebral disc regeneration: new strategies for the aging spine

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Background: Stem cell based intervertebral disc (IVD) regeneration is quickly moving towards clinical applications. However, many aspects need to be investigated to routinely translate this therapy to clinical applications, in particular, the most efficient way to deliver cell to the IVD. Cells are commonly delivered to the IVD through the annulus fibrosus (AF) injection. However, recent studies have shown serious drawbacks of this approach. As an alternative we have described and tested a new surgical approach to the IVD via the endplate-pedicles (transpedicular approach). The Purpose of the study was to test MSCs/hydrogel transplantation for IVD regeneration in a grade IV preclinical model of IDD on large size animals via the transpeducular approach with cell dose escalation.

Methods: Adult sheep (n=18) underwent bone marrow aspiration for autologous MSC isolation and expansion. MSC were suspended in autologous PRP and conjugated with Hyaluronic Acid and Batroxobin at the time of transplant (MSCs/hydrogel). Nucleotomy was performed via the transpedicular approach in four lumbar IVDs and that were injected with 1) hydrogel, 2) Low doses of MSC/hydrogel, 3) High doses of MSC/hydrogel, 4) no injection (CTRL). The endplate tunnel was sealed using a polyurethane scaffold. X-ray and MRI were performed at baseline and 1,3,6,12 months. Disc macro- and micro-morphology were analyzed at each time point.

Results: The MRI index showed a significant decrease in the untreated group, the disc injected with hydrogel and those injected with low MSC dose compared to healthy discs in all time points. The discs treated with high dose of MSC showed maintenance of the MRI index compared to the healthy disc. Morphologically, the grade of degeneration evaluated using the were in agreement with the grades observed at the MRI.

Conclusions: An effective dose of autologous MSC ($1x10^7$ cell/ml) delivered via the alternative transpedicular approach regenerates the NP in a preclinical model of grade IV IDD maintaining the AF intact This preclinical study has high translational value as large animal model with the long fallow up were used, MSCs were expanded in GMP facility simulating the clinical scenario, and the hydrogel were composed of clinically available drags and materials. This approach represent a new valeable strategy to regenerate grade IV degenerated disc of the aging spine.

0173 An intervertebral disc organ culture model mimicking proinflammatory and degenerative disease condition

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Introduction:

Establishment of a proinflammatory and degenerative intervertebral disc (IVD) organ culture model can be instrumental in deciphering the role of therapeutic agents in a relevant and controlled environment. The aim of the current study was to 1) establish a proinflammatory and degenerative IVD organ culture model, by combining TNF-α intradiscal injection, detrimental loading and limited nutrition; 2) simulate the early onset of degenerative disc disease (DDD).

Materials & methods:

Bovine caudal IVDs with endplates were cultured under 2 different loading and medium conditions: (1) physiological loading (0.02-0.2 MPa; 0.2 Hz; 2h/day) and high glucose (4.5 g/L) medium (Phy); (2) degenerative loading (0.32-0.5 MPa; 5 Hz; 2h/day) and low glucose (2 g/L) medium (Deg). In the inflammatory groups, TNF-α was injected into the nucleus pulposus (NP) tissue (100 ng / IVD). Conditioned medium was collected for nitric oxide (NO) and Glycosaminoglycan (GAG) analysis. After 4 days, disc tissue was harvested and gene expression was analyzed using real-time PCR. After 11 days, cell viability was evaluated by lactate dehydrogenase (LDH) / ethidium homodimer staining of disc cryo-sections. One-way ANOVA or Kruskal-Wallis tests were used to assess differences between 4 groups (n=6-10/group), as appropriate. $P<0.05$ was considered statistically significant.

Results:

TNF-α combined with Deg condition up-regulated NO and GAG release from IVD. Gene expression analysis of disc tissue demonstrated a decrease of type I collagen (COL1) in AF tissue, caused by Deg condition or TNF-α injection. TNF-α injection combined with Deg condition induced an up-regulation of interleukin-6 (IL6) and matrix metalloproteinase 1 (MMP1) in NP tissue, and interleukin-1β (IL1B) in AF tissue. After 11 days of culture, cell viability dropped markedly in IVD treated with TNF-α and Deg condition.

Conclusion:

TNF-α injection alone causes an inflammatory effect on the organ cultured IVDs, as indicated by higher GAG and NO release into conditioned medium. Under combined TNF-α injection and degenerative culture condition, an upregulation of catabolic and inflammatory marker gene expression was observed in disc tissue, together with enhanced GAG and NO release, as well as cell death, which indicate an accelerated inflammatory and degenerative effect. Disc ECM content did not change significantly after short term culture. However, gene expression level of COL1 was decreased in AF tissue, which may lead to less collagen content after longer term culture. To conclude, a proinflammatory and degenerative organ culture model was established by TNF-α intradiscal injection, detrimental loading and limited nutrition to mimic the early course of DDD. This model will be of high interest for screening of therapeutic agents in further pre-clinical studies.

Acknowledgements: Funded by SET.

0174 Controlled release of celecoxib from polyesteramide microparticles in a canine pre-clinical intervertebral disc degeneration model

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Introduction: Low back pain affects 80% of the people worldwide at least once in their lives. Low back pain is often related to degeneration of the intervertebral disc (IVD). The process of IVD degeneration and its clinical representation in dogs resembles the human situation. Pro-inflammatory mediators such as the COX-2 mediated prostaglandin E2 play an important role in the sensitization to pain. Oral COX-2 inhibitors are effective in reducing low back pain, although they are associated with side effects. Moreover, it is not known how much of the orally administered drug reaches the avascular IVD. Therefore, local delivery of drugs directly into the IVD could be a suitable treatment strategy for back and neck pain. We have previously demonstrated safety of intradiscally injected Poly-Ester-Amide Microparticles (PEAMs) in a canine model with long term follow up of 6 months. The aim of the present study was to find the optimal loading dose of locally administered celecoxib in a controlled release system based on PEAMs, in a canine model with induced IVD degeneration as a first step towards clinical translation.

Methods: IVD degeneration was induced in lumbar IVDs of beagle dogs by puncture with an 18G needle and partial nuclectomy by aspiration. IVDs that were not punctured served as controls. Four weeks thereafter, dogs received intradiscal injections with PEAMs, loaded with 0.23 mg/mL or 7 mg/mL celecoxib or unloaded microparticles (sham), with n=6 discs per treatment. At 16 week follow-up period, MRI was performed. Post mortem, after CT scan of the spinal column, IVDs were harvested for macroscopic and histopathological evaluation and biochemical analyses (PGE2-, DNA-, collagen-, and glycosaminoglycan content).

Preliminary results: At 16 weeks after intradiscal injection with 40µL unloaded PEAMs (sham), low (0.23 mg/mL) or high (7 mg/mL) dose celecoxib, no adverse effects were visible on imaging modalities (MRI, CT) or macroscopic evaluation. The macroscopic and MRI scores were significantly higher in the IVDs where degeneration was induced compared to control IVDs. The IVDs treated with the PEAMs containing low and high dose CXB did not statistically differ from either non-induced and sham groups. Total- and PGE2/DNA levels were significantly increased in both the NP and AF of the sham IVDs, compared to non-induced IVDs. PGE2 levels of the AF decreased after treatment with celecoxib. Sustained release of celecoxib, regardless of the dose, resulted in a significantly increased glycosaminoglycan content of the nucleus pulposus. Intradiscal injection of PEAMs had no effect on glycosaminoglycan-, total collagen- or DNA content of the annulus fibrosus.

Discussion & conclusions: Celecoxib incorporated in PEAMs was safely administered intradiscally in experimental dogs. Ongoing quantitative MRI analyses will give more detailed results on radiologic changes. Follow up studies in a large animal model with naturally occurring clinical IVD disease as a preclinical model for translation to humans are warranted to determine the efficacy of celecoxib loaded PEAMs in inhibiting low back pain.

0175 Elucidation of the human foetal notochordal cell phenotype to define stem cell differentiation in intervertebral disc regeneration strategies

Stephen Richardson, Ricardo Rodrigues-Pinto, Andrew Berry, Neil Hanley, Judith Hoyland

The University of Manchester, Manchester, UK

While the human embryonic, foetal and juvenile NP is composed of large vacuolated notochordal cells, these morphologically distinct cells are lost with skeletal maturity and are replaced by smaller NP cells. Notochordal cells are thought to be fundamental in maintaining IVD homeostasis and, hence, their loss in humans may be a key initiator of degeneration, leading ultimately to back pain. Therefore, it is essential to understand the phenotype of human notochordal cells to enable the development of novel biological/regenerative therapies. Thus the aim of this study was to isolate notochordal and sclerotomal (annulus fibrosus and vertebrae precursors) cells from foetal human spines and perform microarrays to elucidate the notochordal cell phenotype and regulatory pathways which can be used to direct appropriate stem cell differentiation towards a NC phenotype.

Human foetal spines (7.5-14 WPC, n=5) containing notochordal and sclerotomal cells were micro-dissected from adjacent tissues and cells were isolated enzymatically. Cells were labelled with a PE-conjugated anti-CD24 antibody and viable CD24+ notochordal cells and CD24- sclerotomal cells sorted using fluorescence activated cell sorting (FACS). RNA was extracted, globally amplified to cDNA and sorting accuracy validated using qPCR for previously identified notochordal markers including CD24 and brachury. Affymetrix cDNA microarrays were performed to analyze differential gene expression and differential expression was confirmed using qPCR Interactive pathway analysis (IPA) was used to identify networks, pathways and upstream master regulators of differentially expressed genes.

FACS allowed isolation of a small population of CD24+ve notochordal cells (mean 10.4% CD24+) and a larger population of CD24-ve sclerotomal cells (mean 60.9% CD24-), with cells falling between the CD24+ and CD24- gates being discarded to ensure specificity. qPCR confirmed higher differential expression of the notochordal cell markers CD24 and T in the CD24+ve cells compared to CD24- cells. Hierarchical clustering and PCA mapping revealed distinct differences in the gene expression profile of CD24+ and CD24- cells. Top notochordal markers were CD24, STMN2. RTN1, PRPH and CXCL12 and top sclerotomal markers were WISP3, CHST11, SERPINA3 and CHAD. IPA identified connective tissue development as the top network; importantly IL-1 receptor antagonist (IL-1RN) and noggin were identified as master regulators of notochordal cell phenotype.

This study has, for the first time, identified the phenotype of human foetal notochordal cell and identified a number of pathways and upstream regulators that may be used to direct stem cell differentiation to notochordal cells. In particular, IL-1RN and noggin are of interest as master regulators of notochordal cell function, suggesting vital roles for these molecules in IVD development and homeostasis which requires further study.

0176 Direct versus pre-cultured injection in transplantation of cryopreserved human intervertebral disc cells in a canine disc degeneration model

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INTRODUCTION A multitude of studies have indicated the potential of cell transplantation towards a treatment against degenerative disc disease. Nevertheless, the optimal cell source remains elusive. Injected cells should possess the capacity to survive under the harsh hypoxic, acidic, and mechanically loaded conditions of the intervertebral disc (IVD), while actively producing extracellular matrix. Additionally, issues with limited cell sources and required pre-culturing, limits the translation to an affordable and practical clinical cell therapy. Therefore, this study aims to assess the injection of cryopreserved IVD cells directly from their cryopreserved state or pre-cultured after thaw. Thawed IVD cells were evaluated in a beagle model and compared with cell transplantation of 2 weeks pre-cultured condition in their capacity to limit or improve degeneration associated disc height reduction and maintain histological features.

METHODS: Ten female beagle dogs under complete anesthesia, were subjected to induction of disc degeneration by aspiration of the NP from IVD L3/4. L4/5, and L5/6. 2 weeks after NP aspiration, injection of cryopreserved human IVD cells encapsulated in 0,1% sodium hyaluronate gel was injected. Each dog received a 100-µL injection of saline [Sham], cell suspension directly from cryopreserved state [Frozen], and 2-weeks pre-cultured cell suspension [Cultured]. Additionally, the recipients were randomly divided into a low dose ($1x10^6$ cells/mL, n=5) and high dose group ($10x10^6$ cells/mL, n=5). The different treatments were injected into the aspirated discs. At day of treatment, 2 weeks prior-, and every 4 weeks after cell injection, x-ray radiographic images were obtained. Blood values and body weights were assessed throughout the experiment. 12 weeks after cell transplantation, canine subjects were sacrificed and the manipulated discs plus control disc L6/7 were explanted. Discs were decalcified and stained by HE and Safranin-O/Fast green. Disc height index (DHI) was determined according to methods of Masuda *et al.* Histological sections were scored by adapted scoring methods of Bergknut *et al (*Safranin-O/Fast green staining was used instead of Alcian blue/Picrosirius red staining to assess proteoglycan matrix abundance). Significance was determined by ANOVA followed by Tukey`s multiple comparison. Differences of $p<0.05$ were considered significant.

RESULTS: Weight measurements and blood values showed no relevant alterations during the study. (Data not shown) DHI remained stable for healthy control discs, while the sham control discs showed a significant and progressive deterioration. DHI reduction is limited by injection of both pre-cultured, low and high cell dose injection, and increases for both frozen conditions. Histology scoring via Bergknut *et al* system demonstrates a significant increase for the low dose frozen condition. Moreover, both high dose conditions show an increase in score compared to the sham. Histologic images clearly signify a reduction in safranin-O staining in the nucleus pulposus for the Sham controls and a more fibrous matrix on HE staining. Overall, the treated discs exhibit retained safranin-O staining and a more clear distinction between nucleus pulposus and annulus fibrosus compared to the sham control.

DISCUSSION: The present study demonstrates that, in a large canine animal model, cryopreserved human IVD cells are able to limit induced disc degeneration. The injection of $0,1x10^6$ cells directly from their cryopreserved state, results in a significant increase in DHI and histological features compared to the sham control. These data suggest that the injection of cryopreserved human IVD cells is able to halt progressive degeneration and display a potential for IVD regeneration. Finally, injection of cells directly from their cryopreserved state appears to be an effective approach for cell transplantation.

0177

Andreas Emmendörffer

0178

Jonathan Souquet

0179 Stretch and scale: tissue engineering from lab to industry

Suzanne Mithieux, Anthony Weiss

University of Sydney, Sydney, NSW, Australia

This presentation will focus on a university-based inventor's journey from lab discoveries, innovation and technology translation, founding a company, building management and investment, to the company's clinical trials.

Elastagen is a medical company with an advanced pipeline of products based on recombinant human tropoelastin, the building block of elastin. Elastagen, through its founding scientist Prof Weiss and his team at the University of Sydney, has acquired a unique and dominant position in the tropoelastin and elastin field.

Elastagen is the first and only provider of full length, scalable, commercial cGMP clinical grade human tropoelastin. Furthermore, Elastagen has pioneered the scientific understanding of tropoelastin and the elastagenesis process and developed and patented a range of approaches to formulate tropoelastin for application in tissue repair.

Elastin is a critical physical and biological component of human tissues, such as the skin, lungs, arteries, and elastic ligaments, and is comprised of multiple copies of the protein tropoelastin.

Elastin is produced in the body predominantly during fetal development and by the time we reach adulthood the ability of the body to express tropoelastin and regenerate new elastin is minimal. The application of tropoelastin and/or elastin as a medical biomaterial has to date been limited due to the inability to extract the protein in a functional form from animal tissues or produce it synthetically.

Tropoelastin has huge advantages for a range of medical applications, including its unique physical elastic properties and its ability to support cell growth and tissue repair. In addition, as a recombinant human biomaterial we have tested and addressed the biocompatibility profile and immune compatibility of tropoelastin-based polymers. Elastagen's platform technology has potential applications in a number of areas including skin rejuvenation, scar remodelling and tissue repair.

Elastagen was founded following the acquisition of intellectual property rights to the elastin technology which was developed by Prof Weiss at the University of Sydney. The Company has since successfully attracted capital from a number of leading Australian and overseas life science venture capital groups. Elastagen's operations have expanded from its Australian base to include production in Europe and clinical operations in the UK.

0180 Enabling cell therapies with bioreactors: an SME perspective to commercialisation in life science

Ruud Das

Scinus Cell Expansion BV, Bilthoven, The Netherlands

Cell therapies represent an exciting area of regenerative medicine, with applications in numerous diseases. Success of these therapies not only depends on their clinical effectiveness, but also on their ability to be produced in a cost-effective manner. Bioreactor technology can enable cost-effective cell therapy production by lowering costs for labour, clean rooms and consumables. At the same time, process automation can increase safety and reliability of the culture process by offering improved monitoring and control.

Scinus Cell Expansion develops bioreactor technology (pictured above) for cell therapy production. To ensure successful commercialisation of such novel technology, careful consideration needs to be given to user needs, commercial opportunities and clinical requirements. Here we discuss the SME-approach to establishing a solid pre-commercialisation position, including the importance of intellectual property rights, securing financing and strategic collaborations.

0181 Scaffold materials for cartilage resurfacing

Farshid Guilak

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Osteoarthritis is painful and debilitating joint disease that is characterized by pain and degenerative changes in the articular cartilage and other joint tissues. While a number of techniques have been developed over the years for the treatment of small cartilage defects, there have been few attempts at tissue-engineered therapies for end-stage osteoarthritis. Some of the major considerations for this approach include the identification and characterization of an abundant and accessible cell source as well as the design of biologically and mechanically functional scaffolds that can withstand joint loading. Here we describe the engineering of large, anatomically-shaped cartilage constructs using different stem cell sources (e.g., adipose stem cells, mesenchymal stem cells, or induced pluripotent stem cells), combined with mechanically functional cell-instructive scaffolds based on three-dimensional weaving of biocompatible fibers. As each fiber is selected individually, this textile processing technique allows site-specific functionalization of proteins or virus to facilitate the formation of complex inhomogeneous tissues from a single cell source. Furthermore, the development of custom-designed cells using new genome-editing techniques can be used to create self-regulating “smart” cells or constructs for drug delivery to enhance joint repair or combat inflammation in arthritis.

0182 Donor cell variation and its role in stem cell therapy

Brian Johnstone

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While adult stem cells, including bone marrow-derived mesenchymal stem cells (MSCs) and tissue-derived articular cartilage progenitors (ACPs), are possible cell candidates for autologous tissue regeneration, there exists substantial heterogeneity across populations of cells from adult human donors. Generating clonal populations of MSCs is technically very challenging. Among the few successful examples, clonal MSC populations derived from individual human donors demonstrate intra-clonal heterogeneity with respect to proliferative efficiency, differentiation capacity, and phenotype. In contrast to MSCs, ACPs are clonable, but intra-donor variation has only been defined at the level of colony-forming efficiency and intra-clonal variation remains undefined. Without standardized cell isolation and differentiation protocols in articular cartilage tissue engineering, generalized comparisons across and within cell populations from adult human donors, especially when pooled from multiple donors, may hinder our ability to identify subsets of cells with which to effectively generate autologous tissue applicable to adult patients. Not all adult human stem cells are equivalent, and there exists a range of chondrogenic potential for cells derived from different biologic donors as well as for cells derived from clonal populations from a single donor. This may have consequences for understanding the response of stem cell populations to biological stimuli; for example, the discrepancies in reports on the effects of oxygen on MSC hypertrophy. We have shown that the chondrogenic capacity of stem cells influences the cell's response to culture in a physiologic low oxygen environment: in physioxia, both MSCs and ACPs of high chondrogenicity demonstrate upregulation of the articular chondrocyte phenotype and downregulation of the hypertrophic phenotype. Only ACPs, however, demonstrate a consistent attenuation of hypertrophy in the physioxic environment, and these cells may overcome historical challenges of MSC hypertrophy in tissue engineering applications. Based on our investigation, we suggest that the variation in the reported results for the hypertrophic response of MSCs in lowered oxygen tension may be due to the disparity of the intrinsic chondrogenic capacity of these cells at baseline: highly chondrogenic cells significantly downregulate genes of hypertrophy, but cells of lower chondrogenic potential are much less responsive. Thus, individual stem cell populations intended for allogeneic or autologous tissue repair should be characterized *in vitro* to identify highly chondrogenic cells prior to application in tissue-engineered therapies.

0183 Gene therapy approaches to cartilage repair

Chris Evans

Mayo Clinic, Rochester, MN, USA

Gene transfer is a useful technology for the local delivery of gene products, both protein and RNA, in a sustained and potentially regulated fashion. In the context of cartilage repair, such products include morphogens, growth factors, transcription factors, anti-inflammatory molecules and various species of non-coding RNA. Viral or non-viral vectors can be used for gene transfer to sites of cartilage repair in an in vivo or ex vivo fashion.

There are several different strategies for using gene transfer to promote cartilage repair, two of which will be described in greater detail in this talk.

One involves the use of autologous bone marrow clots to which have been added vectors encoding growth factors. This has shown promise in pre-clinical studies in rabbits.

The other uses a line of allogeneic, human, chondrocytes that have been genetically modified to express high levels of transforming growth factor beta. This product, known as Invossa TM, has completed Phase III trials for osteoarthritis in Korea and Phase II trials in the US. It has been used in Phase II clinical trials for cartilage repair in Korea.

0184 Engineering of blood and lymphatic vascular beds

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Ludwig Boltzmann Institute for Experimental and Clinical Traumatology, Vienna, Austria

INTRODUCTION: Physiological human vessel systems comprise two separate vascular beds. Whereas oxygen and nutrient supply is ensured by microcapillaries transporting blood to tissues, the lymphatic system guarantees removal and drainage of protein-rich interstitial fluid back to the blood circulation. Consequently, most approaches to regenerate or engineer vascular structures have to consider both vascular systems. In our group, we co-culture endothelial cells from different vascular beds together with mesenchymal stem cells in a fibrin matrix [1,2].

METHODS: Endothelial cells (EC) are routinely isolated from different tissues (peripheral blood - ECFCs, umbilical vein - HUVEC, foreskin - HDMEC, fat) and characterized. Blood vascular and lymphatic ECs (LEC) are then retrovirally infected with cDNAs encoding for fluorescence proteins and subsequently embedded together with mesenchymal stem cells derived from adipose tissue (ASC) in a fibrin matrix. Vascular tube formation is followed up for several weeks and network characteristics (tube lengths, junctions) are measured.

RESULTS:

Co-embedding of ECs from blood vascular origin together with ASCs in a fibrin matrix resulted in a network-like structure with endothelial cell-cell junctions (CD31) and pericyte coverage (NG2). Different parameters, like fibrinogen concentration, cell numbers as well as the fibrinolysis inhibitor aprotinin influenced the final vascular density. When LEC were cocultured with ASC, network formation could be observed as well, typically dependent on the addition of the lymphangiogenic growth factor VEGF-C. Tricultures of BEC, LEC and ASC resulted in the formation of two separate vascular networks, reflecting the morphology of blood and lymphatic vascular beds.

DISCUSSION & CONCLUSIONS: Vascular tissue engineering approaches have so far mainly focussed on the blood vascular system. Besides successful generation of microcapillary networks, the supplementation with lymphatic structures is of utmost importance to ensure drainage of the interstitial fluid. Our co-culture system of blood and lymphatic ECs with mesenchymal stem cells in a biodegradable matrix provides a feasible approach towards this goal.

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0185 Indirect neovascularization by endogenous growth factor recruitment via heparin-binding in an ELR-based hydrogel

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The targeting of soft tissue engineering goals is nowadays being focused on the development of complex approaches that aim to act on a specific tissue in a controlled manner, in order to induce a therapeutic effect without adverse effects. This work addresses ischemic conditions treated by supporting the neovascularization of the affected area. To favour this event in a restrained way, avoiding angiomas, we have developed an ECM-mimicking hydrogel based on elastin-like recombinamers (ELRs), conferred heparin retention, biodegradability and cell adhesion. The proangiogenic outcome is driven by indirect retention of growth factors at the site of application of the scaffold. These bind to heparin (1, 2), a polysaccharide with various roles in the human body. Our system acts in a safe way as the growth factors involved in the promoted angiogenesis are not supplied extrinsically, but recruited from the blood at the site of action, avoiding an excess of growth factor activity. For the purpose of this study, a novel ELR, containing a heparin-binding domain, has been recombinantly expressed in *E. coli*, purified by inverse transition cycling (ITC) (3) and chemically modified in order to carry an azide group, subsequently involved in a catalyst-free click chemistry reaction, bringing to the covalent crosslinking of the hydrogel (4). The scaffold has been characterized by various techniques from a biological and mechanical point of view. The other ELRs included in the platform contain cell adhesion and matrix metalloproteinase cleavable sequences, supporting cell colonization and proliferation within the system. *In vitro* assessment of the heparin-binding capacity of the novel ELR, as a non-crosslinked coacervate and within the hydrogel has shown successful results. Subsequent vascular endothelial growth factor (VEGF) release from the scaffold showed the expected retention of the molecule. *In vitro* cytocompatibility in 2D and 3D experiments confirmed endothelial cell adhesion and proliferation in the hydrogel. *In vivo* tests were performed to confirm the expected neovascularization. In conclusion, a device showing cytocompatiblity and an indirect and safe proangiogenic effect has been developed in order to approach ischemic diseases as an alternative to systems that require growth factor supply, which can derive to possible adverse effects.

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0186 Effective vascularization and efficient bone formation in osteogenic grafts requires VEGF dose control

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INTRODUCTION: Spontaneous vascularization of clinically relevant, large-size bone grafts based on bone marrow-derived mesenchymal stem cells (BMSC) is insufficient and requires therapeutic stimulation to ensure progenitor survival and bone formation. Vascular endothelial growth factor-A (VEGF) is the master regulator of angiogenesis. However, we found that, while its sustained over-expression by genetically modified human BMSC effectively improved vascularization of osteogenic grafts, it also impaired bone formation through excessive osteoclast recruitment. Recently we found that delivery of VEGF for a limited duration of 4 weeks in the form of recombinant protein covalently bound to a fibrin hydrogel prevented excessive bone resorption while ensuring increased vascularization (Burger et al. unpublished results). Here we sought to investigate the role of VEGF dose on the coupling of angiogenesis and bone formation, in order to define a VEGF therapeutic window for vascularized tissue-engineered bone.

METHODS: Recombinant VEGF was engineered with a transglutaminase substrate sequence (TG-VEGF) to allow cross-linking into fibrin hydrogels. Osteogenic constructs were prepared with primary human BMSC seeded on hydroxyapatite granules in a fibrin hydrogel containing 4 different TG-VEGF concentrations (0.1, 1, 10 and 100 µg/ml) and optimized to ensure the controlled release of the factor over 4 weeks. Control grafts were generated with BMSC only or retrovirally transduced BMSC that constitutively express VEGF linked to the cell-surface marker truncated CD8 (VICD8). Histological analysis 1, 4 and 8 weeks after ectopic subcutaneous implantation in nude mice was used to determine vascularization (CD31 immunostaining), bone formation (H&E and Masson Trichrome) and osteoclast recruitment (TRAP staining).

RESULTS: All VEGF doses effectively increased vessel density up to 5-fold already after 1 week and vascularization persisted at all later time-points. After 4 and 8 weeks, bone tissue development was enabled by 0.1 µg/ml of TG-VEGF as efficiently as with naïve BMSC alone. However, higher doses progressively impaired bone formation and 100 µg/ml caused a similar reduction as with VEGF-expressing genetically modified BMSC. The loss of bone formation correlated with increased osteoclast recruitment.

DISCUSSION & CONCLUSIONS: These data suggest that VEGF effects on promoting vascularization and bone resorption can be uncoupled by short-term delivery of recombinant VEGF protein. However, VEGF effects on bone resorption are dose-dependent and a therapeutic window exists that enables both rapid vascularization and efficient bone formation. This could provide a clinically applicable strategy with several attractive features: 1) no genetic modification; 2) homogeneous and tunable factor doses; 3) limited and controllable duration of factor delivery.

0187 glycosignatures from self-regenerating, neonatal and adult myocardial tissues are key targets for investigation for cardiac regeneration

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INTRODUCTION: Understanding heart regeneration mechanisms represents the most studied paths to meet the increasing need for a novel treatment for myocardial infarction. Our approach includes a comprehensive glycoprofiling for physiologically relevant carbohydrate structures implicated in the development of a functional contractile cardiac apparatus. We hypothesize that a consistent glycan expression across regenerative, neonatal, and adult models could reveal a distinct trend for cardiac regeneration. METHODS: Lectin histochemistry and lectin microarray analyses were performed on heart ventricle samples from zebrafish (n=30) after cryoinjury [1] and from neonatal and adult healthy rats. H&E, Masson's Trichrome, Wheat Germ Agglutinin (WGA) and *Sambucus nigra* agglutinin (SNA-I) were used in combination with immunohistochemistry for Nkx 2.5 and tropomyosin-1 markers. Labelled membrane extracts were incubated on a 48-lectin microarray and imaged using a G2505 microarray scanner (Agilent) [2]. All statistical analyses were performed using Minitab Express™. RESULTS: Sialic acid expression, specifically □□2,3)- and □2,6)-linkages were clearly expressed in the growing vasculature structures in zebrafish after the induced damage (Fig. 1A-D) and confirmed in both the neonatal and adult fully functional microvessels. A consistent glycosignature was observed across both left and right ventricles in different strains of healthy rats (Fig. 1E). A high expression of sialic acid was evident in the cell membrane of cardiomyocytes (Fig. 1F) and also in neonatal (6 days after birth) hearts.

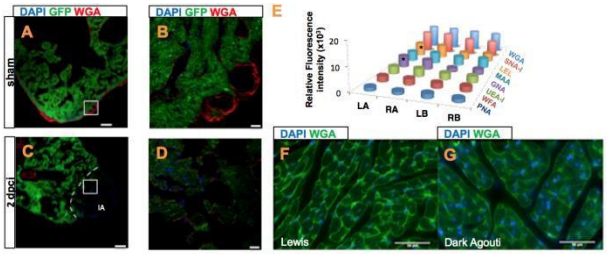

Fig. 1: Sialic acids expression in zebrafish 2 days after cryoinjury and glycoprofile of left and right ventricles across different strains of healthy rats. Confocal imaging showing WGA staining in uninjured zebrafish heart (sham: A,B) and 2 days post cryoinjury (2 dpci: C,D). In C the injured area (IA) is evidenced. A,C scale bar 100 µm. B,D scale bar 10 µm. Relevant lectins binding quantification from lectin microarrays showing glycans expression across left (L) and right (R) ventricles in two strains of healthy rats (E) and WGA staining in rat myocardial tissue (F,G). Scale bar 50 µm. n=3 t-student's test *p<0.05

DISCUSSION & CONCLUSIONS: Glycosylation expression in the self-regenerating zebrafish myocardium showed a clear pattern of development from the injured area, particularly in terms of the presence of □□2,6)-linked sialic acids in the newly forming microvasculature structures. Moreover, □□□2,3)-linked sialic acids, which are present in ion pumps, as well as *N*-acetylglucosamine residues were detected in the cardiomyocytes cell membranes and validated by lectin microarray analysis across the regenerative, neonatal and adult states, suggesting their early-stage relevant role in the preservation of healthy cardiac contractile functionality.

0188 Promoting vascularization in biomimetic microenvironmentsvia synergistic integrin/VEGF signalling

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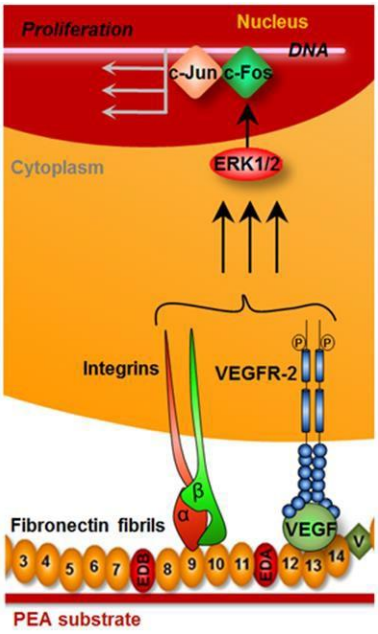
Because sufficient blood supply is essential for tissue regeneration, extensive research has been done towards stimulating vascularization. However, despite this effort it is still challenging to engineer systems that would be clinically applicable as vascularization is a complex interplay between cells and extracellular matrix dependent on strict spatiotemporal distribution of signalling molecules. We have engineered a system that stimulates vasculogenic process based on unique properties of synthetic biocompatible polymer poly (ethyl acrylate) (PEA) to spontaneously stimulate in vitro fibrillogenesis of extracellular matrix protein fibronectin (FN). It allowed us to design a very simple environment for efficient presentation of two FN domains that are crucial for cell adhesion (integrin binding site) and vascular endothelial growth factor (VEGF) binding. Cells adhere to FN-VEGF functionalized surface that allows VEGF receptor to be activated in close vicinity of an integrin, so VEGF receptor and integrin can work in synergy supporting processes such as differentiation and proliferation and thus enhance vascularization by using minimal concentrations of GFs (Fig.). Firstly we characterized the system for VEGF binding and FN domain availability using ELISA, and we also tested VEGF binding specificity by immunoreaction with gold nanoparticles that were visualized by AFM. The vasculogenic response of human endothelial cells seeded on PEA-FN-VEGF interfaces was significantly improved compared to soluble administration of VEGF at higher doses. Early onset of ERK1/2 phosphorylation iun endothelial cells was increased only when VEGF was bound to FN nanonetworks on PEA, while soluble VEGF did not influence ERK1/2 phosphorylation levels at early time point. Experiments with mutant FN molecules with impaired integrin binding site (FN-RGE) confirmed the initiation of vasculogenic response via combined integrin/VEGF signalling. In vivo experiments using 3D PEA scaffolds coated with FN and VEGF implanted in the murine fat pad demonstrated pro-vascularization signalling by enhanced formation of new tissue inside scaffold pores. We conclude that this material system promoting synergistic integrin/VEGF signalling which is highly effective for low vascularization events both in vitro and in vivo using platform for concentrations of VEGF represents a promising clinical applications in regenerative medicine.



0189 Role of HIF-2α-regulated genes and mitochondrial function in human microvascular endothelial sprouting during prolonged hypoxia in vitro

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Recently we have shown that human microvascular endothelial cell (hMVEC) sprouting is severely hampered under prolonged hypoxia, which is partly explained by induction of HIF-2α-specific target genes (Nauta et al., 2016a; Nauta et al, 2016b). Four down-stream target genes of HIF-2α; ARRDC3, MME, PPARG, and RALGPS2 directly influenced hMVEC sprouting during prolonged hypoxic culturing. Silencing of HIF-2α or the above mentioned genes with si-RNA partially restored the inhibition of endothelial sprouting pointing to a HIF-2α-dependent mechanism.

As hypoxia also interferes with the functioning of mitochondria, the potential role of mitochondria in endothelial sprouting was investigated. For that, hMVECs were cultured at 1% oxygen for 14 days which resulted in a reduced gene expression of mitochondrial enzymes (reduction of ~10-20%) and an increase in transcription of glycolysis-related genes (~50% of the genes). The exposure to prolonged hypoxia also reduced mitochondrial volume density by 40±16%, which was accompanied by a 45% lower basal respiration capacity. While a 24h exposure to hypoxia did not alter the production of mitochondrial reactive oxygen species (mitoROS), prolonged hypoxia caused a reduction of mitoROS by 59±8%. Blocking mitochondrial ATP synthase by oligomycin did not affect endothelial sprouting showing that mitochondrial ATP generation is dispensable for endothelial sprouting. However, the contribution of reduced mitoROS production during the reduced sprouting of hMVECs under prolonged hypoxia cannot be fully excluded and is now subject of investigation.

The manipulation of specific downstream targets of HIF-2α provides a new, but to be further evaluated, perspective for restoring reduced neovascularization in several pathological conditions, such as diabetic ulcers or other chronic wounds, for improvement of vascularization of implanted tissue-engineered scaffolds.

0190 Self-assembled peptide hydrogels for tissue engineering applications

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Biomaterials that provide a true 3D support network for human cells are ideal candidates for various tissue engineering and regenerative medicine applications. Of these biomaterials, hydrogels are particularly good at mimicking the extracellular matrix and provide an in vivo-like niche. The challenge is to find hydrogels that provide both biological and physical cues for the maintenance of cell phenotype and stem cell differentiation.

Self-assembling peptide hydrogels are able to self-assemble into complex supramolecular structures after exposure to the appropriate conditions or stimuli. The simplicity of the process and subsequent nano-fibrous architecture produces hydrogels that are highly suitable for the 3D culture of cells and a variety of tissue engineering and regenerative medicine applications. Fine tuning of various properties including gel stiffness allows the tailoring of gels to particular applications.

We discuss here the use of peptide hydrogels for various applications including intervertebral disc, bone and oesophagus. For all of these applications the ability of the cells to produced the correct composition of extracellular matrix when cultured within the hydrogels is key.

For intervertebral disc we have found the upregulation of nucleus pulposus specific genes, including restoration of expression after dedifferentiation, and also the deposition of key extracellular matrix proteins and proteoglycans.

For bone tissue engineering applications we have demonstrated mineralisation of human osteoblast cultures and mesenchymal stem cells when encapsulated into the hydrogels. Finally we show that peptide hydrogels are capable of supporting the activity and function of oesophageal cells leading to re-epithelialisation during in vitro culture.

These examples show some of the wide ranging applications that self-assembling peptide hydrogels are highly suitable for.

0191 Textural, macromechanical and microrheological properties of HA/collagen scaffolds and their influence on cell culture

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INTRODUCTION: In this study, we have used the cryogelation technique [1] to produce macroporous hybrid scaffolds composed of HA and collagen (Coll) using ethylene glycol diglycidyl ether (EGDE) as chemical crosslinker. We have investigated structural, macro-mechanical and local viscoelastic properties of the matrix as well as viscous properties of the pores using Multiple Particle Tracking (MPT) microrheology. Accurate characterization of HA/Coll networks is of high importance as collagen content and matrix stiffness affect cell behaviour, as we have observed for the cultivation of 3T3 fibroblast cells.

METHODS: MPT is performed in native and cell-laden scaffolds by monitoring the thermally driven motion of inert polystyrene microspheres that have been evenly distributed within the pores or the network. The resulting trajectories are then transformed into mean square displacement (MSD) traces from which local viscoelastic moduli G´ and G´´ are calculated using the generalized Stokes-Einstein relation. Textural properties, such as resilience, springiness and cohesiveness are characterized according to Texture Profile Analysis (TPA) procedure in two cycles of uniaxial compression and decompression. Young's modulus as well as fatigue behavior were characterized in cyclic compression.

RESULTS: We have shown how cryogel composition influences swelling capacity, network architecture, as well as macro and micro-mechanical properties. In particular, it has been found that the pore size (100-200 µm) allows fibroblast cells to migrate into the gel and that nutrient supply is appropriate for cell growth in the pores. Pore size seems not to be affected by an increase in Coll concentration but their shape changes from nearly round to needle-shaped. Additionally, we have observed a decrease in swelling capacity and an increase of the Young´s modulus in the hydrated state $E_{(hyd)}$ with increasing collagen content. Cyclic compression tests performed with up to 80% strain deformation and 50 cycles have shown that HA single component gel structure is more robust, compared to a HA/Coll hybrid, probably due to higher matrix flexibility. Finally, increasing the collagen content slows down in-vitro degradation of the gels. From a microrheological point of view, MPT measurements performed in the matrix have shown a slight decrease of the tracer particle motion when increasing the collagen content, indicating an increase of the local matrix strength. At the same time on the micrometer scale a much more heterogeneous structure was observed for the hybrid scaffolds as well as a slower relaxation time of the chains corresponding to a decrease of chain mobility.

DISCUSSION & CONCLUSIONS: The characterization of matrix local mechanical and heterogeneity properties is possible by means of MPT measurements and latter properties are crucial for the development of new tissue engineering constructs. We have shown, that measurements in cell laden scaffolds, that will help to understand how local matrix elasticity affects living cells, are possible. We have observed normal motility and viability of the cells in scaffolds containing tracer particles, as well as normal mobility of tracer particles.

0192 Dynamic hydrogel based on Hyaluronic Acid: from viscosupplementation material to cell scaffold applications

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Hyaluronic acid (HA) is one of the most important bio-polymers in medicine. It is a ubiquitous component of all vertebrates which is fundamental for creating a suitable environment for cells survival, proliferation, motility and differentiation. It contributes to connective tissues homeostasis and provides them firmness and mechanical properties. HA can be chemically modified to specific medical applications, some of which are already very well established and commercially successful. Chemically modified HA with specific properties are replacing applications that formerly were prerogative of unmodified HA (e.g., viscosupplementation, ophthalmic surgery medical devices), and pharmaceutical pipelines contain several combinations of HA with drugs, or use of chemically modified-HA-based scaffolds in Tissue Engineering. Carboxyl group on HA is the most suitable for chemical modification, and allows HA functionalization maintaining its biological properties. The formation of amides is particularly convenient for HA ligation through an in vivo non-labile bond. Similarly to carbodiimides, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium (DMTMM), which was initially developed for peptide synthesis, proved to be very efficient to functionalize HA polymers. Our group has developed a new generation of dynamic hydrogels based on thiolated polymers cross-linked via gold or silver ions. The resulting gold(I)-thiolates species (Au-SH) are exchanging permanently with disulfides which confers mobility to the network via perpetual rearrangement. Characteristic self-healing and frequency-dependent stiffness properties for dynamic hydrogels were observed for low molecular weight polymers. Here, we present the first example of dynamic hydrogel based on HA cross-linked via Au-SH species. First, high molecular weight HA 1.8 MDa was cross-linked with diamine compounds containing disulfide bridge using DMTMM coupling agent. The resulting hydrogel was further reduced with dithiothreitol (DTT) in order to obtain HA functionalized with thiol groups (HA-SH). Addition of gold ions to HA-SH aqueous solution resulted in the formation of a dynamic hydrogels. The rheological properties of the material could be easily changed by varying the concentration of HA-SH, the amount of Gold(I) added and pH. For example, highly cohesive material was obtained at 0.5 wt.% HA-SH cross-linked with 1 mol.% of Au(I) ions. Such material appeared to have similar properties as Synvisc,-One®, a commercial viscosupplementation material currently used to treat osteoarthritis. On the other hand, increasing the amount of HA-SH to 1 wt.% resulted in a stiffer hydrogel. Interestingly, the same hydrogel at pH > 8 appeared to be much weaker and easily injectable. This change in hydrogel stiffness was completely reversible for several cycles. Finally, mesenchymal stem cells (MSCs) were able to survive when incorporated into the dynamic. Addition of denaturalized bone powder into the 3D network allowed the cell to proliferate by increasing the bioactivity of the dynamic hydrogel. Thus, a handful of applications could be anticipated for HA-SH based dynamic hydrogel, ranging from medical device as viscosupplementation material to scaffold for tissue engineering.

0193 Stem cell therapies for intervertebral disc degeneration - targeting the degenerate microenvironment

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Aims: Low back pain (LBP) affects 80% of the population at some point in their lives with 40% of cases attributed to intervertebral disc (IVD) degeneration. An attractive treatment strategy for IVD degeneration is with the use of mesenchymal stem cells (MSCs) in combination with a biomaterial scaffold, to regenerate the matrix and restore disc height. We have previously reported the development of a synthetic Laponite® cross-linked pNIPAM-co-DMAc (NPgel) hydrogel, which was able to induce differentiation of human MSCs (hMSCs) towards the nucleus pulposus (NP) cell phenotype without the need for additional growth factors *in vitro* [1]. However, translation of this treatment strategy into a clinical application is dependent on the survival and differentiation of hMSC to the correct NP cell phenotype within the degenerate IVD. Here, we investigated the viability and differentiation of hMSCs incorporated within the NP gel, to produce an appropriate matrix within a degenerate microenvironment.

Methods: Human MSCs were encapsulated in NPgel, cultured for 4 weeks under 5%O_2 with ± calcium (2.5mM and 5.0mM $CaCl_2$), IL-1β and either individually or in combination to mimic the degenerate microenvironment. Cell viability was assessed by Alamar blue and immunohistochemistry (IHC) for caspase 3. Matrix deposition was assessed histologically using Alcian blue for proteoglycans, Masson Trichrome for collagen and Alizarin red for calcium deposition, alongside IHC for collagen type II, aggrecan and collagen type I. To determine whether MSCs displayed a catabolic phenotype under degenerate IVD conditions the expression of matrix degrading enzymes MMP13 and MMP3 was also assessed via IHC.

Results: Stem cell viability was maintained within the hydrogel systems for the 4 weeks investigated under all degenerate conditions. NP matrix markers aggrecan and collagen type II were expressed even under degenerate conditions. Interestingly MMP expression by hMSCs was upregulated by calcium but not by pro-inflammatory cytokines and TNF. Moreover, where cells were cultured under increased calcium concentrations, increased expression of collagen type I and calcium deposition was observed.

Impact: A number of hydrogel systems have shown potential to deliver MSCs and promote their differentiation towards NP like cells and thus have the potential to regenerate an NP like matrix. However to date these studies have been performed *in vitro* under non-degenerate conditions, yet it has not been shown if stem cells can proliferate and differentiate within the hostile microenvironment of the degenerated disc. Here, we have shown that stem cells can survive for up to 4 weeks in NPgel when exposed to the catabolic milieu associated with the degenerate disc *in vitro*. In addition, we have shown that stem cells continue to express the matrix proteins Aggrecan and Collagen type II associated with NP cell phenotype under the degenerate conditions. Furthermore, we have shown that by mimicking the degenerate microenvironment, the differentiation capacity of the stem cells may be altered from the phenotype observed under non-degenerate conditions. Interestingly, we have demonstrated that calcium plays a key role in MMP expression and Col I expression, and thus calcification could potentially accelerate differentiation towards a degenerate NP cell phenotype. This could have key implications in terms of successful treatment outcomes in relation to the severity of degeneration where calcification is observed in highly degenerate tissue. Interestingly however, the treatment with catabolic cytokines did not affect the expression of markers by the MSCs within NPgel.

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0194 Development of a novel bio-synthetic gelatin-poly(ethylene glycol) hydrogel for pre-vascularized osteogenically differentiated tissue analogues with enhanced structural stability

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Introduction: Matrigel remains an often used culture matrix for engineering biologically complex tissue analogues. This phenomenon highlights the need for new, alternative matrices that do not have the limitations of this mouse sarcoma-derived matrix. A major challenge in soft hydrogel development is presented by a conflictive need of low polymer concentrations for maximal cell movement, while having structurally stable hydrogels. Especially over long-term culture periods, soft biological hydrogels degrade or contract because of intensive cell-matrix interactions. In this study, we introduce and characterize a novel gelatin-poly(ethylene glycol) (PEG) hydrogel, which allows for pre-vascularization of osteogenically committed tissue analogues while providing excellent physico-chemical and structural properties. **Material and Methods:** As previously described, 8-arm PEG was functionalized with the specific glutamate (Gln) acceptor sequence NQEQVSPL [1]. Gelatin, which natively contains lysines was used as substrate. Gelatin and PEG-Gln were mixed in various ratios to establish the optimal crosslinking window by mass loss and swelling studies. Mutual cell-material interactions were studied by encapsulating human multipotent mesenchymal stromal cells (MSCs) and human endothelial colony forming cells (ECFCs). In whole mount constructs, capillary-like structures were identified by CD31 immunohistochemical staining, network stabilizing cells by α-smooth muscle actin (αSMA) and early osteogenesis by expression of alkaline phosphatase (ALP). Green fluorescent protein (GFP)-labelled ECFCs were used to track network formation over culture time. **Results and Discussion:** Gelatin-PEG-Gln hydrogels were successfully optimized towards high crosslinking efficiency for low polymer concentrations. The gels retained their original moulded shape after 20 days of MSC-ECFC co-culture (Fig.1A). After 6 days, ECFCs showed extensive pre-vascular structure formation (Fig.1B), which were stabilized after 10 days by αSMA expressing supporting cells (Fig.1D). Osteogenic differentiation was observed in the same hydrogels that were positive for ALP activity (Fig.1E).

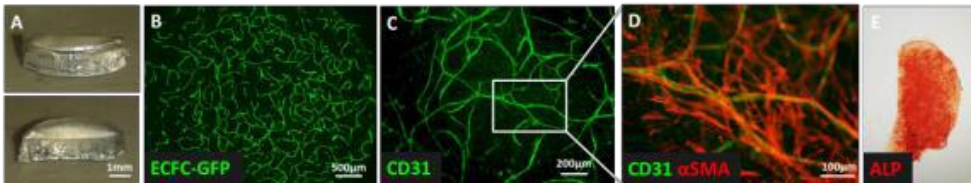

Figure2: MSC-ECFC co-cultures in gelatin-PEG-Gln hydrogels. (A) Hydrogels retained their moulded shape after 20 days of co-culture. After 6 days, ECFCs formed networks (B). After 10 days of co-culture, ECFCs formed highly connected capillary-like structures (C), stabilized by pericyte-like cells (D). In these hydrogels, also high ALP expression was observed after 10 days of MSC-ECFC co-culture (E). **Conclusion:** A 3D culture matrix that allows high cell bioactivity while retaining hydrogel shape-stability over a long culture period was developed**.** This hydrogel enabled differentiation of cells into the vasculogenic, osteogenic and pericyte-like lineage**.**

0195 Allyl glycidyl ether modified gelatin as a versatile bioink platform for biofabrication of tissue engineered constructs

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Biofabrication is a rapidly growing field of research that concerns the automated generation of three dimensional (3D) cell-material hybrid constructs with stratified tissue organization. One of the main drawbacks currently is the lack of suitable materials that can be bioprinted together with cells. A standard system is gelatin methacryloyl (GelMA), where mechanical stability of the printed construct is achieved through free radical polymerization directly after printing.

The aim of this study was to present an alternative modification of gelatin, enabling hydrogel formation based on thiol-ene click chemistry. This dimerization reaction allows for a more controlled network formation and prevents the formation of non-degradable polymers. Therefore, allyl glycidyl ether modified gelatin (GelAGE) was synthesized to fabricate hydrogels in combination with dithiothreitol and further characterized with respect to their mechanical and physico-chemical properties. The versatility of this system for biofabrication purposes was validated via stereolithograpy (SLA) and 3D Bioplotting.

The modification of gelatin was performed under alkaline conditions in the presence of allyl glycidyl ether to obtain hydrogel precursors with varying degress of functionalization. The effect on degradation and the molecular weight distribution of the synthesis parameters of gelatin was assessed by GPC analysis. Moreover we demonstrated the excellent cell compatibility (>80 %) of this system over a wide allyl:SH ratio together with different photoinitiators (based on UV or visible light). Mechanical properties were superior (139 ± 22 kPa) to comparable GelMA hydrogels (42 ± 6 kPa). Different GelAGE precursors were used to obtain constructs via SLA (Fig.1 A) and 3D Bioplotting (Fig.1 B, C), including the plotting of live cells (Fig 1D).

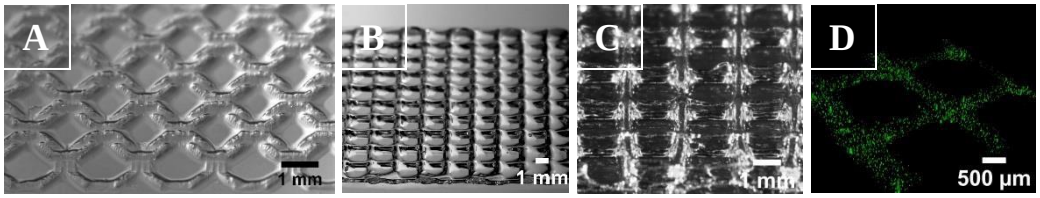

Figure 1. Images from constructs obtained by stereolithography of 10 wt.-% GelAGE (A) and bioplotted constructs of 30 wt.-% GelAGE (B, 4 layer and C, 8 layer). Representative live/dead confocal microscope image of plotted porcine chondrocytes after day 23 *in vitro* culture (D).

An alternative modification of gelatin to commonly used GelMA bioinks was demonstrated and their applicability as a platform for different biofabrication techniques could be demonstrated.

0196 New rAAV-based engineering approaches for cartilage regenerative medicine

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Articular cartilage has a limited ability for self-healing. Cartilage lesions resulting from trauma or in osteoarthritis can not restore an original hyaline structure and mechanical integrity despite various clinical treatments. Tissue engineering and gene therapy approaches have long been employed in experimental and translational cartilage regenerative medicine. While advances have been made with these procedures, little is known on the possibility of combining them to enhance cartilage repair. The effective and safe recombinant adeno-associated viral (rAAV) vectors have emerged as best adapted systems for human gene therapy. Combining tissue engineering to rAAV gene transfer is an attractive strategy to address the remaining obstacles linked to the clinical use of these vectors for cartilage regeneration.

0197 Nonviral gene transfer for cartilage regeneration

GUN-IL IM

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Gene transfer has been used experimentally to promote chondrogenesis and cartilage regeneration. While it is controversial to apply gene therapy for nonlethal conditions such as cartilage defect, there is a possibility that the transfer of therapeutic transgenes may dramatically increase the effectiveness of cell therapy and reduce the quantity of cells that are needed to regenerate cartilage. Single or combination of growth factors and transcription factors has been transferred to mesenchymal stem cells or articular chondrocytes using both nonviral and viral approaches. The current challenge for the clinical applications of genetically modified cells is ensuring the safety of gene therapy while guaranteeing effectiveness. Viral gene delivery methods have been mainstays currently with enhanced safety features being recently refined. On the other hand, efficiency has been greatly improved in nonviral delivery. Our group has been using nonviral gene transfer of SOX trio genes to enhance chondrogenesis from stem cells. In this talk, general overview and recent update on nonviral gene transfer to enhance cartilage regeneration will be introduced as well as the author's own data and experience.

0198 Recombinant adeno associated virus mediated gene transfer and cooverexpression of transforming growth factor beta and sox9 promote the metabolic and chondrogenic activities in human bone marrow aspirates

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INTRODUCTION: Transplantation of genetically modified bone marrow concentrates is an attractive approach to conveniently activate the chondrogenic differentiation processes as a means to improve the intrinsic repair capacities of damaged articular cartilage [1]. **METHODS**: Here, we examined the potential benefits of co-overexpressing the pleiotropic transformation growth factor beta (TGF-beta) and the cartilage-specific transcription factor *sox9* using recombinant adeno-associated virus (rAAV) vectors upon the chondroreparative processes in human bone marrow aspirates. Aspirates were aliquoted in 96-well plates (100 µl/well) and immediately transduced with the rAAV vectors (rAAV-*lacZ*: 20 or 40 µl) or co-transduced (rAAV-hTGF-β /rAAV-FLAG-h*sox9*: 10 or 20 µl each vector) with each aliquot (8 x 10^5 functional recombinant viral particles, MOI = 10 ± 3). Transgenic expression, cell proliferation, biosynthesis, chondrogenesis and hypertrophy were all evaluated. **RESULTS**: Successful TGF-β/*sox9* combined gene transfer and overexpression via rAAV was achieved in fresh, chondrogenically induced human bone marrow aspirates for up to 21 days, the longest time point measured, leading to increased proliferation, matrix synthesis, and chondrogenic differentiation relative to control treatments (reporter *lacZ* treatment, absence of vector application), especially when co-applying the candidate vectors at the highest vector doses tested. Optimal co-administration of TGF-β with sox9 also advantageously reduced hypertrophic differentiation in the aspirates. **DISCUSSION and CONCLUSION**: Our results first indicate that combined TGF-β/*sox9* gene transfer (especially at the highest vector doses employed) allowed for the sustained expression of SOX9 as previously noted with single rAAV-FLAG-h*sox9* transduction and to a durable production of TGF-β relative to the control conditions, in the range of those achieved when providing rAAV-hTGF-β alone [1]. The present data further show that prolonged, effective co-overexpression of TGF-β and *sox9* led to increased levels of cell proliferation, matrix biosynthesis, and chondrogenic differentiation in the aspirates over time (at least 21 days). Equally important, combined TGF-β /*sox9* transduction advantageously delayed premature hypertrophic differentiation in the aspirates versus control treatments, again in good agreement with the known anti-hypertrophic activities of SOX9 that may counterbalance possible hypertrophic effects of TGF-β [1-4]. These findings report the possibility of directly modifying bone marrow aspirates by combined therapeutic gene transfer as a potent and convenient future approach to improve the repair of articular cartilage lesions. **ACKNOWLEDGEMENTS**: This research was funded by grants from the National Natural Science Foundation of China (NSFC, No. 81672183 and 81541134), the Research and Development funds of Peking University People's Hospital (No. RDB2015-10 and RDY2016-15), the Osteoarthritis Research Society International (OARSI 2015 Scholarship Award), Beijing Joint Care Foundation (2016 Young Investigators Scholarship), and the German Osteoarthritis Foundation (*Deutsche Arthrose-Hilfe e.V.*).

0199 RAAV-MEDIATED MODIFICATION OF HUMAN ARTICULAR CHONDROCYTES VIA DELIVERY IN POLYMERIC PEO-PPO-PEO MICELLES TO OVEREXPRESS TGF-B

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INTRODUCTION: Recombinant adeno-associated viral (rAAV) vectors provide attractive tools for the durable treatment of human osteoarthritis (OA), a gradual, irreversible joint disease. Still, the adapted use of these vectors *in vivo* is impaired by some physiological barriers including the presence of neutralizing antibodies against viral capsid elements in a majority of patients [1,2]. Delivery of rAAV via poly(ethylene oxide) (PEO) and poly(propylene oxide) (PEO-PPO-PEO) polymeric micelles is a novel, effective approach to overcome such hurdles, leading to a high and sustained expression of marker genes (E. coli *lacZ*) [3,4]. Here, we tested the feasibility of targeting human OA chondrocytes (hOACs) via rAAV using PEO-PPO-PEO polymeric micelles to overexpress the potent chondrogenic factor TGF-β. **METHODS:** rAAV-hTGF-β, carrying a 1.2-kb human transforming growth factor beta 1 (hTGF-β) cDNA controlled by the CMV-IE promoter/enhancer was packaged, purified, and titrated as previously described [5]. Poloxamer (Pluronic®) PF68 and Poloxamine (Tetronic®) 908 (BASF) were prepared in sucrose 10% at 4ºC and mixed with rAAV (2% v/v final copolymer concentration) [3,4]. hOACs (passage 3; 3,000 cells/well in 96-well plates) were isolated from unprocessed human OA cartilage, incubated with the rAAV/PF68 or rAAV/T908 mixtures (20 or 40 µl), and maintained for up to 10 days at 37ºC. Control conditions included similar amounts of rAAV in sucrose 10% (positive control) or sucrose 10% vehicle alone (negative control). Transgene (TGF-β) expression was monitored by TGF-β ELISA and immunohistochemical analysis. Cell proliferation was quantified using the Cell Proliferation Reagent WST-1 and cytotoxic events were evaluated via the Cytotoxicity Detection kit (LDH). The proteogycan contents were monitored by alcian blue staining and spectrophotometrically estimated after solubilization with 6 M guanidine hydrochloride [4]. Each condition was performed in duplicate in two independent experiments. The t-test and one-way ANOVA were applied to evaluate differences between groups with $P \leq 0.05$ considered significant. **RESULTS:** An evaluation of the TFG-β expression levels by ELISA revealed the effective rAAV-hTFG-β1-mediated gene transfer especially when the vector was provided via PF68 and T908 polymeric micelles compared with free vector treatment (up to 1.6-fold increase; $P \leq 0.04$). The same tendency was observed by immunodetection of TGF-β (up to 1.6-fold increase; $P \leq 0.15$), exhibiting also a vector-dose dependent effect especially after 10 days of treatment (up to 1.7-fold difference between 10 and 20 µl, $P \leq 0.01$). Overexpression of TGF-β resulted in an increase in proliferative activities of hOACs especially upon delivery of rAAV via polymeric micelles (up to 1.8-fold difference, $P \leq 0.002$ compared with the negative control). No cytotoxic events were noted in any condition tested, with viability always above 90% ($P \geq 0.05$). Higher amounts of proteoglycans were always detected upon delivery of rAAV-hTGF-β via polymeric micelles (up to 1.5-fold difference, $P \leq 0.002$ compared with the negative control). **CONCLUSIONS**: Delivery of rAAV via PEO-PPO-PEO polymeric micelles allows for the safe, effective, and prolonged overexpression of the chondrogenic factor TGF-β in hOACs. Administration of therapeutic rAAV via PEO-PPO-PEO copolymers may be a powerful strategy to increase the residence time of the vectors at a target site for improved cartilage regenerative processes. **ACKNOWLEDGMENTS**: Supported by grants from Deutsche Forschungsgemeinschaft (DFG RE 328/2-1 to ARR, HM, MC) and MINECO (SAF2014-52632-R and MAT2013-40971-R; Spain).

0200 Gene-activated scaffolds for cartilage defect repair can enhance cartilage formation while simultaneously inhibiting hypertrophy

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INTRODUCTION: Tissue engineered scaffolds developed within our lab (1) have shown much success in healing cartilage defects in both goats and horses when combined with micro-fracture treatment to encourage bone marrow mesenchymal stem cell (MSC) infiltration (2,3). To further accelerate the repair process and to ensure controlled differentiation of MSCs, localised short-term genetic modification of the cells *in vivo* may enhance their regenerative capacity and provide a novel treatment to ensure stable hyaline cartilage formation. Gene-activated scaffolds have been optimised within our lab to allow for controlled, sustained release of reporter transgene *in vitro* (4). The aims of this study were firstly to investigate potential gene candidates which effectively induce MSC-mediated cartilage formation while simultaneously inhibiting hypertrophy, and secondly, to incorporate these genes into scaffolds to establish an optimal gene-activated scaffold for cartilage regeneration.

MATERIALS AND METHODS: Genes encoding a range of proteins/transcription factors known to be involved in chondrogenesis were delivered to bone marrow-derived MSCs using chitosan nanoparticles (NPs) (4) and expression of the downstream proteins was monitored using ELISA and qRT-PCR. MSCs in pellet culture were then transfected with each gene or combination of genes to investigate if over-expression of these genes resulted in increased chondrogenesis as determined by histology and sulfated glycosaminoglycan (sGAG) assay. The chitosan-DNA NPs were then incorporated into collagen hyaluronic acid scaffolds (5) and the chondro-inductive ability of the gene-activated scaffold was assessed by quantifying sGAG and histology after 14 and 28 days in culture. The ability of the scaffolds to inhibit hypertrophy was assessed using immunohistochemistry for collagen type II and type X expression and qRT-PCR was used to assess the expression of aggrecan and/or VEGF.

RESULTS: The chitosan NPs were capable of efficiently delivering therapeutic genes to MSCs, up-regulating encoded growth factor expression when compared to controls ($p<0.001$). Transfected MSCs cultured in pellets for 21 days produced significantly more sGAG than untransfected controls ($p<0.01$) with the triple combination of Sox 5, 6 and 9 being the most efficacious ($p<0.001$), inducing MSCs to produce 8 µg of sGAG per pellet *versus* 2 µg in untransfected cells. Gene-activated scaffolds positively induced chondrogenesis in MSCs after 14 and 28 days in culture, with cells producing significantly more sGAG than untransfected controls ($p<0.001$). Again the Sox trio combination of genes induced the MSCs to produce approximately 70 µg of sGAG, significantly higher than all of the other groups ($p<0.01$) Furthermore, collagen type X and VEGF expression levels were low in the Sox trio-activated scaffolds after 28 days in culture, while collagen type II and aggrecan expression was up-regulated, indicating that the MSCs were forming stable articular cartilage and not undergoing endochondral ossification. Conversely, a TGF-β3-activated scaffold stained positively for collagen X, in line with published results which state that TGF-β3 supplemented media can induce chondrocyte hypertrophy in long-term culture (6).

DISCUSSION: The combinatorial gene-activated scaffold system developed in this study resulted in more cartilage-like tissue formation than previous studies delivering recombinant proteins, without the adverse side effects. Chondrogenic differentiation of MSCs was significantly enhanced following transfection with the Sox trio, not only over the other groups, but this result also represents a 50% increase in sGAG production when compared to scaffolds that were soak-loaded with TGFβ3 recombinant protein, the most commonly used protein to stimulate MSC chondrogenesis (7). Unlike TGF-β3 however, the Sox trio-activated scaffold did not induce hypertrophy. These cell free gene-activated scaffolds may offer a novel way to control bone marrow derived MSC differentiation and subsequent stable hyaline cartilage formation when used in conjunction with micro-fracture treatment. This is currently being tested *in vivo* with a viewto generating 'off-the-shelf' product for repair of chondral defects.

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0201 Modulation of Runx2 and pRb during in vitro chondrogenesis and osteogenesis of human mesenchymal stromal cells

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Introduction In the field of tissue engineering for musculoskeletal disorders mesenchymal stromal cells (MSCs) are an interesting research focus for their ability, under specific *in vitro* culture conditions, to generate chondrocytes and osteoblasts. However, the molecular mechanisms behind the fate choice of MSCs are still not completely revealed. Here, we studied the effect of the modulation of Runx2 and pRb during *in vitro* chondrogenesis and osteogenesis of MSCs.

Methods Adenoviral vectors (Ad) were generated to overexpress Runx2 or Rb1 and to knockdown Runx2. Human bone marrow-derived (hBM-MSCs) were transduced with a single hAd vector (Runx2 overexpression, Rb1 overexpression, Runx2 knockdown) or with a combination of two hAd vectors (Runx2+Rb1 overexpression and Rb1 overexpression+Runx2 knockdown). The Ad-transduced hBM-MSCs were plated for standard chondrogenesis and osteogenesis differentiation assays. Safranin O-Fast green staining was used to confirm proteoglycan deposition within chondrogenic pellet cultures, while the osteogenic potency of the hBM-MSCs was assessed by ALP and Alizarin red staining. Quantitative RT-PCR for Runx2 and Rb1 was performed at day 0, 14, 21 and 28 for both Runx2 and Rb1 and for genes related to chondrogenic and osteogenic differentiation of MSCs.

Results When Runx2 was overexpressed or knocked down, or when Rb1 was overexpressed, the proteoglycan content of pellet cultures under chondro-permissive culture conditions was reduced. Conversely, the proteoglycan content increased compared control when Rb1 was overexpressed under basal culture conditions. When Rb1 overexpression was combined with either Runx2 overexpression or knockdown then the positive effect on proteoglycan content in basal conditions was abolished.

Rb1 overexpression was also able to increase the ALP activity of cells in both basal and osteogenic medium. This effect was not affected by the concomitant overexpression of Runx2 but the overexpression of Rb1 was not sufficient to overcome the reduction of ALP activity due to knockdown of Runx2. Similarly, mineralised matrix production by hBM-MSCs was enhanced by the overexpression of Rb1 under basal culture conditions but this was not sufficient to rescue the reduction in mineral deposition caused by Runx2 knockdown. The overexpression of both Runx2 and Rb1 further enhanced mineralisation in both basal and osteogenic conditions.

Conclusions The requirement of Runx2 during both chondrogenic and osteogenic differentiation of MSCs is demonstrated, together with the potential of pRb to support MSC differentiation in the absence of *in vitro* osteogenic and chondrogenic clues. This provides new insights for understanding the molecular mechanisms orchestrating the fate choice of MSCs *in vitro*.

0202 The role of tissue-specific stem/progenitor cells in tissue engineeringand regenerative medicine

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it is now beginning to be recognized that several post-natal tissues contain “tissue-specific stem/progenitor cells” (TS/PCs, often loosely called “mesenchymal stem cells”). While TS/PCs of different origins have similar cell surface characteristics due to their fibroblastic nature, they have distinctive transcriptomes, and differentiation capacities based on rigorous assays. As an example, skeletal stem cells (SSCs), a subset of cells within the bone marrow stromal cell (BMSC) population, have the ability to reform cartilage, bone, hematopoiesis-supportive stroma (a defining feature) and marrow adipocytes upon in vivo transplantation with an appropriate scaffold, but cannot spontaneously form myotubes. On the other hand, TS/PCs with the same cell surface phenotype from muscle spontaneously form myotubes, but cannot form bone. Although more tissues need to be investigated, it appears that TS/PCs are pericytes in the post-natal organism (cells on the abluminal surface of blood vessels that provide stability). Their location may be the result of a common developmental process whereby embryonic blood vessels growing into developing tissues incorporate committed cells in their path as pericytes, where they remain quiescent until liberated from the blood vessel wall in response to injury or the need for tissue turnover. TS/PCs faithfully recreate the tissue from which they are isolated and represent valuable tools not only for use in tissue engineering, but also for studying tissue dynamics in health and disease.

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0203 Bioactive coating system with self-antimicrobial and immunomodulatory properties

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All implantable biomedical systems face several risks once in contact with the host tissue : i) excessive immune response to the implant (1); ii) development of bacterial biofilms and iii) yeast and fungi infections. A multifunctional surface coating which can address all these issues concomitantly would significantly improve clinical outcomes. We develop here for the first time a coating that address these three issues simultaneously. We hypothesized that polyarginine (PAR), a synthetic highly cationic polypeptide, can act on macrophages to control innate immune response because arginine is an important component of macrophage metabolism. Moreover, PAR is susceptible to act as an antimicrobial agent due to its positive charges. We developed a new polyelectrolyte multilayer films (2) based on PAR and hyaluronic acid (HA). The PAR/HA films have a strong inhibitory effect on the production of inflammatory cytokines released by human primary macrophages subpopulations (3). This could reduce potential chronic inflammatory reaction following implantation. Next, we show that PAR/HA films were very effective against S. aureus for 24h (4). The PAR/HA films can be easily further functionalized by embedding antimicrobial peptides, like catestatin (CAT), a natural host defense peptide. This PAR/HA+CAT film proved to be effective as an antimicrobial coating against yeast and fungi. The cytocompatibility of the PAR/HA films was assessed with human umbilical vein endothelial cells (HUVECs). This all-in-one system that limits strong inflammation and prevent pathogen's infections constitutes an original strategy to coat implants in an active way.

0204 Engineering immunomodulatory niches

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Antigen presenting cells (APCs) such as macrophages and dendritic cells (DCs) are sentinels of the immune system that act as a bridge between the innate and adaptive immune systems and play a central role in orchestrating immune responses against foreign materials. The activation status of APCs determines the outcome of immune responses following implantation of biomaterials, towards either inflammation or healing. A large number of biomaterials are used in the fabrication of implantable devices and drug delivery systems. Typically immune responses against foreign materials lead to chronic inflammation and fibrosis resulting in either destruction or segregation of foreign bodies. Physico-chemical properties of materials have a critical role in initiating pro- or anti-inflammatory immune responses. Thus, the ability to control biomaterials surface attributes provides a powerful tool for modulating the phenotype and function of immune cells with the aim of reducing detrimental pro-inflammatory responses and promoting beneficial healing responses. Here we will discuss how different components of the immune system respond to foreign materials and how, through engineering surfaces with immune-modulatory properties, it may be possible to harness the immune system in order to reduce inflammation and promote healing.

0205 Programing of anti-inlfammatory macrophages for implants and regenerative medicine using self-standing release systems with a phenotype-fixing cytokine cocktail formulation

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Nondegradable metallic and polymeric implants are used as mainstream solutions for the regenerative medicine. After implant installation pro-inflammatory macrophages induce acute reactions to trauma and foreign material, that has to be followed by the resolution of inflammation and healing phase and restoration of homeostasis. However, chronic inflammation is the most common complication leading to implant intolerance and failure. Macrophages are highly plastic cells that define the reactions of the immediate tissue microenvironment on the implanted material. Frustrated phagocytosis and well as secretion of mixed pro-inflammatory and pro-fibrotic cytokines are key macrophages-mediated processes that lead to the chronic inflammation around implants. The prevention of implant-induced chronic inflammation by programming of macrophage phenotype is a promising strategy to improve implant acceptance. The aims of such programming include controlling the phenotype of local macrophages by long-term fixation of their healing activities and suppression of inflammatory reactions. Herein, we describe the development of a cytokine cocktail formula (M2Ct) that induces stable M2-type phenotype in hum primary macrophages characterized by the significant suppression of induced pro-inflammatory reactions and increased secretion of anti-inflammatory cytokines (M2Ct). The positive effect of the M2Ct was demonstrated in an *in vitro* wound healing model. Using model for induction of inflammation by LPS we demonstrated that the M2Ct phenotype is stable for at least 12 days. However, in the absence of M2Ct components in the medium macrophages underwent rapid pro-inflammatory re-programming upon IFNg stimulation. In order to overcome the plasticity of macrophages and stabilize their phenotype, for direct application of this cocktail on implants and in tissue engineering in vivo; the loading and release of the cytokine cocktail from a self-standing, transferable Gelatin/Tyraminated Hyaluronic acid based release system was developed. The cytokine cocktail demonstrated its anti-inflammatory activity in controlled release conditions. Our data suggest that the direct application of a potent M2 inducing cytokine cocktail in a transferable release system can significantly improve the long term acceptance of implanted materials, and can be further developed to improve functionality of biomedical devices such as electrodes, in vivo biosensors, engineered organ and tissue substitutes and implants.

0206 Mapping macrophage phenotypes in an experimental osteoarthritis model to eventually improve joint tissue regeneration

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Purpose Macrophages have been shown be able to influence cartilage degeneration and regeneration *in vitro*. They can acquire various phenotypes that may range from pro-inflammatory (M1) to anti-inflammatory or repair (M2), each with their own effect on cartilage. One of the challenges when aiming at cartilage regeneration *in vivo*, is that the regenerative process will likely has to occur in an inflamed or osteoarthritic environment. Due to their plasticity, macrophages residing in the synovial lining of joints are interesting targets, since modulating their phenotype may benefit tissue regeneration. The aim of this study was to map the presence of macrophage phenotypes in an OA mouse model as this may provide valuable information for interventions aiming at joint tissue regeneration.

Methods OA was induced in the right knees of male C57BL/6 mice using the collagenase induced-osteoarthritis model (CIOA; single 10U collagenase injection). The left knees received saline and served as controls. The mice were euthanized 1, 3, 7, 14, 28 or 56 days after induction of OA (n=9 per time point/model). The knees were stained with thionin to assess cartilage damage, presence of osteophytes and synovial thickness. To assess various macrophage phenotypes, iNOS was used as a marker for the presence of pro-inflammatory macrophages, CD206 as marker for tissue repair macrophages, and CD163 as marker for anti-inflammatory macrophages. Associations between macrophage markers were assessed by Spearman rho correlation tests.

Results Induction of OA was confirmed by the presence of cartilage damage, synovial thickening and osteophytes. Cartilage damage was significantly increased in the CIOA knees than in the control knees and progressed from day 14 onwards. Osteophytes started to appear from day 3. Synovial thickening was seen at day 3, 7 and 14 after OA induction, and the thickness of the synovium reduced from day 14 onwards. The pro-inflammatory macrophages, as indicated by the presence of iNOS, were significantly more present from day 3 than in the controls, and remained elevated till day 28. The tissue macrophages, as indicated by CD206, were elevated from day 3 to 28 than at day 1, yet not significantly different than the controls throughout the experiment. The anti-inflammatory macrophages, as indicated by the presence of CD163, were already abundantly present at day 1 and were significantly reduced by day 14 and onwards. The presence of $iNOS^+$ and $CD163^+$ cells in the CIOA knees had a strong positive association with each other (ρ=0.709; p<0.001), while $CD163^+$ cells did not associate with $CD206^+$ cells.

Conclusion Macrophage-mediated inflammation negatively affects regeneration of joint tissues. By mapping the macrophage phenotypes in an OA knee joint over time, we have gained knowledge that may benefit new strategies that are focussing on macrophage phenotype modulation and aiming to improve joint tissue regeneration.

0207 Mesenchymal stem/stromal cells immunomodulatory role through a paracrine mechanism in degenerated IVD

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Low back pain (LBP) is one of the highest causes of disability worldwide, most frequently associated with intervertebral disc (IVD) degeneration, and to which mesenchymal stem/stromal cells (MSCs)-based therapies are emerging as promisor alternative treatments. IVD degeneration is often associated with an inflammatory process, characterized by increasing levels of inflammatory mediators, as IL-1β and TNF-α, that is often disregarded in multiple studies and in the end, might challenge the success of MSCs-based therapies for LBP. Here we investigated the effect of the pro-inflammatory/degenerative IVD environment on the regenerative and immunomodulatory potential of human bone marrow (BM)-derived MSCs, using an *ex vivo* model from bovine origin.

Bovine IVD organ cultures of nucleus pulposus (NP) punches were stimulated with needle-puncture and culture medium supplementation with 10 ng/mL IL-1β to induce a pro-inflammatory/degenerative environment. MSCs were posteriorly co-cultured on the top of transwells, placed above NP punches. The effect of MSCs in IVD was analyzed by screening of: 1) cell viability and MSCs migration; 2) the ECM remodeling of IVD organ cultures; 3) MSCs response to the pro-inflammatory environment and 3) gene expression profile NP cells after IVD co-culture with MSCs. Analysis were performed after 48h and 2 weeks of MSCs/IVD co-culture.

The pro-inflammatory/degenerative IVD conditions did not affect MSCs viability, but promoted cell migration, although very few MSCs were found in the IVD tissue. The pro-inflammatory/degenerative IVD conditions promoted IL-6, IL-8, MCP-1, TIMP-2, IL-4 and PGE2 production by MSCs, while reducing TGF-β1. Moreover, the presence of MSCs did not stimulated ECM production (namely type II collagen and aggrecan) in neither basal nor inflammatory conditions, but down-regulated bovine pro-inflammatory gene expression levels (*IL-6*, *IL-8*, *TNF-α*) in IL-1β-stimulated IVDs.

Overall this study shows an immunomodulatory effect of MSCs in degenerated IVD and suggests that this is based on a cytokine feedback loop.

0208 Foreign body on a chip to study implant host immune responses

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Understanding and modulating foreign body responses represent a critical challenge for implants due to their intrinsic immunogenicity in contact with biological tissues. However, studying these responses can be further complicated by the conventional in vivo models, which do not readily allow for convenient monitoring of foreign body interactions with the microenvironment. The oversimplified planar cultures of immune cells in vitro, on the other hand, usually fail to reproduce the complexity involved in the innate immune system featuring multi-scale, staged responses. Here we aimed to developed a biomimetic microfluidic platform termed foreign body on a chip with built-in complexity in modelling the cascade of events during foreign body responses to metal implants. Specifically, a three-layer bioreactor was designed and fabricated, where the bottom chamber contained tissue-like matrices of crosslinked gelatin methacryloyl (GelMA) surrounding an assembly of titanium microbeads, a top vascular chamber constantly perfused by a flow of suspended monocytes, and a porous membrane seeded with a confluent layer of endothelial cells in between the two chamber functioning as the vascular barrier. This setup precisely models the implant microenvironment in the human body where the implants are interfaced directly with surrounding tissues as well as blood vessels containing vascular cells on the walls and immune cells within the blood flow. Indeed, it was observed that under suitable flow conditions and upon releasing of cytokines such as monocyte chemotactic protein 1 (MCP-1) from the GelMA in the tissue chamber mimicking inflammation, the circulating monocytes could become activated and actively transmigrate through the endothelial barrier to reach the tissue chamber to further differentiate into the M2 pro-inflammatory phenotype. Our prototype foreign body on a chip platform provides a novel strategy to study foreign body responses on implants in a biomimetic manner, which may be expanded to a variety of scenarios where the interactions of immune cells with non-native substances/materials require to be investigated.

0209 Designing materials for regenerative medicine

Molly Stevens

Imperial College London, London, UK

An important aim of regenerative medicine is to restore tissue function with implantable, laboratory-grown constructs that contain tissue-specific cells that replicate the function of their counterparts in the healthy native tissue (1,2). In this talk I will describe our recent work in the development of implantable scaffolds and in the development of state of the art technologies for monitoring and elucidating the cell-material interface (3,4,5).

0210 Bio-instructive materials discovery

Morgan Alexander

The University of Nottingham, Notts, UK

The range of biomaterials found in the clinic today are dominated by materials chosen on the basis of their availability and mechanical properties rather than positive interactions with surrounding cells and tissues. It would be desirable to design our way forward from this situation to new biomaterials. Unfortunately our understanding of the bio-interface is poor, with only isolated cases where a good understanding of cell-material interactions can be cited, and fewer still where material-tissue interactions are well characterised and understood. This paucity of information on the mechanism of biomaterials interactions with the body acts as a roadblock to rational design. Consequently we have taken a high throughput screening approach to discover new bio-instructive materials from large chemical libraries- this approach can be described as engineering serendipitous discovery.[1] These new candidate biomaterials provide a starting point for development of new medical devices and provide opportunities to study their mechanism of action to provide new information to tackle the rational design roadblock.

This screening approach has been used to identify bio-instructive materials in the discovery of polymers with application in expansion of pluripotent human embryonic stem cells and the identification of substrates on which to mature cardiomyocytes.[2,3] Other screening campaigns using macrophage differentiation have identified bio-instructive materials with pro- and anti-inflammatory characteristics with great potential in modulating the human immune system in novel therapies and devices.[4] Materials resisting bacterial attachment and biofilm have also been identified and will be presented, with early data on the investigation of their mechanism of biofilm formation resistance.[5] Work to integrate and expand this range of bio-instructive materials will be previewed, including movement to 3D screening.

0211 Injectable and tunable hydrogels enhance stem cell retention and improve diabetic wound healing

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Stem cells have shown substantial promise for various injuries and diseases in preclinical and clinical trials. However low cell engraftment rates significantly limit the clinical translation of stem cell therapeutics. Numerous injectable hydrogels have been developed to enhance cell retention. Yet the design of an ideal material with tunable properties which can mimic different tissue niches and regulate stem cell behaviors remains an unfulfilled promise.

Here, an injectable PEG-Gelatin hydrogel was designed with highly tunable properties, from a multifunctional hyperbranched polyPEGDA polymer and a commercially available thiolated gelatin. Due to a high ratio (around 50 mol%) of the pendant acrylate functional groups on the polymer chains, spontaneously gelation occurs within about 2 minutes under the physiological condition. Murine and human adipose-derived stem cells (ASCs) were encapsulated into the hydrogel, which supported the ASCs growth, maintained their stemness and enhanced regenerative cytokine secretion. The hydrogel mechanical properties, bio-degradability and cellular responses can be finely controlled by changing hydrogel formulation. Animal study showed this *in-situ* formed hydrogel significantly improved cell retention *in vivo*, enhanced angiogenesis and accelerated wound closure using a diabetic excisional wound healing murine model.

These data suggest that injectable PEG-Gelatin hydrogel can be used for regulating stem cell fate and behaviors in 3D culture, delivering cells for wound healing and other tissue regeneration applications.

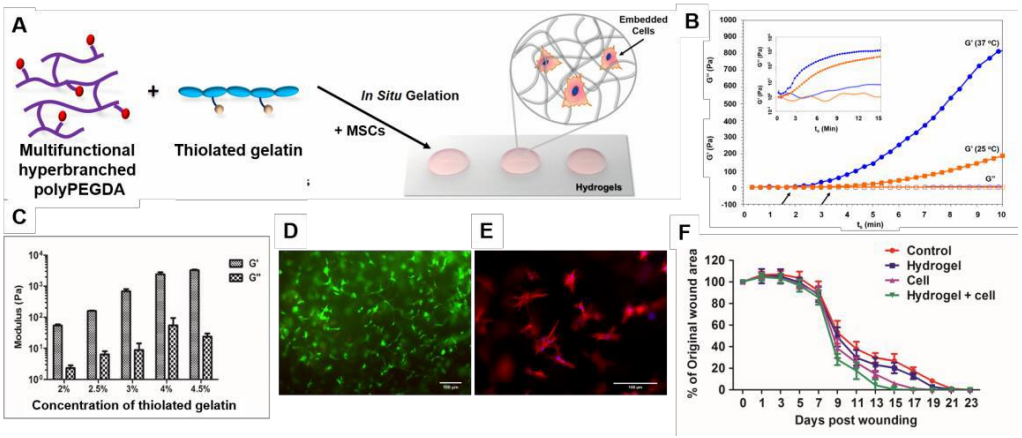

Figure: A) Schematic representation of the injectable PEG-Gelatin hydrogels. **B)** Real time cross-linking rheological measurements. **C)** Tunable mechanical properties of the hydrogels. **D)** LIVE/DEAD® Staining of encapsulated hASCs after 14-days culturing in the hydrogels. **E)** Cellular morphology of encapsulated hASCs in the hydrogel. **F)** Wound closure curves for diabetic wound healing studies.

0212 A site-specific delivery platform for all trans Retinoic Acid in pulmonary regeneration applications

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Background: Limitations associated with current treatment of chronic obstructive pulmonary disease (COPD) has resulted in the need for new and efficient systems to enable delivery of therapies to, and extend the duration of action at, damaged pulmonary alveolar tissue. The drug molecule all-trans Retinoic Acid (atRA) has previously shown potential in alveolar regeneration (1) but achieving site specific delivery is challenging. To address this need, a methylcellulose (MC) based thermoresponsive hydrogel incorporating atRA loaded solid lipid nanoparticles has been developed, which has the potential to enable loco-regional delivery of atRA to the lung using minimally invasive devices.

Methods: atRA-SLNs were prepared via the emulsification-ultrasonication method, for encapsulation of the molecule. SLN formulations were optimised based on particle size (Z-ave), zeta potential (ZP), polydispersity index (PDI) and encapsulation efficiency (EE). atRA-SLN morphology and size was confirmed by transmission electron microscopy (TEM). atRA release kinetics from the SLNs was determined using a Franz Diffusion apparatus and quantified via HPLC. SLNs were then dispersed in a thermoresponsive MC, collagen and beta glycerophosphate (βGP) hydrogel and their effect on the rheological properties of the gel determined. Assessment of the toxicity of the atRA-SLN formulation was investigated on both primary cells (hMSC) and cell lines (A549 and Calu 3). Immunomodulatory effects of the atRA-SLN formulation and the atRA-SLN-hydrogel formulation on a human respiratory cell line (A549) *in vitro* was determined using ELISA.

Results: atRA-loaded SLNs were 150-200 nm in size, had a zeta potential of -19.25 mV and polydispersity index of 0.28, as determined via Zetasizer Nano ZS, Nanosight NS300, and confirmed by TEM. Average encapsulation efficiency was 78.82%. atRA release from SLNs was sustained over a 14-day time period. MC can form a thermoresponsive hydrogel in combination with collagen and βGP which undergoes sol-gel transition at approx. 37^{o}C, and demonstrates a strong 3-dimensional structure. atRA-loaded SLNs form a stable colloidal dispersion when added to the developed hydrogel and do not impact gel thermoresponsivity. Toxicity studies of atRA-SLNs on hMSCs, A549 lung epithelial cells and Calu3 cells showed good biocompatibility and proliferation of cells. Assessment of immunomodulatory effects of the atRA SLNs showed a significant decrease in pro-inflammatory IL-6 levels post treatment.

Conclusion: We have demonstrated that all-trans Retinoic Acid (atRA) can be encapsulated within SLNs, which are biocompatible, and exert an anti-inflammatory effect on the A549 respiratory cell line. atRA-loaded SLNs may also be suspended in a MC/collagen/βGP which shows a sustained release profile and negligible toxicity to respiratory cells *in vitro*. Results to date indicate the potential modulation of an inflammatory environment and therapeutic applicability of this formulation *in vivo*.

0213 Wound dressings with organic electronic ion pumps for anti-fibrosis therapy

Anne Géraldine Guex[1], David J Poxson[2], Daniel T Simon[2], Giuseppino Fortunato[1], René M Rossi[1], Katharina Maniura[1], Markus Rottmar[1]

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Fibrosis and scar formation is a medical condition observed in various settings, including skin wound healing, cardiac deterioration after a myocardial infarction, liver cirrhosis or systemic sclerosis. Currently, there are no effective treatments for fibrotic disorders and no approved anti-fibrosis therapies. The sustained presence of myofibroblasts, followed by increased collagen deposition and reduced elasticity, has been identified as the main cause of fibrosis and scarring. While the transition from benign wound healing to pathological scaring is a timely orchestrated event that comprises several complex phases, it has been hypothesised that decreasing the pH level of wounds increased healing rates and wound closure.[1]

Highly specific and controlled H^+ delivery to the site of interest is thus an interesting concept for the treatment of chronic wounds. Due to their unique design and operation, organic electronic ion pump (OEIP)[2] devices have the potential to provide localised proton delivery at high dosage precision, without the draw-backs associated with current biomaterials-based drug delivery technologies.

In first experiments, current-controlled proton delivery from an OEIP to a modified cell culture media was evaluated. A local pH change at the OEIP outlet from 7.4 to ~4.4 (based on methyl red pH indicator) was observed. This has then been translated to human dermal fibroblast (HDF) cell culture, having a free-standing OEIP with its delivery outlet aligned with the bottom of the cell culture well. Current/voltage monitoring over 16 hours provided evidence for stable proton delivery.

This approach is now translated to HDF cultures on electrospun membranes, with the OEIP outlet being integrated within the fibrous architecture to develop an advanced wound dressing.

OEIP based technology does not only allow for the controlled release of protons, but can be adapted for delivery of small molecule drugs.

0214 Advanced treatment for arthritic diseases based on the capture and inactivation of interleukin-6 by biofunctionalized polymeric nanoparticles

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Arthritic diseases, such as osteoarthritis and rheumatoid arthritis, are associated with synovium inflammation (synovitis). Several pro-inflammatory cytokines, especially tumor necrosis factor-α (TNF-α) and interleukins (IL), are important mediators of inflammation and articular cartilage destruction, supporting a potential possibility of anticytokine therapy in these diseases. IL-6 is one of the key regulators of the inflammatory response. Thus, human monoclonal antibodies against IL-6 may prevent its action, and consequently reduce inflammation after intra-articular (IA) injection. Indeed, several clinical trials have already demonstrated positive outcomes over disease progression. Although these treatments are very attractive, they are associated with limited efficacy because of the rapid clearance of antibodies by the synovium. A solution to overcome this problem is using nanoparticles (NPs) as a substrate to protect and extend the action of the antibodies. Natural-derived polymers, like chitosan (Ch) and hyaluronic acid (HA), are biocompatible and biodegradable polysaccharides, being HA a natural component of the extracellular matrix of articular cartilage. Therefore, biodegradable polymeric NPs represent a good candidate for IA administration.

In the present work we propose natural biodegradable polymeric NPs biofunctionalized with immobilized antibodies that selectively capture and inactivate the pro-inflammatory cytokine IL-6, reducing synovium inflammation. Ch-HA NPs were successfully prepared by polyelectrolyte complexation and further stabilized through carbodiimide chemistry (ethyl(dimethylaminopropyl) carbodiimide (EDC)/N-hydroxysuccinimide (NHS)). The particle size and zeta potential of the NPs were optimized. Stable NPs with 121.8 ± 2.4 of particle diameter, 0.11 ± 0.01 of polydispersity index and +25.12 ± 1.86 mV of zeta potential were produced with 0.25 mg/mL of initial polymers concentrations, at pH 5 and with 50/200 mM of EDC/NHS concentration. The anti-IL-6 antibody was immobilized at the surface of Ch-HA NPs. After determining the maximum antibody immobilization ability (7 µg/mL), the capacity to capture the recombinant IL-6 was evaluated. The efficacy was around 94-97%. Biological assays demonstrated not only the cytocompatibility of the produced NPs with human articular chondrocytes (hACs) (Fig 1) and human macrophages, but also the benefits of the capture and inactivation of IL-6 after stimulation with monocyte-derived macrophage conditioned medium. In conclusion, it is foreseeable that these NPs will overcome the limitations of the abovementioned treatments, since such NPs will increase the therapeutic efficacy due to their subcellular size, non-toxicity and high stability, being a promising approach for the local and sustained treatment of arthritic diseases.

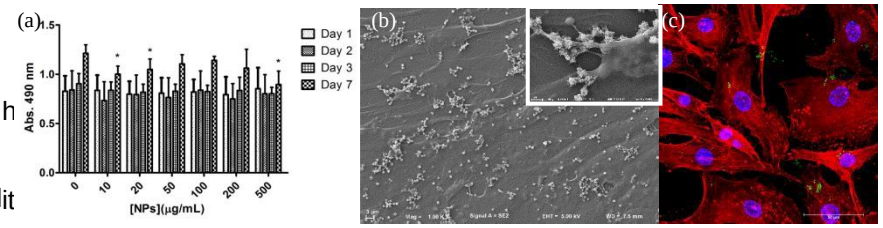

Fig 1. performance of t h with NPs (a) cell viabilit (b) SEM micrographs, and (c) confocal microscopy with NPs at 50 µg/mL.

0215 The ethics of biomedical innovation

Annelien Bredenoord

University Medical Center Utrecht, Utrecht, The Netherlands

Recent advances in biological and medical research, such as regenerative medicine (RM) and stem cells, organoid technology, next generation DNA sequencing, genome editing, reproductive technologies, biobanking and Big Data, continuously raise ethical and societal challenges: how to translate biomedical innovations from basic research into clinical care and society in an ethically sound way?

Translating RM interventions into clinical trials and society takes time and careful research. For example, today only one orthopedic RM treatment, specifically the treatment for focal knee cartilage defects, is approved for market use. In the meanwhile stem cell clinics are taking advantage of the hopes and expectations of stem cells and already offer a variety of unproven stem cell interventions to patients in return for payment. As the amount of (early) clinical studies in this field is expected to rapidly expand in the near future, ethics parallel research is needed to discuss the interwoven scientific and ethical challenges involved in the translation of these technologies into patients and society. Ethics parallel research means that ethicists evaluate the ethical issues of a novel biomedical intervention parallel or even proactive as the field develops and not end-of-pipeline. In this way science, technology, ethics, society and politics 'co-produce' each other rather than the traditional view that these fields have clearly demarcated roles. "Co-production" means that scientists, clinicians, ethicists, patients, and other members in society generate new knowledge, treatments and medical technologies together in a dynamic interaction for optimal alignment of output and needs. It is particularly important for regenerative medicine: no innovation without social and public support.

RM is highly promising but at the same time faces several layers of cross-cutting complexity, not only technological but also with respect to the introduction into clinical trials, patient care and society.

In this presentation I will address three trends in the ethics of biomedical innovation that are highly relevant for RM:

(1) The shift from clinical research on people to research on their tissues, cells and data.
(2) The shift from traditional drug trials to novel technologies and health care interventions.
(3) The shift from a protection paradigm to a participation paradigm.

0216 Engineered tissues and stem cells - what are the practical hurdles for clinical translation?

Nicole Rotter

Ulm University Medical Center, Ulm, Germany

Engineered tissues and stem cells have the potential to solve significant clinical problems related to damaged tissues and organs by providing readily available off-the-shelf solutions. Ideally, in order to prevent donor site morbidity and long time intervals between the need for treatment and the actual treatment such therapies might be based on allogeneic cells or bioactive scaffolds that have the potential to induce functional tissue regeneration. With respect to clinical translation, more complicated options include in vitro engineered tissues as well as therapies based on autologous cells, which need to be harvested from the affected patient and prepared in vitro for further application. Important clinical examples which are adressed by engineered tissues and stem cells are cartilage defects in the head and neck region as well as articular cartilage defects.

The pathway from in-vitro to preclinical studies into first-in-human trials is long and extremly difficult as a close interaction between clinicians and basic researchers is required from the very beginning of an therapeutic idea until its clinical application. Furthermore, the progression of first-in-human trials into larger, long term trials and into daily clinical routine requires a multitude of further steps, including regulatory and financial aspects, besides the actual clinical problem.

This talk will discuss the way from bench to bedside with respect to clinically practical hurdles with respect to specific applications of cartilage in the head and neck area.

0217 The challenge of making the newly created Swiss Biobanking Platform sustainable and reliable

Sabine Bavamian, Christine Currat

Swiss Biobanking Platform, Lausanne, Switzerland

Statement of the problem

Development of a national coordination platform for Biobanking activities is an initiative responding to the needs of researchers in terms of quality, access, transparency and interconnectedness of biobanks in Switzerland and abroad. Even though such a platform is essential to position Switzerland at the forefront of biomedical and biological research, sustainability is the key issue. For promoting access and sharing, harmonization of processes with a high-quality perspective is essential. However, changing practice is a long and breath-taking process, as well as changing minds in terms of willingness to share.

Proposed solution

Building trust through the development of a customer-oriented network of biobanks is the ambition of Swiss Biobanking Platform (SBP) by becoming the privileged partner for biomedical researchers.

SBP is developing a specific biobank quality standard fragmented into modules. This approach is aiming at corresponding to any type of biobanks covering harmonized processes from general consent to the return of results. SBP will then be positioned as a certification body delivering audits with a module strategy allowing the delivery of the standard in a “one fits all” strategy without any discrimination to any small or poorly funded biobank.

In terms of interconnectedness, SBP will provide a central IT solution that will help implementation of harmonized processes through the development of a specific SBP module. This strategy has the advantage to equip main biobanks with an IT solution at a lower price, to build a reliable collaboration with a LIMS provider and to give incentives in participating to SBP network.

In terms of access, SBP is developing a “One-stop-shop” model for samples in Switzerland with the ambition to deliver an efficient process through a SBP catalogue. Access and benefit sharing guidelines will be developed with a part on samples’ pricing that needs to be harmonized in Switzerland. SBP has already established a working group with pharma industries and academic partners to develop the model.

In terms of acceptability, SBP is supporting commitment through public engagement with a need on good governance issues with fair and transparent distribution of samples.

Conclusions

SBP strategy is to enhance sustainability of biobanks in Switzerland with the integration of the three financial, operational and social dimensions. Fostering common good, quality, economies of scale and trust are key issues to build up a reliable and efficient network with potential return of investments for individual biobanks.

0218 Legal issues for personalised TERM – Can law cope with the speed of development?

Brigitte Tag

University of Zurich, Zurich, Zurich, Switzerland

This lecture will provide an overview of the different legal regulations and challenges concerning personalised TERM. Personalized medicine stands for a development which will have a long-lasting impact on the health care system. This is also true concerning Tissue Engineering and Regenerative Medicine. In a nutshell it can be said that the challenges and chances related to personalized medicine are remarkable. Personalized TERM will require and generate a significant increase of information. This assumes a fundamental change in existing directives and standards. Closely related to this there will be a big challenge to explore and identify genes and molecular structures as well as drawing the right conclusions from it. These developments make us hope and worry at the same time, primarily due to the following points: the storing and reliable handling of the data to be collected, readiness of research institutes to collaborate and ultimately legally and ethically adequate regulations concerning bio-bancs. Legal issues in personalized TERM principally refer to two thematic fields: on the one hand, there is the use of personalized TERM in the context of medical diagnosis and therapy and, on the other hand, within the context of medical research. Both fields often partially overlap. Particular attention is to be paid to the handling of data and samples gathered during research activities, more precisely speaking - it depends on whether it is genetic data or biological material.

0219 Development of 3D printed silica-gelatin sol-gel hybrid scaffolds for cartilage tissue engineering - effects of material geometry on cartilaginous matrix formation

Siwei Li, Maria Nelson, Molly Stevens, Julian Jones

Imperial College London, London, UK

There are increasing interests in "in situ" cartilage regeneration, combining micro-fracture surgical procedure with the use of scaffolds for cell ingrowth and guidance towards tissue formation. Our group has developed a unique silica-gelatin sol-gel hybrid material, which has nano-scale co-networks of organic and inorganic components that allow tailorable degradation rate and mechanical properties. Utilising 3-D printing technology, the present study aims to create silica-gelatin scaffolds with various pore structures and, investigate their effects on chondrogenic differentiation. The silica-gelatin hybrid sol was synthesised using a TEOS based sol-gel process with GPTMS as coupling agent. The printed 3-D scaffolds were characterised using SEM, micro-CT and mechanical testing prior to *in vitro* biocompatibility screening in accordance to ISO-10993 standards. ATDC5 cells were seeded onto scaffolds with average pore sizes between 250 and 1000 μm and, analysed for cell attachment, proliferation and cartilaginous matrix formation following culture for up to 21 days. The scaffolds contained open and interconnected pores (Figure 1). The Young's moduli when wet improved significantly to a level comparable to the range of human articular cartilage as the pore size reduced to 250 μm.

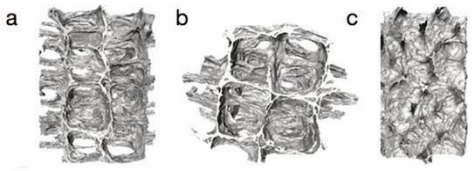

Figure 1. Representative 3D reconstructed X-ray image of silica-gelatin scaffold. (a) YZ view, (b) XY view and (c) XZ view.

The hybrid material demonstrated excellent biocompatibility. Cytoskeletal constituents Vimentin and F-actin were immunolocolised in ATDC5 cells following 72-hours of culture. WST-1 assay demonstrated improved rate of cell growth in scaffolds with larger pores (500 and 1000 μm), however, the expression of fibrocartilage marker Type-I Collagen was up-regulated. Cells in large pores likely experienced environments close to monolayer culture and gradually lost chondrocytic phenotype in dedifferentiation. Scaffolds with smaller pores (250 μm) significantly up-regulated the expression of hyaline cartilage specific Type-II Collagen and the formation of sulphated glycosaminoglycan (Figure 2).

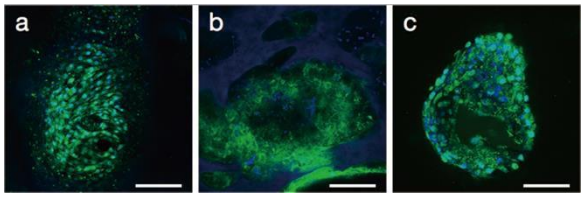

Figure 2. Immunohistochemical analysis of day-21 cell seeded silica-gelatin constructs. The expression of (a) Sox9, (b) Collagen Type II and (c) Aggrecan demonstrated hyaline cartilaginous matrix formation in scaffolds with 250μm pores. Scale = 100μm.

The present study demonstrated that scaffold microstructure plays a key role in cell behaviour and matrix formation and, silica-gelatin sol-gel hybrid material and its 3-D scaffolds are promising for cartilage regeneration applications.

0220 Engineering the fibroblast growth factor-18 for increased affinity to the cartilage extracellular matrix to delay knee osteoarthritis

Priscilla Briquez[1,2], Xavier Quaglia[1], Jeffrey Hubbell[1,2]

[1]Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland, [2]University of Chicago, Chicago, USA

Introduction: Osteoarthritis (OA) is one of the leading causes of disability in the world, affecting more than 10% of people aged over 60 years old. Despite extensive researches, clinical therapies to treat OA remain very limited, and mainly focus on pain management and total joint arthroplasty. Consequently, chondrogenic drugs are highly sought to protect patient's cartilage from degeneration. Particularly, the fibroblast growth factor (FGF)-18 has been identified as a promising therapeutic against OA due to its role in chondrogenesis and adult cartilage repair [1]. In our study, we engineered FGF-18 to increase its affinity to the cartilage extracellular matrix, aiming to improve its delivery kinetics and so its therapeutic efficacy.

Material & Methods: We increased the affinity of FGF-18 by engineering it with a super-affinity ECM-binding domain derived from the placental growth factor-2 [2], and produced it as a recombinant protein in CHO cells. We used ELISA-based and cellular *in vitro* assays to compare the affinity and bioactivity of engineered FGF-18 to the wild-type FGF-18. We further analyzed *ex-vivo* the affinity of FGF-18 variants to bovine cartilage explants. To assess the therapeutic potential of FGF-18 variants, we used a surgically induced medial meniscus destabilization OA model in mice, performing *in vivo* contrast-enhanced micro-computed tomography and histological analyses to monitor cartilage degeneration.

Results & Discussion: We successfully produced and purified engineered FGF-18 variant. We found that engineered FGF-18 displayed enhanced affinity to cartilage matrix components, notably to chondroitin sulfate glycosaminoglycans. Additionally, we confirmed that the retention of engineered FGF-18 on bovine cartilage explants was significantly higher than for wild-type FGF-18. However, we found that the chondrogenic bioactivity of engineered FGF-18 was reduced, although not abolished, compared to wild-type.

In vivo, we found that intra-articular injections of engineered FGF-18s significantly preserved the cartilage glycosaminoglycan content compared to wild-type FGF-18, and showed positive trends toward delayed onset of cartilage OA. Thus, our data open the path toward the further use of engineered FGF-18 as a protective drug to preserve cartilages of osteoarthritic patients.

0221 Extracellular matrix-modulating enzyme expression and proteoglycan degradation are regulated by oxygen tension during chondrogenesis of bone marrow-derived stromal cells

Martin Kraehnke[1], Thomas Boeck[1], Eva Heusler[2], Lorenz Meinel[2], Rainer Meffert[1], Torsten Blunk[1]

[1]University of Wuerzburg, Department of Trauma, Hand, Plastic and Reconstructive Surgery, Wuerzburg, Germany, [2]University of Wuerzburg, Institute for Pharmacy and Food Chemistry, Wuerzburg, Germany

Objectives: Oxygen tension is well acknowledged to impact chondrogenic differentiation of human bone marrow-derived stromal cells (BMSCs). The beneficial influence of hypoxia on the synthesis of cartilaginous extracellular matrix (ECM) synthesis has been repeatedly demonstrated, however, so far very little is known on the effect of hypoxia on ECM degradation. Therefore, the aim of this study was to investigate, under normoxic and hypoxic conditions, the expression and activity of ECM-modulating enzymes such as matrix metalloproteinases (MMPs) and assess the resulting impact on ECM development during BMSC chondrogenesis.

Methods: BMSCs were cultivated either in pellet culture ($2x10^5$ cells per pellet) or in silk fibroin scaffolds (Ø 3 mm, height 4 mm, pores 315-710 µm; $8x10^5$ cells) for 21 days. Chondrogenic medium was supplemented with TGF-β1 (10 ng/mL), and pellets and constructs were subjected to normoxic (21% O_2) or hypoxic conditions (2% O_2). Development of cartilaginous ECM was analyzed histologically and immunohistochemically and by common biochemical quantitative assays for glycosaminoglycans (GAG) and collagens. The impact of oxygen tension on expression of various ECM-modulating enzymes (MMP1, MMP3, MMP13, ADAMTS4, ADAMTS5, TIMP1, TIMP3) was analyzed on mRNA and protein level. Furthermore, a FRET-based general MMP activity assay was performed, and specific ECM degradation products (e.g., neoepitope DIPEN after aggrecan cleavage by MMPs) were analyzed by immunohistochemical staining and subsequent quantification (software ImageJ).

Results and Conclusion: In BMSC pellets, culture under hypoxic conditions led to improved chondrogenesis with increased GAG and collagen content after 21 days, as compared to normoxic conditions. After 5, 10, and 21 days, high and low oxygen tension differentially affected MMP expression patterns, as determined by qRT-PCR. Distinctly lower MMP13 and MMP3 expression levels were detected under hypoxic conditions ($p \leq 0.05$), while TIMP3 expression was increased ($p \leq 0.05$). Reduction of MMP13 expression was confirmed on protein level using immunohistochemistry and subsequent quantification (normoxia 240 ± 150 relative immunolabeled area (RIA) and hypoxia 37 ± 18 RIA ($p \leq 0.01$)). Strikingly, a strong decrease of the MMP-generated aggrecan neoepitope DIPEN was detected under hypoxic conditions by immunohistochemical staining (10.7 ± 4.3 RIA for normoxia; 2.9 ± 1.5 RIA for hypoxia ($p \leq 0.01$)). This observation was further supported by reduced general MMP activity under hypoxia. All key findings of the pellet culture were corroborated in BMSC-silk fibroin constructs, with hypoxic conditions leading to increased GAG content, increased TIMP3 and decreased MMP13 expression, and distinctly reduced aggrecan neoepitope DIPEN. In two independent 3D culture systems, our results indicate a new mechanism of action of hypoxic conditions during chondrogenesis of BMSCs. Hypoxia appears to not only support ECM synthesis, but also to protect cartilaginous ECM from degradation by shifting the ratio of MMP/TIMP gene expression and especially by attenuation of MMP activity contributing to a higher proteoglycan content in the ECM.

0222 The role of cell death in mesenchymal stem cell therapy for osteoarthritis

Patrizio Mancuso, Mary Murphy, Frank Barry

REMEDI, Galway, Ireland

INTRODUCTION - Osteoarthritis (OA) is a degenerative disease of the articular cartilage with a strong inflammatory component. Treatments are currently lacking, with a heavy economic impact on healthcare systems worldwide. Mesenchymal stem cells (MSCs) promote cartilage regeneration when injected locally. However, the retrieval yield of cells administered in pre-clinical models is extremely low, suggesting that they undergo apoptosis or cell death. Apoptotic cells have an anti-inflammatory effect, by secretion of immunomodulatory cytokines or by indirect action following phagocytosis from macrophages. The aim of this study is to investigate the fate and effect of MSCs when locally administered in an animal model of OA and to assess the role of cell apoptosis using an in vitro system.

METHODS - Collagenase-induced OA (CIOA) was performed on C57BL/6 mice and 2×10^5 GFP^+ MSCs were intra-articularly (IA)-delivered in the animals. 3 days later, knee joints were digested into a single cell suspension and GFP-MSCs were sorted using the BD FACS Aria II. Conditioned medium (CM) of retrieved cells was tested on murine macrophages. Immune activation was measured as expression of MHC-II and secretion of interleukin (IL)-6. Apoptosis of MSCs was induced in vitro by treatment with camptothecin (CPT), staurosporine (STS) or dimethyl sulfoxide (DMSO). Apoptosis was evaluated by flow cytometry using anti-Annexin V/Sytox Blue; activation of caspases was measured by FLICA assays. Murine splenocytes were cocultured with apoptotic MSCs and their proliferation was measured by quantification of Cell Trace Violet.

RESULTS - 1.63% of injected cells were successfully retrieved by cell sorting. Sorted cells proliferated in culture retaining morphology and differentiation potential. Their CM significantly suppressed activation of macrophages, with greater effects seen with OA-induced MSCs. All tested compounds induced apoptosis in a time-dependent fashion, with activation of Caspase 3/7; STS was the most potent inducer. MSCs significantly prevented the proliferation of murine splenocytes -this effect was reduced by DMSO treatment but enhanced by CPT and STS.

CONCLUSIONS - MSCs can be administered and retrieved from murine knee joints. Retrieval yield is low, consistent with previous studies. The effect on macrophages is paracrine as produced by their conditioned media, without cell-to-cell contact.

IA-injected MSCs may contribute to local modulation of joint inflammation. Engrafted MSCs retrieved from the OA joint were licenced to produce an immunosuppressive milieu that decreased macrophage activation ex vivo. In vitro, apoptotic cells were more immunosuppressive than viable MSCs. This suggests that apoptosis may contribute to the known therapeutic effects of MSCs in the mouse OA knee.

Whether apoptosis plays a role in licensing surviving MSCs will be further investigated.

0223 Improving mechanical functioning of micro-fibre reinforced hydrogels for cartilage repair

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Designing implants approaching the unique mechanical properties of articular cartilage remains a challenge in tissue engineering. Implants should withstand high compressive loads adequately distributing forces throughout the implant and at the same time, guide ECM formation and cell differentiation with zonal variation. Recently, Visser *et al*. reported on reinforcement of gelatin–methacrylamide (GelMA) hydrogels with a box-structured micro-fibre scaffold obtained by direct melt electrospinning (MEW) [1]. Although abundant matrix formation was observed in these novel composite constructs and they reached compressive properties similar to those of articular cartilage, they were not able to guide zonal tissue formation and may not ensure adequate mechanical integrity after implantation. We hypothesize that this may have been due to a lack of a superficial tangential zone (STZ) -like structure that could improve mechanical functioning of cartilage engineered constructs.

Therefore, in this study different micro fibre scaffold architectures were designed to reinforce a GelMA hydrogel system, *i.e.* a densely distributed crossed fibre mat (STZ), fibres in a uniform box structure (deep zone, DZ) and a combination with construct height of 10% STZ and 90% DZ (STDZ). Such scaffolds were electrospun by a special custom-built MEW device, and then embedded in GelMA hydrogel (Ø5/8 (E_{UC}/E_{IND}) x 1 mm cylinders). Construction of composite phase materials was evaluated by micro-CT, and equilibrium elastic moduli were assessed by unconfined compression (E_{UC}) and macro-indentation (E_{IND}, indentor radius = 2 mm), mimicking congruent and in-congruent joint loading. Elastic moduli of articular cartilage in osteochondral cores harvested from porcine knee joints were also evaluated, with and without the superficial tangential zone.

The experiment demonstrated the possibility to directly produce melt electrospun microfibre-scaffolds with a well-defined and sophisticated zonal organization that cannot be obtained by any other fibre fabrication technique. The DZ composites were stiffer under pure compression than STZ constructs (E_{UC}: 155±40 vs. 24±6kPa), but were not significantly different under indentation (E_{IND} : 89±18 vs. 85±5kPa). Interestingly, reinforced gels with a zonal fibre architecture (STDZ) improved the construct's elastic moduli approximately 2.5x under UC and more than 5x in IND, when compared to the DZ reinforcement.

An explanation for these observations is that the inclusion of a thin superficial tangential zone reinforcing layer enables to recruit a larger volume of the deep reinforced region to carry compressive loads, and therefore more efficiently transfer the loads from directly loaded regions throughout all of the construct when in-congruent surfaces are compressed. This biomimetic nature of the scaffolds are being confirmed with comparison to the measured moduli of native cartilage with and without the STZ. Furthermore, we plan to examine the 3D deformed structure of the loaded composites implants using microCT to confirm out loading mechanism hypothesis. This novel layered architecture may also contribute to the zone-specific differentiation of the cells. [1] Visser *et al.* Nature Commun 2015

0224 Establishment of a hydrogel-based antimiR-221 delivery system for in situ guidance of cartilage repair

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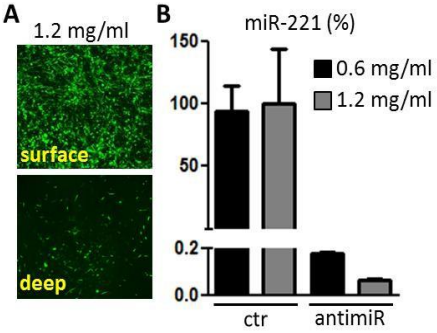
Human Mesenchymal Stem Cells (hMSCs)-based therapy is regarded as a promising approach for cartilage repair. However, important challenges still need to be addressed, e.g. cost of the procedures, delays due to *in vitro* culture and safety issues. Interestingly, novel strategies able to promote endogenous cartilage repair may allow to circumvent these obstacles. Such approaches aim to recruit endogenous MSCs to the injury, subsequently inducing *in situ* chondrogenesis and tissue repair. To this purpose, bioactives stimulating cell recruitment and guide chondrogenesis need to be selected and delivered into the defect, in combination with a scaffold. Previously, we showed that antimiR-mediated silencing of miR-221 in hMSCs functions as potent pro-chondrogenic signal, enhancing production of cartilage by hMSCs *in vivo*. Here we focused on the establishment of a hydrogel-based delivery system to inhibit miR-221 *in situ*, for potential application in endogenous cartilage repair.

We selected a miR-221 specific LNA-mixmer (antimiR-221) as tool for gene silencing, due to the intrinsic stability and efficacy of LNA-based molecules. A collagen I-based hydrogel was chosen as scaffold. We first assessed whether hMSCs could infiltrate the collagen hydrogel, by labeling the cells with the fluorescent dye CFDA-SE and seeding them on top of the scaffolds formed with different concentrations of collagen (0.6÷2.4 mg/ml). The constructs were cultured *in vitro* for 3 days in the presence of the chemokine PDGF-BB. Confocal imaging revealed that hMSCs could quickly invade hydrogels with low concentrations of collagen (≤1.2 mg/ml) under the influence of PDGF-BB (Fig.A). To evaluate whether silencing of miR-221 could occur within the scaffold, hMSCs and antimiR-221 were loaded in the hydrogels (≤1.2 mg/ml), that were then solidified and cultured *in vitro*. Remarkably, miR-221 expression was already abolished after 3 days of culture and not recovered for at least 14 days (99.7% and 99.2% inhibition, respectively). Similar suppression of miR-221 was achieved in the absence of the carrier, suggesting that the inhibitor had intrinsic ability to penetrate the cells. No silencing was observed when a scrambled mixmer was used. In the effort to better mimic transfection of endogenous cells, the hydrogels were loaded with antimiR-221 and hMSCs were seeded on top. Interestingly, after 7 days of culture miR-221 was knocked-down in hMSCs colonizing the hydrogels (99.8% inhibition; Fig.B).



In summary, collagen hydrogels formed with low concentrations of collagen (≤1.2 mg/ml) and loaded with antimiR-221 allow hMSCs ingrowth and mediate miR-221 silencing in colonizing cells, suggesting that our system is applicable for *in situ* transfection. Future studies will aim to implement the delivery system with a chemokine, in view of potential *in vivo* use for endogenous cartilage repair.

0225 BMP9 is a potent chondrogenic factor for articular cartilage-derived chondroprogenitors

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Introduction: Chondroprogenitors in cartilage tissues have been proposed as the ideal cell source for generating hyaline cartilage suitable for tissue implants. Chondrogenesis of epiphyseal chondrocytes and mesenchymal stem cells has been extensively described and shown to be optimally induced in medium with transforming growth factors (TGF□). There have been no comparable studies to determine the optimal chondrogenic medium for articular chondroprogenitors. The unique developmental history of chondroprogenitors led us to hypothesise that there are specific context-dependent differentiation factors for this cell type. To determine the optimal chondrogenic medium for articular chondroprogenitors we quantitatively assessed the effect of a panel of known chondrogenic factors on these cells using high-density pellet culture.

Methods: Immature chondroprogenitors were isolated from the metacarpophalangeal joint of 7-day-old bovine steers. Chondrogenesis assays were conducted by pellet culture using a base medium consisting of DMEM:F12, insulin-transferrin-selenium, ascorbate, 10% heat-inactivated foetal bovine serum, HEPES and gentimicin. $5x10^5$ chondroprogenitors were cultured for 21 days in basal medium or supplemented with chondrogeneic factors; ethanol, C-natriuretic peptide, chelerythrine chloride, dibutyryl-cAMP, concanavalin A, dexamethasone, TGF□-1, -2, -3 and BMP-2 and -9.

Results: Of the panel of chondrogenic factors tested, BMP9 was the most potent inducer of differentiation. Histological analysis showed that BMP9 treated pellets were exceptionally large and stained uniformly for the dye, whereas other treatments produced patterns of high and low staining across pellets. These data correlated with the amount of sGAG and hydroxyproline within pellets, and by gene expression within pellets using ACAN and COL2B primers. DNA quantitation showed that BMP9 pellets alone did not increase cell number from the initial seeding density, indicating that the majority of cells within BMP9 pellets had responded to differentiation cues. Periodic gene expression analysis of BMP9-induced differentiation of chondroprogenitors revealed that ACAN expression peaked at 3 days in culture and COL2B at 7 days. Collagen type X gene expression was minimal, in absolute terms 1 transcript for every 400 of COL2B. We also found that combinations of chondrogenic factors were not as effective as using BMP9 alone.

Discussion: The data shows that BMP9 is significantly more effective as a chondrogenic factor for articular chondroprogenitors than TGF□growth factors. The fact supplementation of BMP9 with other chondrogenic factors has no additive or synergistic effect argues that it may be a specific, context-dependent factor for chondrogenic induction. Early in development, cartilage growth is faster than in post-natal phases and BMP9-stimulated growth may be important in these early growth stages as opposed to homeostatic tissue maintenance later in life. This idea is supported by studies that describe differential responses of immature and mature cartilage to BMP9. In conclusion, BMP9 is a potent chondrogenic factor for immature articular chondroprogenitors suggesting a role during early development phases of tissue growth. This work will lead to optimised chondrogenic protocols specific for cartilage tissue engineering using chondroprogenitors, enabling rapid and scalable neo-cartilage production.

0226 Mechanobiology of extracellular matrix: from cells to tissues

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Major transformations of extracellular matrix are tightly associated with the progression of a wide range of diseases, including cancer and fibrosis, yet how the reciprocal crosstalk between extracellular matrix and cells drives these processes remains unknown. Despite major progress in our understanding how proteins act as mechano-chemical switches and how the mechanobiology of cells regulates their fate, our knowledge about the mechanobiology of tissues in health and disease is lacking behind, mostly due to the lack of probes to either measure cell traction forces in living tissues or the mechanical strain of extracellular matrix fibers. As fibronectin is a key player in tissue growth and repair, we are developing peptide probes to assess fibronectin fiber strains in tissues and illustrate for the first time that the fiber strain in tumor stroma is significantly altered. To quantify mechanotransduction processes, from 2D cell cultures all the way to living tissues, the availability of novel probes to document and track ECM fiber strain in histological samples and living tissues is thus highly relevant and urgently required.

0227 Cell geometry regulates temporal regulation of gene expression during macrophage activation

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Macrophage M1 activation is a multi-step process. Even though the chemical regulation of this multi-step process has been extensively studied, the role of physical factors in co-regulating the temporal gene expression, activation and hence functional output is largely unexplored. We found actin dependent correlative changes in macrophage's cell and nuclear geometry during proinflammatory M1 activation. Macrophages increases their spreading area very rapidly upon experiencing the activation signal along with nuclear size, which were largely reversible with longer time scales. This motivated us to ask whether controlling cell geometry and hence nuclear architecture could tune their activation response. Using micropatterning, quantitative imaging, knock out studies complemented with pharmacological inhibition studies, we provide evidence for cell geometry regulated nuclear signaling events which impinges on temporal regulation of gene expression and hence functional response during macrophage activation. Our finding that controlling macrophage spreading, as enforced by spatial restriction, can downregulate their inflammatory response and might thus have major physiological significance in wounds sites and in tightly packed tissues, including fat tissue and tumor stroma.

0228 Strain-controlled organ culture of bone-ligament-bone human-derived anterior cruciate ligaments – an *ex-vivo* model to investigate degenerative and regenerative therapy

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Introduction:

Injuries of the anterior cruciate ligament (ACL) are among the most common ligament injuries.[1] Unresolved challenges in primary repair of ACL ruptures demanded the establishment of a culture system to study the regenerative capacities. The present study introduces an *in-vitro* culture model for human ACLs. Moreover, this study evaluates whether culturing ACLs in a strain-controlled dynamic loading bioreactor system is superior to a free-swelling static loading culture model.

Material and Methods:

A bioreactor to harbor and mechanically stimulate full human cruciate ligaments was designed and manufactured. Fresh and full ACLs with attached femoral and tibial bone were obtained from full-knee prosthesis surgery with written consent of the patients (ethically approved). ACLs were maintained for 7 days free-swelling in high glucose DMEM supplemented with 5% fetal calf serum (FCS) under either static or dynamic loading applying 7% dynamic strain with 0.2Hz. ACL cell activity and viability was determined close to the tibial region (T), the mid region (M) and close to the femoral region (F) by performing a resazurin salt cell activity assay and applying 3D stacks of confocal laser scanning microscopy (cLSM) on cells treated with a LIVE/DEAD staining kit. Additionally DNA content (Hoechst), collagen content (hydroxy-proline = HYP assay) and RT-qPCR, to check for relative gene expression of ligament specific markers, were screened.

Results:

Large differences in relative gene expression between each ACL sample were observed, indicating an inter-individual variability. Among the 3 different ACL zones of dynamically loaded ACLs, DNA content was higher in tissue close to the F zone than in the M and T zone. This trend was also noticed for cellular mitochondrial activity on day 1 (1480.9±415.4 vs. 844.7±155.8 RFU, respectively) as well as on day 7 (973.8±229.6 vs. 790.8±39.6 RFU, respectively). There was a trend that cell viability was higher in ACL cells cultured under dynamic loading conditions compared to cells cultured under static loading conditions (78.80±19.52% vs. 76.61±3.4% respectively, measured after 7 days). After 7 days of dynamic culturing, ACL-fibroblasts still expressed their ligament-specific genes (data not shown).

Discussion:

ACL cells are able to survive for 21 days and to maintain their phenotype in organ culture as shown by qPCR. DNA and cellular activity revealed that the ACL is an inhomogenous tissue in terms of cell density and cell phenotypes. The interest in tissue-engineered solutions grows leading to improved mechanobiological models of ACL culture, that allow testing of different regenerative approaches.

0229 The effect of implant surface texture design on peri-acetabular bone ingrowth: A multiscale mechanobiology based numerical analysis

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The fixation of uncemented acetabular prosthesis largely depends on the biological fixation. Despite a number of animal studies investigating the influence of implant surface texture designs on osseointegration, there are considerable differences in the implant shape, bone quality and physiological loading conditions of human joint replacements and those animal studies. Therefore, further studies involving physiological peri-prosthetic mechanical environment are required to investigate the influence of implant surface texture designs on peri-acetabular bone ingrowth. The objective of this *in silico* study is to investigate the influences of different implant texture design factors on bone ingrowth around a cast-in beaded acetabular prosthesis. The novelty of this study is the comparative Finite Element analysis of 3D microscale models (Fig. 1) representing the implant-bone interface [1]. The study proposes a statistical framework wherein the patient-specific mechanical environment (host bone material property and implant-bone relative displacement) is considered along with sequential mechanoregulatory algorithm and design of experiment. Bead height, bead diameter and inter-bead distance were chosen as three variables influencing the osseointegration. The bone ingrowth process was found to be inhibited due to an increase in inter-bead spacing from 200µm to 600µm and bead diameter from 1000µm to 1500µm and a reduction in bead height from 900µm to 600µm. The bead height exhibited a pronounced influence on bone ingrowth. Amongst the interaction effects, the combination of bead height and bead diameter was found to have a predominant influence on bone ingrowth process. A combination of low inter-bead spacing (P=200µm), low bead diameter (D=1000µm) and high bead height (H=900µm) facilitated peri-acetabular bone ingrowth. An increase in available surface area was also found to promote osseointegration. Hence, such an implant surface texture design seemed to improve the acetabular component fixation.

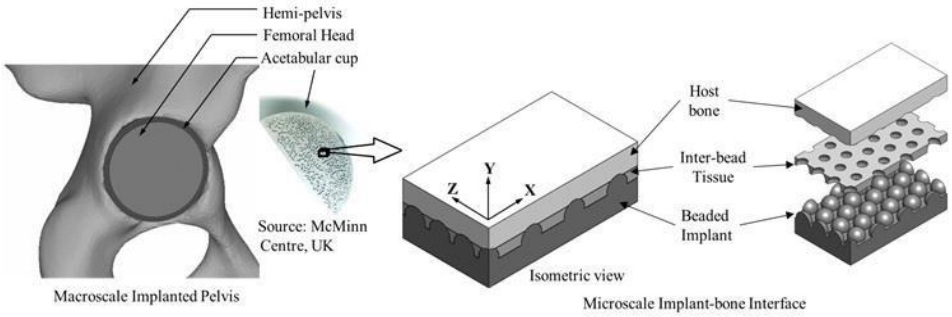

Fig.1: Macroscale implanted pelvis model and microscale implant-bone interface model

0230 The effect of defect size on local mechanical environment in an in vivo mouse femoral defect model

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INTRODUCTION: Mouse models are often used to study novel biomaterials and bone tissue engineered scaffolds in an early *in vivo* setting. However, the local mechanical environment within the defects created in such models is not well understood. As the bone healing process is mechanically sensitive, the beneficial effects of a scaffold may be masked by inappropriate mechanics. Furthermore, comparison between materials could be invalid if the mechanical environments are significantly different. The mechanical environment is affected by the size of the defect, stiffness of fixation and load bearing of the mouse. **AIM:** We investigate the role of defect size on the mechanical environment early in the healing process, and hypothesize that, as the healing process is at least partially mechanically regulated, the level of strain energy density (SED) preceding ossification is independent of the defect size. **METHODS:** Two groups of female mice (C57BL/6) underwent a femoral mid-shaft osteotomy stabilised with a MouseExFix (RISystem, Switzerland), one group (n=8) received a defect of 1.45(SD=0.16) mm, the second group (n=10) 0.85(SD=0.09) mm. The mice were scanned weekly using *in vivo* micro-computed tomography (vivaCT 40, Scanco Medical). The images were registered, thresholded (641.9 mg HA/ccm) and sites of bone formation were determined by overlaying the images. Based on the thresholded images micro-finite element models were generated, assuming an elastic modulus of 14.3 GPa for bone and 3 MPa for non-bone. An axial load of 10.5 N and a bending moment of 3.5 Nmm was applied to the fixator-bone construct. Using ROC analysis, the optimal SED level for bone formation and area under curve (AUC) were determined. **RESULTS:** SED is a strong predictor of ossification in the first 3 weeks as demonstrated by a high AUC (Table 1). AUC decreases with the progression of healing corresponding with the shift from repair to remodelling. The level of SED above which ossification occurred was not found to be significantly different between groups in weeks 1, 2 and 4. In the 0.85 mm group bridging was observed radiographically in 4 mice in week 2, this leads to stress shielding of the soft callus and thus lower SED values. **DISCUSSION & CONCLUSIONS:** At sites of ossification, no significant difference in the pre-existing SED was found, thus confirming our hypothesis that the SED preceding and possibly provoking ossification is independent of the defect size. As a large amount of ossification occurred in the second week we recommend that future mouse studies adjust either the defect size or fixation stiffness to achieve a mean SED of at least 0.2 MPa in the defect to create an optimal mechanical environment for healing. **ACKNOWLEDGEMENTS:** Support from EU (BIODESIGN FP7-NMP-2012-262948) and the Swiss National Supercomputing Centre (CSCS).

Table 1	1.45 mm group		0.85 mm group	
	AUC(-)	SED(MPa)	AUC(-)	SED(MPa)
Week 1	0.87 SD=0.03	0.20 SD=0.08	0.83 SD=0.07	0.31 SD=0.18
Week 2	0.87 SD=0.02	0.19 SD=0.05	0.87 SD=0.03	0.22 SD=0.06
Week 3	0.71 SD=0.08	0.18 SD=0.08	0.78 SD=0.09	0.08 SD=0.06
Week 4	0.58 SD=0.15	0.00 SD=0.00	0.66 SD=0.19	0.00 SD=0.00

0231 Hippo effector yap is a key regulator of cell-matrix interaction

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The extracellular matrix (ECM) is the non-cellular constituent of the tissues that, far from being an inert structural scaffold, provides biochemical and biomechanical cues that impact on cell behavior. Several reports have focused on the molecular systems by which the ECM interaction impacts on the Hippo signaling pathway to regulate YAP nuclear shuttling and its consequent co-transcriptional activity[1,2].

In the present work, we describe the mechanism by which the mechanotransducer YAP directly controls through its transcriptional activity both the deposition of extracellular matrix components and the assembly of the inner apparatus of cell-ECM interaction.

In fact, by exploiting ChIP-seq technology and YAP mutants obtained by CRISPR/Cas9 targeted approach, we unveil a number of targets of YAP-DNA binding activity that lead to the formation of membrane complexes devoted to the interaction with ECM including various integrin subunits like ITGA1, ITGA4, ITGAV and ITGB1, talin2, cadherins and catenins. At the same time, YAP binds DNA elements connected to the activation of genes encoding for ECM structural proteins like versican, collagens, laminins, fibronectin and osteonectin or involved in the processing of ECM components, like hyaluronan synthase 3, connective tissue growth factor (CTGF) and metallopeptidases.

As expected, YAP mutant clones underwent a substantial switch in the expression of genes involved in structural ECM composition and remodeling, thus leading to the complete absence of focal adhesions. As a consequence, cells failed to spread, invade and migrate through the surrounding matrix, when challenged in 2D and 3D assays and lose the ability to spread and acquire the given shape, develop tension through the cytoskeleton and exert force against the surrounding ECM.

Consistent with the model of YAP acting as a master of cell-ECM interaction, cell biophysical parameters were partially recovered by the re-expression of ITGAV integrin subunit in conjunction with ITGB3 subunit, two of the proteins being more affected in YAP-defective cells.

In conclusion, YAP functions as an important regulator of the cell-matrix interface, being able to control the expression of crucial genes involved in the composition and arrangement of the extracellular environment, together with key components of cell mechanosome. Moreover, these results pave the way for the design of novel biomaterials controlling cell adhesion and ECM deposition by tuning YAP expression.

0232 Propelling mesenchymal stem cell contributions to tissue regeneration and repair through spheroid formation

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Mesenchymal stem cells (MSCs) have tremendous potential for use in cell-based therapies of tissue repair and regeneration due to their proliferation, multilineage potential, proangiogenic capabilities, immune regulatory and anti-inflammatory potential, and relative lack of ethical concerns. Despite the promise of MSC-based therapies, previous studies have reported that the high death rate and poor engraftment of cells in ischemic conditions reduces the efficacy of stem cell therapy. Compared to individual cells, the formation of MSCs into spheroids enhances their survival, proangiogenic and anti-inflammatory potential. However, the fabrication and delivery of MSC spheroids to promote tissue repair is not a “one size fits all” approach, and effective strategies to optimize spheroid function for these applications are lacking. In this talk, I will highlight ongoing efforts by our laboratory to develop MSC spheroids for use in treating large bone defects and accelerating wound closure.

We form MSCs into spheroids containing 5,000-40,000 cells per spheroid *via* gravity-phased aggregation. Spheroids are entrapped in clinically relevant biomaterials such as fibrin, collagen, or alginate for delivery to large bone defects. MSC spheroids entrapped in RGD-modified alginate exhibit increased angiogenic potential and accelerate bone formation *in vivo*. The characteristics of the biomaterial further instruct spheroid function and accelerate bone healing, providing another opportunity to increase the therapeutic potential of MSCs. Specifically, biophysical properties such as adhesion ligand density and initial substrate stiffness are key parameters in guiding spheroid function. To improve the efficacy of MSCs applied for wound healing, we determined conditions for forming MSC spheroids that simultaneously enhance their proangiogenic and anti-inflammatory potential. We employed a Design-of-Experiments (DOE) multivariable analysis approach to determine the interaction between numerous input variables on MSC spheroids by quantifying secretion of VEGF and PGE_2. We validated our results by stimulating endothelial cells or macrophages with spheroid conditioned media, and then we tested optimized spheroids in a human skin equivalent (HSE) model of wound closure. DOE revealed a combination of variables to generate spheroids that simultaneously secreted potent levels of VEGF and PGE_2. Spheroids were significantly more effective in healing the wounded HSE than individual cells.

MSCs have significant potential to contribute to tissue repair beyond direct differentiation to a more specific phenotype. This talk will highlight some of our pursuits to capitalize on their therapeutic potential using engineered approaches.

0233 Microfluidic oil-free fabrication of multiple 3D platforms to screen cell-material interactions

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The micro encapsulation of mammalian cells within a semi-permeable hydrogel matrix is an attractive procedure for many biomedical and biotechnological applications. Thanks to controlled and automated systems it is possible to encapsulate cells in 3D microenvironments constituted by different biomaterials that can act as models to predict how cells respond. This would allow creating a library of materials to correlate the composition of the artificial extra cellular matrix to specific cell responses. A possible strategy to encapsulate cells is through the adoption of a microfluidic approach to create microdroplets of a hydrogel precursor in a non-miscible continuous phase (oil and surfactant). The droplets must be then crosslinked, removed from the oil and collected. This procedure is laborious given the presence of oil and the droplets are difficult to handle in the successive processing and characterization steps. We hereby propose a new oil-free approach compatible with the most common polymers crosslinking strategies (chemical, ionic, thermal and UV) and that surpass the constraints of the emulsion systems. Fibres embedding different cell laden droplets were fabricated with a flow-focusing microfluidic chip by pulsatile flow of polymeric solutions in the inner channel in anti-phase with the outer channels. The composition of the cell laden droplets was controlled by programmable flow sensors that allow an accurate regulation of the amount of the different components. The length of the droplets embedded in the fibre was also controlled by adjusting the flow rates of the solutions and their pulse periods. The fibres were extruded directly into an isotonic calcium chloride crosslinking bath for polymerisation. Ionically crosslinkable gellan gum was blended with marine derived collagen, chondroitin sulphate and hyaluronic acid at different concentrations to form the cell laden droplets whose compositions linearly change along the axis of the fibre. Alginate was used as the acellular portion of the fiber which allowed its degradation in alginase or dissolution with EDTA to release the individual compartments on demand. Human adipose stem cells encapsulated in the different compartments remained viable up to 21 days of culture mainly due to the width of the droplets, smaller than 400 µm, which does not compromise the diffusion of nutrients and removal of metabolites. Cell-laden fibres were also cultured in osteogenic differentiation medium and the degree of differentiation analysed, after immunocytochemistry, by confocal microscopy. Image analysis methods were employed to assess cell response to the different materials and the results were confirmed by quantitative standard methods after release of the individual droplets from the fibers.

Overall the herein proposed approach allows the fabrication of multiple 3D cell-laden hydrogel-based platforms that can be used for the screening of cell-materials interactions and selection of conditions for the development of improved tissue engineering approaches.

This work was supported by European Research Council grant agreement ERC-2012-ADG 20120216-321266 for project ComplexiTE.

0234 In vitro engineering of a human bone marrow organoid

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Introduction: Human Hematopoietic Stem Cells (hHSC) have the unique capability to give rise to all blood-cell lineages. In adults, hHSC reside in the bone marrow (BM) microenvironment -defined as the hHSC niche- responsible for the tight regulation of hHSC self-renewal, differentiation and blood tissue homeostasis. Improving our understanding of hHSC biology is greatly hampered by the scarcity of hHSC, but also by the lack of suitable in vitro culture systems both maintaining their intrinsic properties and recapitulating their microenvironment. In this study, we target the in vitro engineering of a functional BM tissue capable of maintaining hHSC properties, through the 3D reconstitution of their native microenvironment.

Methods: Human Mesenchymal Stromal Cells (hMSC) were seeded and cultured within porous hydroxyapatite scaffolds mimicking structural and compositional features of bone under direct perfusion flow, supporting cell nutrition and mimicking interstitial flow within bone marrow. After 4 weeks, the resulting 3D stromal cell network was seeded with Cord Blood derived CD34+ cells and cultured one further week under perfusion in serum-free medium. After enzymatic digestion and harvesting of the cells, the capacity of the 3D niche to promote hematopoietic progenitors maintenance and expansion was assessed by quantitative flow-cytometry, based on a CD34+, CD38-, CD45RA- and CD90+ panel of surface markers. Their functionality was evaluated in vitro by standard Colony Formation Unit assay and in vivo by intrafemoral transplantation in sublethally irradiated NSG mice. Imaging was used to decipher structural and cellular composition of the 3D niche.

Results: Stainings of the in vitro generated constructs revealed the successful generation of a complex tissue with compositional and morphological features resembling those of the human BM. This reconstituted “niche” consisted in a heterogeneous multi-cellular environment populated by terminally differentiated osteoblasts and hMSC progenitors directly interacting with human blood cells within the tissue architecture. One week of culture on the 3D osteoblastic niche resulted in a 14-fold increase expansion of phenotypic hHSC. Their functionality was confirmed in vitro by clonogenicity assays, indicating a 39-fold increase of the total number of cells able to generate multipotent (GEmM) colonies. The stem cell properties of the retrieved cells were finally confirmed by the ability to reconstitute human blood tissue at the long-term (27 weeks) following transplantation in sublethally irradiated mice.

Discussion & Conclusion: In this study we successfully validated the engineering of a 3D human bone marrow organoid capable to maintain and expand functional hHSC. This was achieved by the combined use of hMSC, synthetic scaffolds and a perfusion bioreactor system.The system represents an innovative model for the in vitro study of hHSC-hMSC interactions, healthy/malignant hematopoiesis and could ultimately impact on the development of new therapeutic strategies.

0235 Tissue O_2–related hypoxic microenvironment ensures the resistance of adipose-tissue derived stromal cells to acute hypoxic stress

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Mesenchymal stromal cells (MSCs) are of considerable interest due to their intrinsic role in ensuring the physiology and tissue repair and the needs of regenerative medicine. However, the adequate interpretation of experimental data and the effective application of these cells are not possible without the elucidation of the microenvironmental cues governing the MSC biology and the role of these cells in maintaining the integrity and remodeling of tissues after injury. “Physiologic hypoxia or physioxia” - low O_2 tension, is assumed as an important specific feature of MSC local milieu, which is driving MSC functional activity in physiologic and reparative remodeling. Acute hypoxic stress is considered as one of the important hampering factor in tissue damage. We have applied permanent expansion of adipose-tissue derived stromal cells (ASCs) at physioxia (5% O_2) to simulate tissue O_2 level and evaluate the susceptibility/resistance of tissue-O_2-adapted ASC to acute hypoxic stress (less than 1% O_2). After hypoxic exposure ASCs retained high viability, stromal cell morphology, mesenchymal phenotype (CD73+, CD90+, CD105+, CD45-). Mild oxidative damage was unveiled as elevation of ROS and TBA-active products, while no reduction in the activity of antioxidant enzymes catalase and glutathione peroxidase and 20% statistically significant increase in superoxide dismutase (SOD) activity was detected. Expression of *HIF1A* and *HIF3A* isoforms were differently regulated. *HIF1A* displayed transient up-regulation with a maximum after 30 min of acute hypoxic exposure, while the *HIF3A* was significantly up-regulated after 24 hrs. The up-regulation of *ERK7*, *MEK1*, c-fos and down-regulation of *MKK6*, p53,*Cyclins A2,B,B2* was observed after 24h of oxygen deprivation. Acute hypoxic exposure didn’t affect the gene expression of other MAPKs and their kinases, MAPK/ERK kinase interacting proteins, MAPK-activated transcription factors and scaffolding proteins. Tissue-O_2-adapted ASCs displayed high expression of Wnt signaling associated genes: *Dkk1*, *RHOA*, *CSNK1A1*, *SNK2A1*,*GSK*3β, and *PORCN*. *FZD1*, *FZD3*, *SFRP1*, and *SFRP4* genes were upregulated after short-term hypoxic stress, whereas the expression of *LEF1* and *RUVBL1* genes was significantly down-regulated. The data suggested that Wnt signal transduction was suppressed under hypoxic stress, whereas the noncanonical Wnt signaling prevailed under the “physiological hypoxia” (5% O_2). Significant stimulation VEGFα and IL-6 production was detected in ASC conditioned medium. These data allowed us to suppose that physioxia-adapted MSCs are resistant to hypoxic stress and can be effectively involved in the regeneration of tissue damage under significant oxygen deprivation.

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0236 Unravelling the path to create a cell sheet-based model of skin scar-like tissue

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Regardless of the advances in understanding the mechanisms and the pathophysiology behind skin deformities, scaring continues to be an unsolved clinical problem. The underlying wound healing process involves a series of key cells which play different key roles. Fibroblasts are known to suffer the influence of local biochemical (e.g TGF-β1) and biomechanical signaling upon a wound scenario leading to a phenotypical change into myofibroblasts. The latter enhance immature extracellular matrix (ECM) synthesis and generate tensional forces that leads to ECM reorganization. Certain skin pathologies (e.g hypertrophic scars) rise from a dysfunction of this underlying regulatory mechanism which in turn drives myofibroblast persistence in the wound. When trying to study the mechanisms behind scarring human ex vivo samples are many times scarce and most of the current in vitro systems rely on standard 2D cultures of keloid/hypertrophic scar fibroblasts. Taking all of this into consideration we propose the use of cell sheet technology to create an *in vitro* 3D scar model. Herein we report the effect of TGF-β1 in human dermal fibroblast cell sheets as the first step to attain cell sheets with a myofibroblast-like phenotype in which cells are embedded in a scar-like ECM. To further strengthen our concept we performed the stacking of pre-formed cell sheets generating a cohesive 3D scar-like tissue.

Human dermal fibroblast (hDFbs) cell sheets were produced as previously described[1], and stimulated with TGF-β1 (10ng/ml) over 7, 14 and 21 days. Following phenotype and ECM characterization, cell sheets were stacked in order to obtain a 3D structure composed of 2 or 3 cell-sheets. The analysis of key genes (q-PCR) and proteins (Western blot and immunocytochemistry) showed that hDFbs cell sheets, when stimulated with TGF-β1 present an increased expression of α-SMA, fibronectin (FN) ED-A and FN ED-B, characteristic of a myofibroblast-like phenotype. When looking into the expression of scar ECM-associated proteins, hDFbs cell sheets obtained in the presence of TGF-β1 produced higher amounts of fibronectin and collagen I. Stable 3D constructs with a noticeable level of integration after a total of 21 days of culture, were further created upon stacking of the cell sheets obtained after 7days of culture in the presence of TGF-β1.

In conclusion, this work suggested that it is possible to promote the secretion of scar-like ECM in hDFbs cell sheets due to phenotypic changes into myofibroblast-like cells when stimulated with TGF-β1. Cohesive 3D scar-like tissue structures were obtained which opens the possibility to develop a highly accurate *in vitro* 3D scar model to study underlying cellular mechanisms involved in the wound healing deregulation.

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0237 A novel compression-based microbioreactor towards the modelling of osteoarthritis in a chip

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Osteoarthritis (OA) is the musculoskeletal disease with the highest prevalence, and yet no satisfactory therapies are currently available [Bijlsma, 2011]. The development of reliable *in vitro* OA models would, therefore, help in better understanding disease pathogenesis and in developing possible new therapies. In the present study, we present a PDMS-based microbioreactor, which mimics the OA joint environment, enabling the application of both mechanical and biochemical stimuli. In particular, the device is tailored to provide cells, cultured in a 3D hydrogel matrix, with defined compression levels, either 10% or 30%, while allowing the expulsion and intake of fluid that is proper of physiological joints.

The OA microbioreactor builds on a previously reported platform for the generation of 3D cardiac microconstruct [Marsano, 2016]. Briefly it comprises two integrated compartments, separated by a thin, flexible membrane. The upper compartment consists in a central channel hosting the 3D cell-laden hydrogels and divided by two series of overhanging posts from two lateral channels dimensioned for biochemical conditioning. The bottom compartment consists in a pneumatic chamber, which is actuated by a custom-made control unit, programmable in terms of compression frequency, duration and pattern.

The shape of the posts was specifically designed to limit the lateral expansion of the cell-laden hydrogel, thus ensuring its confined compression. To this aim, a computational model was implemented to evaluate the stress and strain fields distribution. A poro-elastic constitutive model was used for the cell-laden hydrogel. The achievement of a pure compression was confirmed by the 25^{th}, 50^{th} and 75^{th} percentiles of the lateral expansion, which resulted, respectively, in 0.7%, 1.6% and 2.2% at most (i.e. in correspondence to a 30% compression level). Deformation experienced by cells was also characterized experimentally, verifying the match with the computational results.

The OA microbioreactor was exploited for investigating the potential of human nasal chondrocytes (NCs) as cell source for treating OA. NCs have been embedded within synthetic PEG hydrogels and cultured within the platform. Pilot tests revealed optimal cell viability and considerable matrix deposition after two weeks of static culture. Furthermore, the effect of different compression levels and regimens caused altered levels of Coll II to Coll I ratios and MMP 13 RNA expression. Combination of mechanical loading with inflammatory environment (i.e. IL-1b, TNFa) is currently under investigation.

In conclusion, the presented platform allows recapitulating in a chip physiological/pathological compression levels experienced by cells in the joint microenvironment, in a controlled fashion and superimposed to biochemical factors. It is thus a promising medium-throughput tool for *in vitro* modelling OA, aiming at further investigating its mechanism and screening over the effect of anti-OA drugs.

0238 Engineering macromolecular self-assembly of hyaluronan with peptides: from matrix design to biomedicine applications

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Hyaluronan (HA) has attracted considerable interest in a wide range of biomedical applications due to its unique physicochemical properties (high molecular weight, hydrophilicity, inherent biodegradability and biocompatibility), associated with its complex interactions with extracellular matrix components and cells. The dynamic flexibility of HA, associated with its simplicity, make it a versatile macromolecular template to create supramolecular biomaterials. Our group is interested in developing HA-based biomaterials (membranes and hydrogels – Fig 1) by using supramolecular crosslinking self-assembling peptides [1-4].

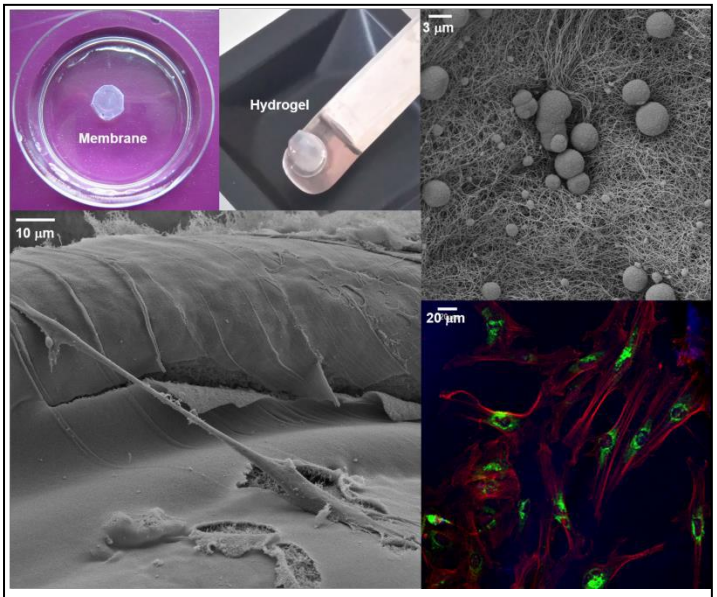

Fig. 1 - Self-assembling HA-peptide matrices

This talk will cover our efforts on the molecular design of peptides for the supramolecular display of HA in 2D and 3D biomaterials and their applications in cell culture and tissue regeneration. This design includes macromolecular recognition elements (e.g. HA-binding motifs) and bioactive signals for cells to adhere, spread or differentiate.

0239 Presentation cancelled on-site

0240 Studying dynamic processes in peptide amphiphile self-assembly using super-resolution microscopy

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Introduction

Peptide amphiphile (PA) hydrogels have been extensively used as a platform for regenerative medicine applications.[1] These hydrogels are composed of entangled cylindrical nanofibers, which are formed by reversible non-covalent interactions. Therefore, we hypothesised that PA molecules exchange between nanofibers, which has important implications in the biological function and future design of these hydrogels.

Results

A PA consisting of a palmitic acid tail, a β-sheet forming segment (six alanines), and negatively charged solubilizing moieties (three glutamates) was synthesized by using fmoc solid-phase chemistry (Palmitoyl-AAAAAAEEE-NH2). Fluorescently labelled PAs were synthesised coupling sulfonated cyanine dyes (Cy3, Cy5) to a sacrificial lysine added at the N-terminus. Circular Dichroism (CD) spectra showed the typical beta-sheet signature. PAs self-assembled in cylindrical nanofibers (diameter ~7nm, length in the µm range), either alone or co-assembled with labelled PAs, as observed by Cryo-TEM. Molecular exchange was studied using stochastic optical reconstruction microscopy (STORM), together with image analysis methodologies previously reported by us.[2] First, non-labelled PA was co-assembled either with Cy3 or Cy5-PA. After, aqueous solutions of single-colour nanofibers were mixed (t=0), and the extent of PA mixing was determined at several time points. Molecular exchange of labelled PAs between nanofibers was detected up to 48h. The distribution of both labelled PAs remained constant throughout the experiments, consistent with an exchange mechanism of single molecules or unordered aggregates smaller than STORM imaging resolution (~50 nm). Finally, the extent of exchange varied considerable for different nanofibers, revealing an unexpected structural diversity. These observations were only possible by the ability of STORM to perform multicolour imaging of individual nanofibers.

Conclusions

We revealed the molecular distribution and dynamic nature of PAs in assemblies. We foresee that this information can be used to generate adaptable hydrogels with improved biomedical function, able to interact dynamically with cells.

Acknowledgments

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0241 Multi-functional self-assembling hydrogels as biomimetic scaffolds for protein delivery and stem cell culture

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Sulfated glycosaminoglycans (GAGs) are known to be involved in the sequestration and controlled release of growth factors (GFs) in the extracellular matrix thus regulating many cellular activities, such as proliferation and differentiation[1]. The sulfate groups typically serve as multiple binding sites for positively charged regions of GFs[2]. Recapitulating this scenario has been an important goal in GF-mediated regeneration. Here, we present a multifunctional hydrogel formed by self-assembly between the negatively charged polystyrene sulfonate (PSS) and a positively charged peptide amphiphile (PA) aimed to create a biomimetic 3D environment for the controlled differentiation of stem cells.

Combining an aqueous PSS solution with a positively charged PA[3] results in the formation of a self-supporting gel (Fig 1A) with a random network of nanofibers (Fig 1C). PSS was shown to direct the nucleation and growth of calcium carbonate minerals by binding to calcium ions[4]. Thus, these gels were investigated for their ability to promote the nucleation of hydroxyapatite and create a mineralized matrix resembling the structure of natural bone.

Incubation in osteogenic medium promotes the formation of calcium phosphate mineral on the nanofibrous scaffold, as confirmed by SEM and EDS analysis (Fig 1C). While, the release profile of the BSA-FITC loaded in these gels (Fig 1D) shows that this acidic protein is completely released from the PSS/PA gels in the first 96 hours (Fig 1E), a more controlled release is expected for basic proteins. When culturing mesenchymal stem cells (MSCs) within these gels, good levels of cell viability are observed when compared to cells growing in 2D monolayer (Fig 1F).

The nanofibrillar structure and the mineralizing ability of PSS/PA gels provide a biomimetic environment for growing stem cells in 3D and an opportunity to stimulate their differentiation into bone cells.

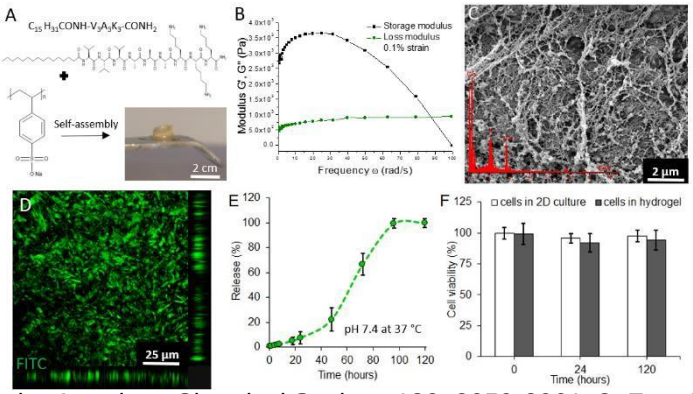

Fig 1 (A) Chemical structure of PA and PSS, and their self-assembly into self-supporting hydrogel; **(B)** Rheological properties of the PSS/PA gel; **(C)** SEM and EDS analysis of the gel after 14 days incubation in osteogenic medium; **(D)** Gel incorporating fluorescently (FITC) labelled BSA; **(E)** BSA release profile; **(F)** Viability of MSCs within the gels.

References: 1. Azevedo and Pashkuleva 2015. Advanced Drug Delivery Reviews, 94, 63-76. **2.** Rawat et al. 2008. Journal of the American Chemical Society, 130, 2959-2961. **3.** Ferreira et al. 2013. Biomaterials Science, 1, 952-964. **4.** Smeets et al. 2015. Nature Materials, 14(4), 394-399.

0242 Artificial bone cells as support for osteoblast cells to enhance biomineralization

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Load-bearing implants such as artificial hip or knee joints require a stable interface with bone tissue. Bioactive glass or bioactive ceramics as bulk implants or as surface coatings have so far proven to be the most successful concepts used in clinics due to their ability to induce osteoconduction or even osteostimulation. However, these ceramic and glass implants are difficult to fabricate and suffer from selective mechanical properties. Here, we aim to improve the osteoconduction of bone-forming osteoblast cells by providing a triggered mineralization at the bone tissue-implant interface. Specifically, we develop a kick-start for osteoblasts to produce extracellular matrix (ECM) and mineralization and, thus, provide a faster integration of implants within bone tissue. Our approach involves the assembly of artificial bone cells, which are composed of ECM components and matrix vesicles (MVs). Both components are important for the initial mechanism of bone mineralization. MVs are 100 nm-sized phosphatidylserine (PS)- and alkaline phosphatase (AP) -containing liposomes secreted by osteoblasts. Calcium phosphate (CaP) crystals are formed within MVs and adhere to the ECM of bone tissue, where further mineralization takes place on collagen fibrils. Our artificial bone cells are assembled in two different ways to form “soft” and “hard” microparticles using droplet-microfluidics to form agarose (hydrogel) microbeads and the layer-by-layer technique to assemble core-shell particles, respectively. Our key requirements are that the artificial bone cells i) have similar sizes to biological osteoblasts, ii) are composed of collagen fibers and iii) contain artificial MVs composed of PS-containing liposomes with encapsulated AP enzymes. AP cleaves phosphate ions from phosphomonoester substrates, which can be added to the cell media and induces CaP crystallization. Furthermore, the particle surface is crucial for cell adherence and cell interaction. Polydopamine, poly-L-lysine, and growth factors are employed as surface coatings. The “soft” and the “hard” artificial bone cells are compared by coculturing them with bone-forming Saos-2 cells (sarcoma osteogenic cells). The rate of mineralization is assessed by quantifying the produced mineral content and the number of cells in presence or absence of the artificial bone cells. We anticipate that our approach has the potential to enhance osteoblast-mediated bone formation and that our artificial cells can be used in different forms and shapes, not only as spheres, but also as surface coating.

0243 Characterization of a cell-assembled matrix (CAM) in the development of a completely biological tissue-engineered vascular graft

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Autologous veins and arteries are the preferred conduits for bypass surgery in small diameter applications but, because patients typically suffer from chronic arterial diseases, autologous vessels are often unavailable due to poor quality or repeated procedures. In these cases, synthetic vascular grafts are the only option. However, the use of these grafts is limited to large- and medium diameter blood vessels replacements because they promote infection, fibrin deposition and thrombus formation, which lead to failure at smaller diameters. To create a biological graft, sheets of cell-assembled matrix (CAM) were obtained by culturing normal human skin fibroblasts during 8 weeks with ascorbic acid and 20% foetal bovine serum. While vascular grafts produced from these sheets rapidly progressed through pre-clinical and clinical studies (McAllister, T.N. *et al.*, Lancet. (2009) ; Wystrychowski, W. *et al*, J. Vasc. Surg. (2014)), a detailed analysis of the CAM itself was never not performed. To characterize the composition and organization of this CAM, we have used mass spectrometry, histology, immunolabeling, transmission electron microscopy (TEM) and second harmonic generation (SHG). The CAM was composed of a complex laminar network of matrix proteins, including a dense collagen network with embedded fibroblasts. Indeed, all the families of collagens were present: fibril-forming collagens (collagen I and III), beaded filament (collagen VI) and FACITs (fibril-associated collagens with interrupted helices) (collagen XII and XIV). Components of elastic fibers were also present (fibrillins-1, fibulins-2, EMILIN-1 (elastin microfibril interface located protein)). Finally, ECM components associated with fibrillogenesis and cell-ECM interactions were also identified (tenascin C, periostin, galectin 3, fibronectin-1, decorin and TGF-beta induced protein). SHG and TEM results showed that collagen fibers were almost exclusively aligned in the plane of the sheet but that their orientation was overall anisotropic within that plane which followed fibroblasts orientation. TEM analysis revealed that collagen fibers diameter was 47 ± 3 µm and that the density varied through CAM thickness (56 ± 30 fibers/mm²) with alternating fiber-dense layers (134 fibers/mm²) and cell-rich layers (9 fibers/mm²), to create a laminar distribution. Previous CAM-based grafts were produced by rolling sheets and promoting their fusion in a bioreactor during an extended culture period. To accelerate the vessel formation process, the next generation of CAM-based grafts will be produced using a textile assembly principle. In that aim, strips (3 – 10 mm) were cut in the sheets and processed to form a yarn. Uniaxial break strength varied from of 40 ± 6 gf to 442 ± 43 gf depending on cell line and processing steps. ECM organization of the yarn will be compared to that of the native CAM to see how the various processing steps affected its morphology. The next step will be to characterize the inflammatory response and the *in vivo* remodeling of human yarns processed in various ways in nude rats. This detailed analysis of CAM organization and composition can provide important clues to understand its mechanical and biological properties both *in vitro* as well as *in vivo*, and will guide our design strategy in the development of the next generation of completely biological tissue-engineered vascular grafts.

0244 The role of tissue engineering in developing preclinical pipelines for treating skin cancer

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The amplification potential of keratinocyte culture methods developed by Rheinwald and Green in 1975 was soon recognized to be highly applicable to clinical practice as sheets of autologous keratinocytes(CEA) were first applied to burns patients in 1981 and subsequently to a variety of chronic wounds. This technology has now expanded as ex vivo gene therapy for hereditary skin diseases notably recessive dystrophic epidermolysis bullosa and such grafts can also be generated using ips technology. However the skin is a complex organ and bears many specialized structures including hair follicle and so much effort has been expended in intervening years to produce complex tissue engineered products including dermal matrices and mesenchymal cells. As the epidermis in CEA is fragile and poorly differentiated, more robust “Skin equivalent “ or organo-typical cultures would have multiple clinical applications. There have been numerous variations on this theme and multiple dermal and skin equivalent models have now also gone into clinical practice.

Keratinocyte skin cancers (KSC) can be cultured in monolayer cultures from primary tissue and when placed in organo-typical culture provide robust in vitro invasion assays. Combinations with cancer-associated fibroblasts will enhance tumour invasion. Classical genetically manipulated animal models vary in their usefulness in studying human disease and this is particularly the case in KSC where TPA-DMBA mice are a good model of mutant RAS signaling, which has been found to be uncommonly mutated in human tumours. As we understand more about the genetic landscape of cSCC, we can design more appropriate models targeting keratinocyte stem cells in hair follicle and inter-follicular epidermis. Patient derived cancer cell lines can be used as subcutaneous xenografts for drug testing. However skin equivalent cultures of cSCC can be used as surface xenotransplants in immunosuppressed mice and recapitulate themorphology and athology of the primary tumour. Therefore a preclinical pipeline can be visualized as primary tumour keratinocyte cultures for high throughput screening leading to assays of invasion in skin equivalent models through to genetically engineered mouse models, with patient-derived xeno-grafts and surface xeno-transplants for drug testing.

0245 Engineering complexity into 3-dimensional in vitro models of cancer: Cancer tissue mimetics and their applications

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Recognition of the heterogeneic nature of the cancer microenviroment has led to a drive away from “flat biology” 2D cell culture to 3D biomimetics [1,2]. This fast developing field is driven by two major imperatives: (i) The requirement for laboratory mimetic models to enable interrogation of genetic, molecular and cellular events in real time to understand cancer biology better; (ii) The demand for test platforms to assess drug responses that are translational and relevant to the clinic. Our aim was to create a biomimetic cancer model suitable for interrogating both disease pathophysiology and treatment efficacy.

We have tissue engineered a 3D in vitro model of cancer (tumouroid) which recapitulates the microarchitecture of solid cancers. Tumouroids comprise a central cancer mass, manufactured by mixing cancer cells and collagen 1 (as the basic extracellular matrix protein) and applying plastic compression to the resulting gel, to increase tissue density to physiological levels. The cancer mass is nested into a larger connective tissue compartment, made of collagen 1, fibroblasts, endothelial cells (to mimic cancer stroma); the two-compartment construct can be compressed to specific parameters.

Applications include: 1. Manipulation and characterisation of key aspects of cancer cell pathophysiology in tumouroids. Specifically, using colorectal cancer tumouroids, an increase in controllable tissue density (up to 10% collagen) changes invasive profiles of the cancer mass, from a pattern of spheroids budding off and invading the stroma, to a pattern of invasive sheets. While the addition of laminin (nM-mM) drives cell-cell fusion of endothelial cells within the stromal compartment to create a primitive vasculature-like network.

2. Response to treatments. Using prostate cancer tumouroids as exemplar, responses to classical chemotherapeutics can be described at the level of the tissue (e.g., metabolic activity assays); the cancer cell (e.g., PSA biomarker production) and at the subcellular level (cell cycle states using FACS). Importantly, by incorporating nanotechnology (e.g., fluorescent quantum clusters) conjugated to drugs, drug fate can be visualised at the cellular level and within the 3D volume, an element of novelty not realised in in vivo models, allowing in depth investigations that can drive and speed preliminary cancer drug screening.

3. Patient derived tumouroids. Incorporation of primary cells from cancer tissues excised at surgery has been approved for different cancer types. The aim is to create platforms for testing drugs (for responders/non-responders) towards stratification of treatment for each patient.

Tissue engineered tumouroids provide a powerful and sophisticated tool for basic and translational research.

0246 Investigating the interactions between breast cancer cells and bone microenvironment by a polyurethane foam 3D *in vitro* model

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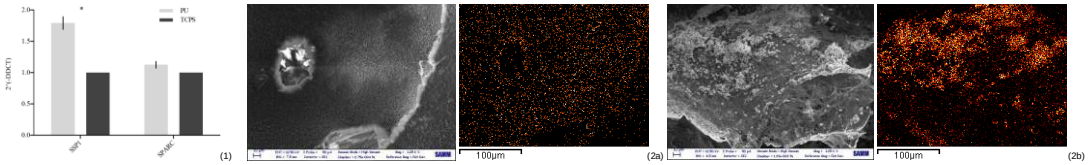
Breast cancer represents the most incident cancer case in women (29%), with high mortality rate. Bone metastases occurs in 20-50% cases and, despite advances in breast cancer research, the interactions between tumor cells and the bone metastatic microenvironment are still poorly understood. *In vitro* 3D models gained great interest in cancer research thanks to the reproducibility, the 3D spatial cues and associated low costs, compared to *in vivo* and 2D *in vitro* models.

In this study, we investigated the interactions between breast cancer stem cells and the bone microenvironment by using a poly-ether-urethane foam (PU) as 3D *in vitro* model.

The PU foam used as an *in vitro* model was synthesised by reacting an optimised poly-ether-polyol mixture with isocyanate MDI prepolymer, using Fe-AcetylAcetonate as catalyst and water as foaming agent[1]. The measured PU foam open porosity (> 70%) proved to be suitable to mimic the trabecular bone structure. PU foam showed good and stable mechanical properties under cyclic compression, even if lower than human trabecular bone. The *in vitro* bone metastasis model was produced by seeding on the PU foam patient-derived human adipose derived stem cells (ADSCs) and by differentiating them into osteoblasts for four weeks; subsequently, breast cancer derived stem cells (MCFS) were co-cultured on the PU foam with differentiated ADSCs for three weeks. Successful ADSCs attachment and elongation was shown by hematoxylin-eosin staining. Moreover, efficient osteoblastic differentiation was proved both by inorganic matrix deposition evidenced by alizarin red staining and RT-PCR (Fig1); a significant increase of osteopontin levels was shown in cells differentiated on PU foam compared to those differentiated on control tissue culture plastics (TCPS), suggesting that the PU foam can recreate a more physiological-like and biomimetic microenvironment for osteoblastic differentiation than TCPS surface.



(1) (2a) (2b)

Tumor cells agglomerates were identified on PU foam co-cultured with ADSC/MCFS by e-cadherin staining. SEM/EDX images showed a homogeneous and well-distributed deposition of Ca and P sub-micrometric particles on PU foam seeded only with ADSCs (Fig2a, Ca in red), confirming osteoblastic differentiation. On the contrary, when breast cancer derived stem cells were co-cultured with differentiated ADSCs, tumor cells agglomerates were observed (Fig2b) and a qualitative desorption of Ca and P particles in some areas of the PU foam pore walls was detected. The PU foam demonstrated to be a suitable model for reproducing a bone biomimetic microenvironment *in vitro*, allowing for the co-culturing of differentiated ADSCs and MCFS, and for the investigation of their interaction.

0247 Investigating the role of monocytes in adoptive T cell-mediated cancer immunotherapy

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The recent breakthrough of immunotherapies currently in clinical trials for cancer treatment has hastened the development of techniques to accelerate the translation of efficient therapies to the clinic. Adoptive T cell-mediated cancer immunotherapy is based on the isolation, genetic engineering and reinfusion into patients of CD8+ T cells that target cancer cells by expressing cancer-specific antigen receptors. Although immunotherapeutic approaches are currently in clinical trials for the treatment of liquid tumours, the use of T cell therapy for solid tumours is slow mainly because of the hostile tumour microenvironment (TME). In fact, the components of the TME as stromal and immune cells, extracellular matrix (ECM), lymphatic vessels and blood vessels are known to limit T cell interactions with cancer cells. In particular, tumour-associated monocytes may play a suppressive role against the T cells but the mechanisms of their interaction is far from clear, especially in the context of engineered T cells. Our work focuses on investigating the role of monocytes on the anti-tumor activity of T cells transduced by viral vector to express the hepatitis B virus (HBV)-specific receptor (TCR) to target HBsAg-expressing human hepatocellular carcinoma (HCC) cells. Importantly, our study is performed on a microfluidic platform that allows us to (i) co-culture multiple cells in 3D to investigate their interactions, (ii) test T cell anti-tumor efficacy in a variety of inflammatory or oxygen conditions similar to those found in the TME and, (iii) screen different ways to engineer T cells. Interestingly, we found that the presence of monocytes together with HCC cells and HBV-TCR-T cells reduced the anti-tumor activity of the engineered T cells and, by blocking programmed cell death–ligand 1 (PD-L1) in the system with an anti-PD-L1 antibody, the efficacy of T cells was partially restored. Therefore, the PD-L1 expressed by the monocytes by engaging the immune checkpoint PD-1 receptors on the T cells may be, at least partially, responsible for the reduced T cells\ activity against the cancer cells. These results from the microfluidics experiments show the possibility to study physical and molecular mechanisms of cancer-immune cell interactions and open the tremendous opportunity to rapidly test and improve the engineered T cell efficacy prior to clinical translation.

0248 Engineered microfluidic 3D human microvascular models identify novel therapeutic targets affecting cancer cell extravasation

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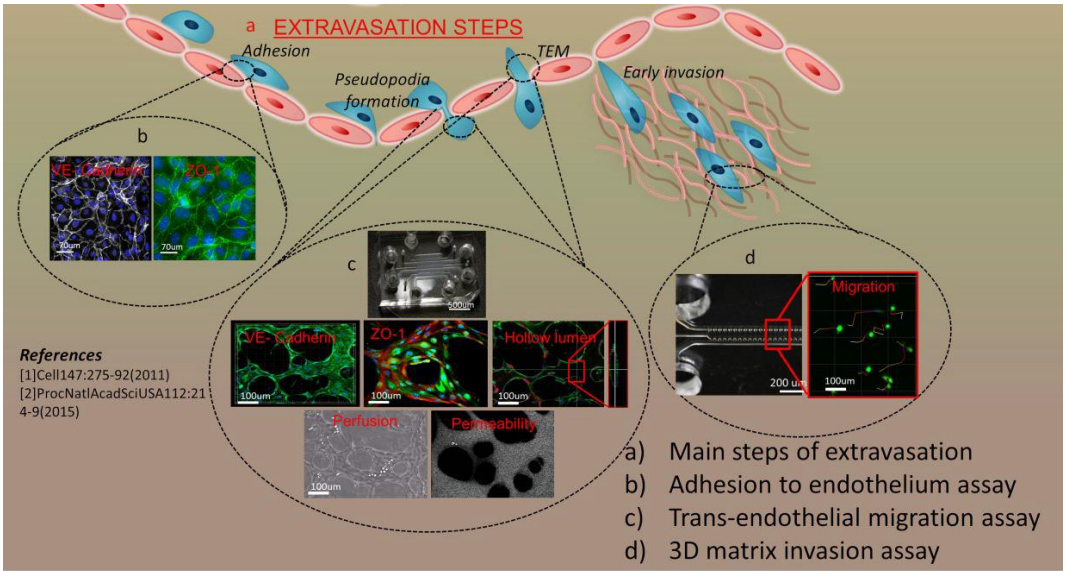
Metastasis are responsible for 90% of cancer related deaths [1]. Understanding the molecular mechanisms underlying cancer cell-perivascular niche interactions will be fundamental to develop new anti-metastatic strategies. Animal studies suggested that metastatic dissemination lies in focal adhesion alterations. Talin-1(TLN-1) and Focal Adhesion Kinase (FAK) are focal adhesion proteins overexpressed in breast cancer and linked to metastatic spread. However, it still needs to be elucidated which is their role in each step of the metastatic cascade. Given the complexity of the interactions at the interface between cancer cells and endothelial wall, it is necessary to go beyond conventional *in vitro* 2D models and analyze through physiologically relevant vascularized 3D models each step of extravasation from adhesion to trans-endothelial migration (TEM) and early invasion. Here, we generated advanced 3D microfluidic models to quantitatively analyze the role of TLN-1 and FAK and their genetic and chemical inhibition in MDA-231 breast cancer cell extravasation [2] (Figure 1). Both KD induced significant changes in cell morphology, cell adhesion to conventional plastic surfaces and cell proliferation. Noteworthy, both TLN-1 KD and FAK KD breast cancer cells showed a significant reduction of adhesion to the endothelial wall ($p<0.001$), TEM ($p<0.05$) and invasion of a 3D matrix ($p<0.001$). Moreover, high resolution imaging demonstrated that TLN-1 and FAK localize to cancer cell pseudopodia. Strikingly, collected data showed the requirement of the structural role of TLN-1/FAK in pseudopodia formation and cytoskeletal organization instead of their activation.

Concluding, our results supported by *in vivo* data ($p<0.05$) demonstrate that TLN-1 and FAK inhibition may represent a novel strategy to tackle the metastatic dissemination.



0249 *In situ* cellular characterization of pancreatic cancer cells in a highly porous 3D scaffolding system

Stella Totti[1], Mark Allenby[2], Susana Brito Dos Santos[2], Athanasios Mantalaris[2], Eirini Velliou[1]

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INTRODUCTION:

Pancreatic cancer, is a highly lethal disease and it is the fifth leading cause of all cancer related deaths in the UK and the eighth worldwide[1]. Advances in tissue engineering point that 3D scaffold systems can better reflect the *in vivo* tumour micro-environment and can guarantee a physiological distribution of oxygen, nutrients and treatment (i.e. chemotherapy, radiotherapy), therefore, being promising low cost tools for pancreatic cancer screening[2,3]. We have previously developed a 3D highly porous scaffolding system which supports growth of pancreatic cancer[2,3]. In particular we have reported long term *ex vivo* cultivation of pancreatic cancer cells –for up to 7 weeks- in a highly porous polymeric scaffolds with appropriate extracellular matrix protein coating (laminin, fibronectin). We have also seen that the pancreatic cancer kinetics are influenced by the extracellular matrix cocktail coating of the scaffolds, especially towards the end of the culturing period[3].

The aim of the current work is to perform *in situ* cellular characterisation of the *ex vivo* 3D tissue, in order to gain insightful information on the cellular development and the cellular properties in 3D.

METHODS:

Different pancreatic cancer cell lines, i.e., AsPC-1, PANC-1, BxPC3, were seeded in highly porous polymeric (polyurethane) scaffolds coated with different extracellular matrix proteins (laminin, fibronectin). The viability of the cells was monitored *in situ*. Evaluation of cell distribution and adhesion within the different scaffolds was conducted with Scanning Electron Microscopy (SEM). *In situ* cell proliferation, extracellular matrix protein distribution as well as oxidative stress distribution (hypoxic vs normoxic regions) took place with confocal microscopy imaging of different scaffold sections. Furthermore, quantitative assessment of the overall extracellular protein production took place through measurement of the protein in the spent medium.

RESULTS & DISCUSSION:

We previously reported the successful long term growth of pancreatic cell lines in 3D scaffolds with the growth kinetics being a function of the extracellular protein cocktail[3]. Confocal microscopy verified that the pancreatic cells were viable and the majority of the population was Ki-67 positive up to the culture endpoint and throughout the whole scaffold. Additionally, the cell population which was closer to the edges of the scaffolds was HIF-1a negative, and a small region of hypoxic cells (HIF-1a positive) was present at the centre of the scaffold. Moreover, a significant amount of extracellular proteins was produced by the cells up to the end of the culturing period.

Our findings indicate that our 3D platform is a promising tool for pancreatic cancer studies as well as treatment screening, as it allows the long term cultivation of pancreatic cancer cells in an environment which enables the 3D development of a culture with physiological properties and environmental stress population areas which mimic closely a solid *in vivo* tumour.

0250 Engineering human heart muscle for in vitro testing and cardiac repair

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The discovery of human induced pluripotent stem cell (hiPSC) technology and improvements in protocols to differentiate cardiomyocytes from hiPSC-derived cardiomyocytes (hiPSC-CM) have opened new perspectives for cardiac biology. A current shortcoming is the limited maturity of hiPSC-CM. We have developed methods to generate 3-dimensional heart muscle strips from hiPSC-CM (engineered heart tissue, hEHT) and showed that CM develop an advanced degree of cardiac maturity, both structurally and functionally. Human EHT show canonical responses to a variety of drugs with known effects on cardiac repolarization, force and contraction kinetics. Viral overexpression of fluorescent calcium and voltage sensors allow parallel assessment of calcium transients, action potentials and force. Expression of channelrhodopsin makes hEHTs sensitive to pacing by blue light and allow long-term pacing. Human EHTs may also serve a patches for heart muscle repair after myocardial infarction. A study in immunosuppressed infarcted guinea pigs showed that hEHT form large human heart muscle grafts within the scar and improved LV function 4 weeks after transplantation. Efforts are underway to translate this concept towards first-in-man application.

0251 3D tissue printing for myocardial repair

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University Medical Center Utrecht, Utrecht, The Netherlands

Cardiac cell therapy suffers from limitations related to poor engraftment and significant cell death after transplantation. Tissue engineering is emerging as a potential therapeutic approach to overcome limitations of cell therapy, like cell retention and survival, as well as to mechanically support the ventricular wall and thereby prevent dilation. Tissue printing technology (TP) offers the possibility to deliver, in a defined and organized manner, scaffolding materials and living cells. The aim of our studies was to evaluate the combination of TP, human cardiac-derived cardiomyocyte progenitor cells (hCMPCs) and biomaterials to obtain a construct with cardiogenic potential for *in vitro* use or *in vivo* application.

Printed tissues demonstrated an homogenous distribution of cells in the scaffold *in vitro*. Cell viability was determined after printing and showed a high degree of viability at 1 week of culturing. Printed hCMPCs retained their commitment for the cardiac lineage and could migrate from the alginate matrix and form tubular-like structures. By applying the 3D-printed patch on a mouse model of myocardial infarction (MI), we tested the therapeutic potential of a matrix that is composed of human cardiac-derived progenitor cells (hCMPCs) in a hyaluronic acid/gelatin (HA/gel) gel. The application of the patch led to a significant reduction in adverse remodeling and preservation of cardiac performance as was shown by both MRI and histology. Furthermore, the matrix supported the long-term in vivo survival and engraftment of hCMPCs, which exhibited a temporal increase in cardiac and vascular differentiation markers over the course of the 4 week follow-up period.

Overall, we developed an effective and translational approach to enhance hCMPC delivery and action in the heart which could be used to enhance the therapeutic effects of local myocardial cell applications.

0252 High throughput generation of quiescent valvular interstitial cell micro-aggregates for biofabrication of heart valve tissue

Annelies Roosens, Inès Puype, Charlot Philips, Ria Cornelissen

Ghent University, Ghent, Belgium

INTRODUCTION The major challenge of working with valvular interstitial cells (VIC) in tissue engineering is the preservation or recovering of their native quiescent state, because VIC phenotype changes could cause progression towards diseases. In this study, a biomimetic approach is used which aims to engineer small volume, high quality VIC aggregates that can be used as building blocks for the biofabrication of heart valve tissue. In addition, we hypothized that this aggregate culture, which mimics more closely the actual *in vivo* microenvironment, can stimulate VIC to a functional homeostatic quiescent state.

MATERIALS & METHODS To form micro-aggregates, $1*10^6$ porcine VIC were seeded in agarose micro-wells (1590 pores, diameter 200µm) and cultured in standard medium supplemented with 250µM Ascorbic Acid 2-phosphate (AA) for 22 days. Aggregate formation, morphology, phenotype and ECM production was analyzed by various techniques; (immuno-) histochemical methods, quantitative biochemical assays and RT-qPCR.

RESULTS Histology showed viable aggregates with normal nuclei and without any signs of calcification. Aggregates stained strongly for GAG and collagen I and reticular fibers were present. ECM formation was quantified and showed a significant increase of GAG, elastin and Col I during the aggregate culture. Cultivation of VIC in aggregates also promoted mRNA expression of Col I/III/V, elastin, hyaluronan, biglycan, decorin, versican MMP-1/2/3/9 and TIMP-2 compared to monolayer cultured VIC. Phenotype analysis of aggregates showed a significant decrease in α-SMA expression, and an increase in FSP-1 expression at any time point. Furthermore, VIC aggregates did not show a significant difference in OCN, Egr-1, Sox-9 or Runx2 expression.

CONCLUSION In conclusion our findings showed that (1) agarose micro-wells are well suited for the large scale production of uniform, high quality VIC aggregates, (2) supplementation of AA to the culture medium before aggregation starts is indispensable, (3) downregulation of α-SMA expression in aggregates is due to the 3D culture, resulting in the stimulation of a quiescent VIC phenotype, (4) VIC aggregates are able to produce their own ECM, resembling the native valve composition.

0253 Myocardium-on-chip derived from human iPSCs with capillary-like flow for personalized therapy

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INTRODUCTION: Cardiovascular diseases (CVDs) have been the world's leading cause of death, responsible for nearly half of all deaths in 2012 [1]. Unfortunately, current *in vitro* and *in vivo* models are incapable of perfectly mimicking human CVDs [2, 3]. In this study, we engineered a microfluidic, 3D, co-culture platform composed of hiPSC derived cardiomyocytes (iCMs) and endothelial cells (iECs) from the same cell line, to mimic a single patient's myocardium. The myocardium-on-a-chip (MOC) consisting of a cardiac muscle chamber and microvasculature channels can be used to study cardiovascular biology and pathology. RESULTS: iCMs displayed an upregulation of cardiomyocyte specific mRNA as well expression of striated troponin-I and connexin-43 expression at cell-cell junctions (Fig. 1A-B). In addition, iCMs displayed synchronized Ca^{2+} flux, and pacing to external electrical stimulations. iECs in single channel microfluidic devices retained CD31 and Ve-cadherin expression and aligned to physiological flow (Fig. 1C). In the MOC, seeded iCMs encapsulated in the cardiac muscle chamber (Fig. 1D) retained spontaneous beating. iECs in the cardiac muscle chamber showed tube formation and iECs in the microvasculature channels aligned to physiological flow (Fig. 1D-F). Both iCMs and iECs retained specific marker expression (Fig. 1G-H). Encapsulated iCMs displayed a 7-day viability above 75%. DISCUSSION & CONCLUSION: In this study we utilized iECs and iCMs from the same cell line to model human cardiovascular physiology *in vitro*. In contrast to other models, the MOC can simultaneously study various aspects of cardiovascular biology including iCM contractility and cardiac/endothelial health following drug treatment through microvasculature channels. Additionally, both iECs and iCMs can be removed from the MOC for additional analyses. The MOC displays great potential as an *in vitro* model that can be used to study cardiovascular phenomena, drugs, and treatment methods at a personalized, patient specific level in a high throughput, inexpensive manner. REFERENCES: [1] Go, A. et al. (2014) [2] Hay, M. et al. (2014) [3] Grosberg, A. et al. (2011) ACKNOWLEDGEMENTS: This work was funded by a Project Development Team in ICTSI NIH/NCRR [Grant No: UL1TR001108], by Advanced Diagnostics and Therapeutics Discovery Grant and by NSF Grant No: 1530884.

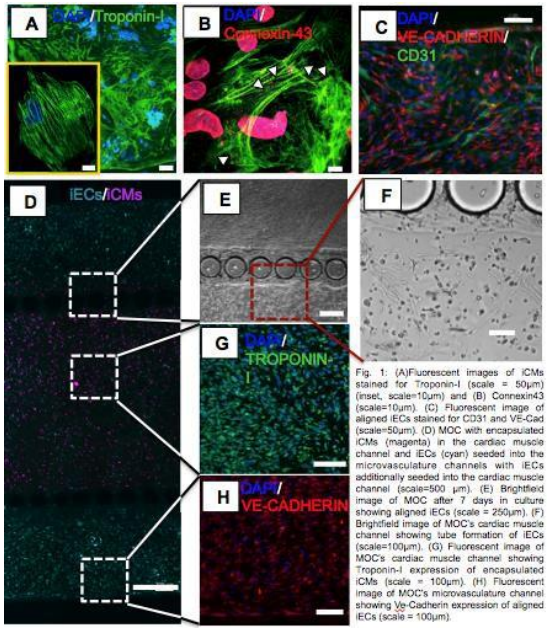

Fig. 1: (A)Fluorescent images of iCMs stained for Troponin-I (scale = 50μm) (inset, scale=10μm) and (B) Connexin43 (scale=10μm). (C) Fluorescent image of aligned iECs stained for CD31 and VE-Cad (scale=50μm). (D) MOC with encapsulated iCMs (magenta) in the cardiac muscle channel and iECs (cyan) seeded into the microvasculature channels with iECs additionally seeded into the cardiac muscle channel (scale=500 μm). (E) Brightfield image of MOC after 7 days in culture showing aligned iECs (scale = 250μm). (F) Brightfield image of MOC's cardiac muscle channel showing tube formation of iECs (scale=100μm). (G) Fluorescent image of MOC's cardiac muscle channel showing Troponin-I expression of encapsulated iCMs (scale = 100μm). (H) Fluorescent image of MOC's microvasculature channel showing Ve-Cadherin expression of aligned iECs (scale = 100μm).

0254 Combined oxygen level and mechanical stimulation in a microfluidic system for modelling pathological myocardial tissue condition

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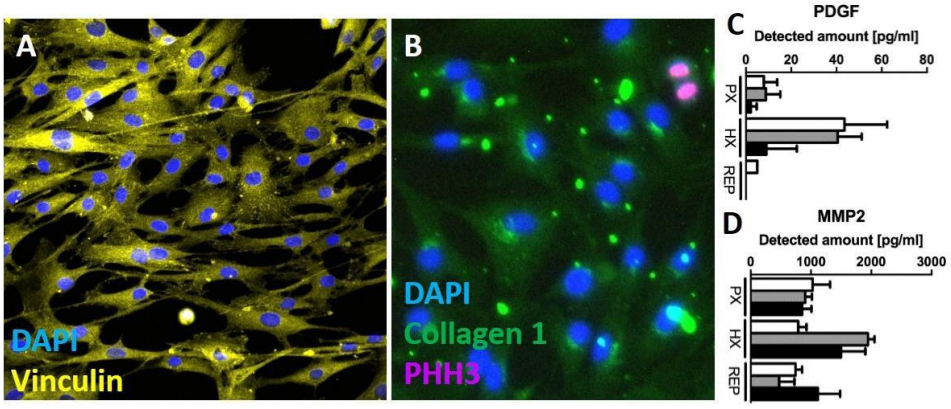
Background - Cardiac fibroblasts (CFs) are key actors in remodeling of the myocardial tissue. Upon pathological conditions such as ischemia, the cellular microenvironment undergoes dramatic changes and instructs a series of cellular responses that initiate and maintain tissue remodeling and inflammation. Previous literature has described mechanical signals and oxygen-sensing by human CFs as key regulators of cellular pathological routes [1]. Nevertheless, a comprehensive model of how tissue environment may induce CF pathological responses is far from being elucidated, partly due to the lack of approaches involving complex and simultaneous environmental stimulation as they happen in physiological tissues.

Methods - Building on a previously described microdevice [2], we engineered an *in vitro* model of myocardial tissue physiology and pathology by developing a multi-layer microdevice for combined application of cyclic mechanical strain (by means of side vacuum chambers) and controlled oxygen changes (by means of a lower conditioning channel). We subjected human primary CFs to combinations of three regimes of mechanical stretch (0%, 2%, 8% strain) and three oxygen conditions (physioxia, hypoxia and hypoxia-reoxygenation) relevant for cardiac physiology and pathology.

Results and discussion - We analyzed cell adhesion (Vinculin, Panel A) proliferation (YAP, PHH3, Panel B), collagen production (Panel B), inflammatory (e.g., PDGF) and remodelling (e.g., MMP2) factors production (Panel C and D). Our findings delineate different mechanisms of human CFs adaptation to different combinations of the above stimuli. We therefore provided a novel characterization of human CFs tissue responses, key to unravel mechanisms of onset and progression of pathological myocardial remodeling, together with a newly proposed microfluidic model for engineering myocardial tissue physiological and pathological conditions.



0255 Elastin-like recombinamers-based hydrogel modulates infarcted myocardium glycoprofile towards a regenerative state

Paolo Contessotto[1], Pierce Lalor[3], Doriana Orbanic[4], Tatjana Flora[4], Audrys G. Pauža[5], Mark Da Costa[1,6], José C. Rodríguez-Cabello[4], Lokesh Joshi[2], Peter Dockery[3], Michelle Kilcoyne[2,7], Abhay Pandit[1]

[1]CÚRAM, Centre for Research in Medical Devices, National University of Ireland, Galway, Galway, Ireland, [2]Advanced Glycoscience Research Cluster, National University of Ireland, Galway, Galway, Ireland, [3]Anatomy, School of Medicine, National University of Ireland, Galway, Galway, Ireland, [4]G.I.R. Bioforge, Ciber-BBN, Universidad de Valladolid, Valladolid, Spain, [5]Institute of Anatomy, Lithuanian University of Health Sciences, Kaunas, Lithuania, [6]University College Hospital Galway, Galway, Ireland, [7]Microbiology, School of Natural Sciences, National University of Ireland, Galway, Galway, Ireland

INTRODUCTION: Recent studies have started to focus on glycosylation expression during the extracellular remodeling after myocardial infarction (MI) [1]. We hypothesize that the evaluation of the remodeling effects of an elastin-based hydrogel and a comprehensive glycoprofiling of myocardial tissue will enable us to tailor a regenerative treatment for the infarcted tissue. METHODS: Lectin histochemistry and lectin microarray analyses were performed on heart ventricle samples from Romanov sheep (n=18) after the ligation of the side branches of LAD. H&E, Masson's Trichrome, Wheat Germ Agglutinin (WGA) and *Sambucus nigra* agglutinin (SNA-I) were used in combination with immunohistochemistry for Nkx 2.5 and tropomyosin-1 markers. Labelled membrane extracts were incubated on a 48 lectin microarray and imaged using a G2505 microarray scanner (Agilent) [2]. Sheep tissues were fixed, processed and imaged for TEM analysis [3]. All statistical analyses were performed using Minitab Express™. RESULTS: The hydrogel-treated group showed lower collagen and higher muscle volume fraction than in the MI-only control group, respectively, 28 days after the induction of MI (Fig.1A-D). Differences were found in the carbohydrate expression by lectin microarray analysis across the myocardial ischemic and border zones. Specifically, sialic acids (Neu) and *N*-acetylglucosamine (GlcNAc) expression was validated using WGA and SNA-I histochemistry.

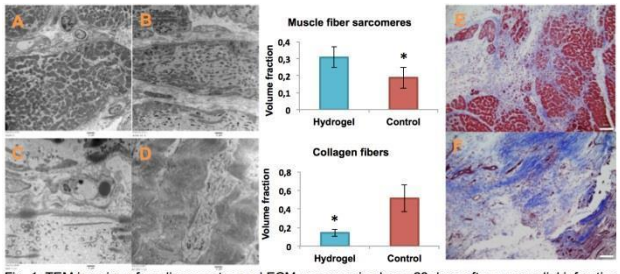


Fig. 1: TEM imaging of cardiomyocytes and ECM response in sheep 28 days after myocardial infarction. (A,B): Muscle volume fraction in hydrogel-treated (A) and MI-only group (B), (C,D): Collagen volume fraction in hydrogel-treated (C) and MI-only group (D). Masson's Trichrome staining of infarcted area in hydrogel-treated (E) and control MI group (F). Scale bar 100 µm. n=4 t-student's test *p<0.05

DISCUSSION & CONCLUSIONS: ELRs-based hydrogel treatment showed a significant positive effect on the myocardial remodeling 28 days after MI induction in sheep, both in the border and in the ischemic zones, together with the clearance effect of immune system cells. The elastin-based hydrogel was injected one week after MI induction in the infarcted myocardial tissue and provided a mechanical barrier against the development of the extensive fibrous scarring that is normally seen after permanent muscle damage. A comprehensive analysis examining glycosylation expression showed possible key players in the MI remodeling process, which could be exploited in a further functionalization of the hydrogel. ACKNOWLEDGEMENTS: Seventh Framework Programme Grant Agreement no.: 317304; Centre for Microscopy & Imaging funded by NUI Galway and PRTLI, Cycles 4 and 5, National Development Plan 2007-2013; Science Foundation Ireland (SFI) and European Regional Development Fund under Grant Number 13/RC/2073

0256 Exploring the potential of endometrial MSCs for veterinary regenerative medicine

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Mesenchymal Stem/Stromal Cells (MSCs) have been used therapeutically in horses for over 15 years, providing an excellent pre-clinical model for humans, in which most MSC therapies are still in clinical trial phase. Equine MSCs are commonly harvested from bone marrow or adipose tissue before expansion *in vitro* for autologous transplantation. The requirement to use surgical procedures to harvest these cells has driven the search for other, less invasive sources of equine MSCs. In that regard, an attractive alternative is the endometrium. In fact, endometrial cells meeting the criteria of MSCs have been to this date harvested and characterized from species including humans, rodents, pig and sheep. Endometrial MSCs are easily accessible and can be readily expanded *in vitro*, and their regenerative potential has already been demonstrated in relation to the reproductive tract and other body systems. To explore the therapeutic potential of endometrial MSCs for veterinary regenerative medicine, we have identified, isolated and characterized stromal cells from equine endometrium which fulfill the definition of MSCs, based on clonogenicity, immunophenotype and ability to differentiate into multiple mesenchymal derivatives including bone, cartilage, fat and smooth muscle. Of note, when compared to MSCs derived from other sources, endometrial MSCs had distinct features including their expression of cell surface markers, ability to differentiate into cartilage and smooth muscle, and cytokine responses to inflammatory stimuli in vitro. We are currently investigating the effects of autologous transplantation of endometrial MSCs into the uterus with a long-term aim of using these cells to enhance uterine regeneration in mares. Moreover, endometrial MSCs may provide a relatively non-invasive alternative to the current therapeutic use of bone marrow and adipose MSCs in the horse.

0257 2D and 3D osteoblast differentiation of equine induced pluripotent stem cells

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Bone fractures with non-traumatic origin occur in Thoroughbred racehorses, with the majority of fractures occurring in the distal limbs; bones subject to high-impact and load during exercise and racing. Severe fracture leads to euthanasia and fracture is the main reason for euthanasia on the racecourse.

In addition to environmental factors, it has been shown that there is a genetic basis for fracture risk in racing Thoroughbreds. Although the causal mutations are unknown, it is possible to calculate an individual horse's relative risk using genome wide information. Identification of horses at high risk of fracture, together with an understanding of the biological pathways which are affected in these horses, has the potential to reduce the incidence of fracture through informed breeding decisions and modulated training regimes.

It can be difficult to treat fractures in horses due to their need to bear weight on all legs during the healing period. Some fractures can be treated conservatively but in delayed union or comminuted fractures surgery is required. However, up to 40% of horses do not return to their previous athletic activity after surgery. Regenerative medicine to improve fracture reunion and recovery would significantly improve horse welfare.

We have previously derived equine induced pluripotent stem cells (iPSCs) and demonstrated them to be self-renewing and capable of differentiating into derivatives of the three germ layers. Here, we have established the methodology to differentiate equine iPSCs into bone forming osteoblasts in 2D culture. We demonstrate that differentiation on an osteoassay surface results in increased levels of calcium deposition, inhydroxyapatite production and alkaline phosphatase activity compared to a normal tissue culture surface.

When cultured in osteoblast differentiation media on a 3D printed thermoplastic polymer scaffold the iPSCs attach, align to the construct and differentiate. They produce a mineralised matrix via the deposition of calcium and inhydroxyapatite onto the structure of the scaffold. The iPSC-derived osteoblast cells produce alkaline phosphatase and express osteoblast specific genes and proteins under these conditions.

Compared to 2D cultures, 3D systems provide more accurate modelling of the physiological and cellular environment of cells to promote and maintain lineage specific differentiation and normal bone architecture. 3D artificial bone therefore has the potential to be used as a bone tissue substitute in regenerative medicine and to understand the genetic basis of fracture risk by using iPSCs generated from high and low risk horses.

0258 Long-term adult feline liver organoid cultures for modeling of hepatic steatosis

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Hepatic steatosis is an emerging health problem in humans, but the disease is also encountered in cats. Research into etiology, pathogenesis and possible therapies is hampered due to the lack of a suitable in vitro cell culture models and (pre)clinical animal models. This study aimed to develop a robust culture system of genetically stable feline liver organoids that mimic in vivo liver progenitor cells which can be differentiated towards functional hepatocytes.

Methods - To establish feline organoid cultures, biliary duct fragments were isolated from cat liver (fresh liver, frozen liver, and fine needle aspirate) and maintained in 3D culture by suspension in Matrigel droplets and R-spondin-1-based culture medium. Characterization of organoid cultures consisted of gene expression profiling with QPCR, immunocytochemistry, and a proliferation assay (EdU incorporation). For induction of hepatocyte maturation, Wnt agonists were withdrawn and Notch signaling was inhibited. For disease modeling of hepatic lipidosis, liver organoids from cats, mice, dogs, and humans were incubated with either vehicle or free fatty acids (FFA) and stained with lipophilic dye LD540. Fluorescence was quantified using flow cytometry. For feline organoids the β oxidation of excess FFA was studied using a carnitine palmitoyltransferase-1 inhibitor (etomoxir) and supplementation of L-carnitine.

Results – Organoids could be cultured successfully from all feline liver samples. Feline liver organoids were highly proliferative and were cultured up to 32 passages. Organoids expressed adult stem cell marker LGR5, hepatic progenitor/biliary markers (K7, K19, and HNF1β), early hepatocyte markers (HNF4α, TBX3, ALB), and had low expression of mature hepatocyte markers (TTR, FAH, CYP3A132). Mature markers increased when organoids were cultured without Wnt agonists in combination with Notch inhibitors, indicating their potential to differentiate towards a hepatocyte-like phenotype. Feline liver organoids showed lipid accumulation when exposed to excess FFA and accumulated more lipids than human liver organoids. Lipid accumulation was enhanced when β oxidation was blocked with etomoxir and was attenuated when medium was supplemented with L-carnitine.

In conclusion, feline liver organoids present the first primary liver cell culture system for cats. Feline liver organoids show a pronounced phenotype in lipid accumulation, making them an interesting model in hepatic steatosis research.

0259 Fetal regeneration - a key to improve adult tendon repair?

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Repair of injured adult tendon tissue leads to formation of scar tissue with inferior biomechanical properties resulting in high re-injury rates and chronic disease. Fetal tissue, in contrast, has the inherent capacity to regenerate without scar tissue formation. The aim of the presented study was therefore to identify key factors responsible for scarless regeneration in the fetus versus fibrotic repair in adults. Recapitulation of developmental processes is discussed as a potential key to tissue regeneration.

The study was carried out in an ovine model. Standardized tendon lesions were surgically induced in adult (aged 2-4 years) and fetal sheep (80 days gestation). Time points for sampling were chosen according to the three stages of healing (inflammatory phase, reparative phase, remodelling phase). Sample comparison was carried out at a gene expression, protein secretion and histologic level with special emphasis on extracellular matrix composition, collagen organization and collagen types, qualitative and quantitative composition of growth factors and cytokines, inflammatory response, chemotaxis, cell proliferation, MMPs and migration as well as senescence.

For proteome analysis a label free bottom-up shot gun proteomics approach applying high resolution orbitrap mass spectrometry was chosen. 30-100mg of tissue were incubated for 6h at standard culture conditions. Secreted proteins were isolated and processed by Nano Flow Liquid Chromatography (Dionex 3000 UHPLC) to separate Peptides which were consequently analysed by mass spectrometry (Thermo QEXACTIVE orbitrap). For accurate quantification of selected proteins, reaction monitoring using triple quadrupole mass spectrometry (Agilent 6490) was employed. Peptides were separated using nano-flow Chip-HPLC (Agilent) and quantified according to isotope labeled internal standards. Protein identification, lable free quantification and statistical data evaluation was performed using and MaxQuant (free software by M.Mann) and Perseus software. For gene expression analysis a newly designed sheep specific whole genome microarray (Affymetrix) was used.

As a result more than 3.000 proteins, assembled from more than 30.000 distinct peptides were identified meeting a FDR < 0,01 at both peptide and protein levels. Time course analysis of proteins revealed significant abundance differences of subsets of proteins at specific stages of healing. From adult samples taken during the inflammatory phase mainly imflammation- related proteins such as Cathelicidins and Serum Amyloid A protein were significantly upregultaed, whereas proteins related to extracellular matrix formation and wound healing such as Periostin, Fibulin-7 and C-Mannose Receptor were significantly up regulated only in samples obtained during the reparative and remodeling phase. Furthermore, tremendous differences between adult and fetal regeneration were observed with regard to inflammation-related processes.

A better understanding of the underlying processes involved in tendon repair versus regeneration may lead to the development of novel therapeutic strategies inducing regeneration rather than scar formation.

0260 In vivo MRI cell tracking of autologous mesenchymal stem cells in an ovine osteochondral defect model

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Introduction: The application of mesenchymal stem cells (MSCs) in treating cartilage damage and osteoarthritis (OA) has been made possible by the immunosuppressive, differentiation and homing abilities of these cells. A non-invasive means of assessing cell integration, tissue regeneration and cellular bio-distribution is fundamental in evaluating the success of this therapy. Previous studies have focused on the development of a standardised SPION (superparamagnetic iron oxide nanoparticle) & MRI (Magnetic resonance imaging) based protocol to image and track stem cells *in vivo*. In this study the suitability of this protocol in evaluating cell homing was assessed in an osteochondral ovine model over 7 days.

Methodology: Autologous ovine MSCs were isolated, expanded and labelled overnight with Nanomag; a 250nm dextran coated SPION using a novel transfection agent; P21-8R. *In vitro* and *ex vivo* MRI detection thresholds were determined prior to *in vivo* studies. Cell viability, proliferation and differentiation potential pre and post Nanomag labelling were also evaluated. A single 8mm diameter osteochondral defect was created in the medial femoral condyle in the left knee joint of each sheep with the contralateral joint serving as the control. $10x10^6$ Nanomag labelled-MSCs (P3) were subsequently labelled with a fluorescent lipophilic dye (DII) and delivered by intra-articular injection at either 1 week or 4.5 weeks post defect creation. Sheep were then sacrificed 7 days post implantation and immediately MR imaged using an ESAOTE 0.2T scanner and validated using a Siemens 3T MRI scanner. Joints were then processed for histology.

Results: *In vitro* and *ex vivo* MRI data demonstrated significant increase in MRI contrast as a result of P21-8R:Nanomag uptake. Cell viability, proliferation and differentiation capabilities were not affected by Nanomag-labelling. *In vivo* MRI data revealed the presence of Nanomag-labelled cells within the synovial joint 7 days' post implantation however, cells were not seen to home to the site of injury when implanted 4.5 weeks after the defect was created. MRI results were validated by histology with the presence of implanted cells visible by fluorescent microscopy. Serum CRP (c-reactive protein) levels were measured by ELISA with no obvious increase in CRP levels observed as a result of P21-8R:Nanomag delivery.

Conclusion: This study clearly demonstrates the potential of Nanomag and P21-8R as an effective means of imaging and tracking cells in an ovine osteochondral defect model. This protocol has great implications in the clinical translation of a wide range of stem cell based therapies.

0261 Functional, bioinspired supramolecular polymeric materials - en route to synthetically meet nature's complexity

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Full integration of synthetic materials into living tissues requires the interplay between synthetic and living materials in a spatiotemporal way. We propose that dynamic reciprocity plays an important role in guiding these interactions. Bioinspired materials based on supramolecular units intrinsically show this dynamic behavior. However, the development of biomaterials with stable bioactivity presentation and with good mechanical properties demands for the incorporation of covalent bonds. Inspired by nature we have combined supramolecular bonds based on hydrogen bonding, pi-pi stacking and hydrophobic interactions, with covalent bonds in order to incorporate biofunctionality on supramolecular thermoplastic elastomers, and to introduce robustness in supramolecular hydrogel systems. Reactive additives have been supramolecularly incorporated in thermoplastic elastomers that can be covalently post-modified with reporter molecules and/or proteins.

These supramolecular thermoplastic elastomeric materials have been investigated for their application as vascular grafts that in-situ can be engineered. Furthermore, these robust materials have also been studied for their use to ameliorate hemodialysis through the development of bilayered supramolecular membranes on which kidney cells can be cultured outside the body. Besides that, the introduction of additional chemical crosslinks in supramolecular hydrogel systems has shown to improve the mechanical properties while self-healing behavior is still displayed. These supramolecular hydrogels have been investigated to deliver drugs to the infarcted heart via catheter injection. We propose that via introduction of covalent bonds in supramolecular systems both biological and mechanical properties can be controlled in synthetic supramolecular polymers in a comparable way as natural polymers in the extracellular matrix control these properties; en route to synthetically meet nature’s complexity.

0262 Engineering physiologically relevant in vitro microenvironments for cell-based therapies

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Cell therapies require in vitro cell expansion to attain therapeutically relevant numbers. Unfortunately, in vitro cell culture is associated with cell phenotypic drift, cell senescence and loss of cells' therapeutic potential. For these reasons, scientific research and technological innovation are focused on creation of functional in vitro microenvironments that would either maintain permanently differentiated cell phenotype or differentiate stem cells towards specific lineage. Indeed, biophysical (e.g. surface topography, substrate rigidity, macromolecular crowding, mechanical loading), biochemical (e.g. oxygen tension) and biological (e.g. growth factor media supplements) tools are under intense research and development. This talk will discuss how these in vitro microenvironment modulators will control cell function in vitro and ultimately enable clinical translation and commercialisation of cell-based therapies.

0263 Hydrogel bioink with unique reactivity for tissue engineering applications

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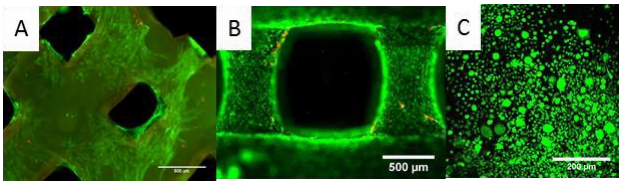
Despite their highly attractive properties, 3D printing of hydrogel materials can be rather challenging. Herein, we present a novel hydrogel material that can be easily processed into three-dimensional scaffolds using different 3D printing technologies.

An acrylate-terminated, urethane-based PEG was prepared by reacting PEG 2000 with isophorone diisocyanate (IPDI) and monoacrylated PEG (336 Da) in a 1:2:2 molar ratio. For melt 3D-printing, pure polymer was used (T_m 38°C). For bioprinting, a 50 wt% solution with 3 wt% Laponite was used.

A first characteristic of this material is its unique reactivity. In contrast to other materials, it can be photo-polymerized in the solid state, as characterized by photo-DSC. This allows for the material to become printed from the melt, similar to other conventional thermoplasts (e.g. poly-(ε-caprolactone)). Material crosslinking can be performed in a separate UV-A curing step (15 mW/cm^2). By applying a gelatin-methacrylamide coating, excellent cell adhesion and proliferation of MC3T3 cells and hMSCs (A) can be obtained. Interestingly, the material is highly reactive, even in the absence of a photo-initiator (PI). Rheology has shown that without a PI, the reaction is slower (about 30% slower for a 50 wt% solution). However, similar moduli were obtained with and without PI after complete curing.

In a final part of the work, extrusion bioprinting was explored as a method to eventually allow printing of cell-laden constructs. To allow shape fixation after printing, a nanosilicate additive (Laponite) was added which results in shear-thinning behaviour due to physical crosslinking. Furthermore, this additive results in excellent cell adhesion on the printed scaffolds up to 23 days using MC3T3 cells (B). In addition, preliminary experiments have shown that L929 cells encapsulated in a solution of polymer and Laponite maintain high viability during 21 days and was further increased by adding 1 wt% gelatin (C).



0264 Computer designed topographical surfaces for instructing cell fate

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Topographical cues have been repeatedly shown to influence cell fate dramatically. Altering the cell shape through surface topographies opens new opportunities for the development of biomedical materials. Unfortunately, the exact mechanism of topographical control of cell behavior remains largely unknown. We have therefore developed a technology in our laboratory to determine the optimal surface topography for virtually any application in the biomedical field. The TopoChip, a micro topography screening platform, enables the assessment of cell response to 2176 unique topographies in a single high-throughput screen. The topographical features were randomly selected from an *in silico* library of more than 150 million of topographies, which were designed from an algorithm that synthesized patterns based on simple geometric elements circles, triangles and rectangles.

In our previous studies, we have demonstrated that these surface topographies exert a mitogenic effect on hMSCs, as well as on cell shape. We also showed that these topographies can be used to modulate the ALP expression in human mesenchymal stromal cells, as well as pluripotency in human induced pluripotent stem cells. We further showed that computational models can be built to predict these protein levels using surface topography parameters.

In the next stage we analyzed a variety of cell shapes recorded during high-throughput screening approach and determined cell morphology on 2176 randomly generated surface topographies. Cell morphology was captured by high-content imaging and we performed image analysis in CellProfiler which generated a large dataset with hundreds of image based cells descriptors. Importantly, we found biologically meaningful clusters of cells based on cell shape features. In total we identified 28 surfaces based on cell shape diversity – the resulting selected surfaces were observed to have distinct designs. These 28 topographies were further used to reveal how different cell shapes induced by topography can affect fundamental cell functions. To investigate this, we have performed various functional assays with hMSCs such as: differentiation, proliferation, migration, apoptosis and protein synthesis. We used these assays to identify surfaces inducing the most unique cell response By performing micro array analysis on cells grown on these surfaces, key target genes involved in surface topography interaction will be identified.

The results of this study will lead to new advances in our understanding of how surface cues can influence cell behavior, enabling the improved design of materials for biomedical applications.

0265 Biocompetent dendrons as extracellular matrix analogues

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Tissue repair and regeneration depend on the control exerted by the extracellular matrix (ECM) as well as on complex growth factor pathways on cell activity. Specific bioligands are exposed by the structural components of the ECM to favour the anchorage of cells through receptors and control their ability to promote tissue remodelling and repair. Biomaterials able to mimic ECM macromolecules can therefore be used as substrates for the handling of cells prior to transplantation as well as tissue engineering components promoting healing of damaged tissues in a number of clinical applications.

Novel biomimetic biomaterials mimicking natural ECM components were designed as hyperbranched peptides tethered with biocues relevant to cell recognition processes. The hyperbranched peptides (i.e. biocompetent dendrons) were synthesised by solid phase peptide synthesis and coupled to linear polymers by mild, aqueous chemistry. The obtained biomaterials were characterised for their physico-chemical properties and tested for their ability to mimic the basement membrane of the adult tissue stem cell niches and blood vessel endothelia.

The results showed that the spacing of the biocues controls of the clustering of the cell integrins leading to cell organisation into tissue-like structures; human bone marrow mesenchymal stem cells (hMSCs) formed 3D spheroids through asymmetric division, while endothelial cells (HUVEC) formed endothelial sprouting that were wrapped by pericytes when HUVEC were co-cultured with hMSCs.

The absence of serum in the culturing conditions shows the potential of these biomaterials to be used as in vitro substrates for the standardised pre-clinical handling of cells and become a surface functionalisation method of biomaterial scaffolds for tissue engineering such as those for osteochondral tissue regeneration.

0266 RNA therapeutics and anabolic gene delivery for tissue engineering and regenerative medicine

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Delayed union/non-union resulting from bone fractures or serious trauma remains a challenging problem for orthopaedic surgeons. These problems have inspired the development of tissue engineering, which combines cells, biomaterials and biological signals, to stimulate tissue regeneration. Over the past decade, gene therapy has converged with bone engineering, by which an increasing number of therapeutic genes are explored to stimulate bone repair. These genes can be administered to cells via in vivo or ex vivo approaches using either viral or nonviral vectors. This presentation will focus on the use of viral vectors for genetic engineering of mesenchymal stem cells for bone regeneration. In particular, emphasis is placed on the applications of baculovirus, an emerging nonpathogenic gene delivery vector, for the delivery of various anabolic genes and miRNA mimics/sponges to repair bone.

0267 Gene transfer for musculoskeletal regeneration

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Gene transfer offers a unique opportunity to modify cellular behaviour. For the repair of traumatic injuries of cartilage and bone transient expression would be desired to initiate the response.

Classical gene transfer is based on the over-expression of a bioactive protein leading to a change in cell function. Such proteins can be secreted molecules, such as bone morphogenetic protein 2, or can act intracellularly as in the case of the SOX9 transcription factor. Alternatively, short hairpin RNA shRNA can be expressed in order to down-regulate the expression of an endogenously produced protein.

As gene therapy products begin to emerge on the market, safety data will begin to accumulate and options to translate this technology to patients are increasing.

Within this presentation, various strategies will be discussed with a translational route in mind. protein over expression and transient gene knockdown will be presented with examples from both bone and cartilage regeneration.

An interactive model combining gene transfer and mechanical stimulation will also be described for cartilage regeneration.

0268 Gene-activated materials for bone regeneration: from standardized bone substitute to personalized 3D-printed blocks

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Effective treatment of patients with bone defects and alveolar ridge atrophy needs the effective materials for bone grafting. Among all the groups of the bone substitutes the gene-activated matrices take special place being a part of the gene therapy that could significantly induce the bone regeneration and improve the healing process.

Firstly, we have developed several variants of standardized gene-activated matrices using different scaffolds (xenogeneic bone matrix, collagen/hydroxyapatite composite material (Col/HA), octacalcium phosphate (OCP)) and plasmid DNA encoding *VEGF* gene which is the active substance of the gene-therapeutic drug "Neovasculgen" (HSCI, Russia) indicated for treatment of the chronic lower limb ischemia. In vivo studies on rabbits cranial bone defect (D=10 mm) model revealed the safety and high osteoinductive capacity of the gene-activated matrices that appeared in newly formed bone tissue formation around their granules in the central part on the defects since 15 days after implantation. In the first clinical case (in frame of the clinical trial NCT02293031) the safety was confirmed and efficacy was shown partly limited by osteoconductive properties of the Col/HA. Now we are carrying out the clinical trial of the gene-activated bone substitute based on OCP scaffold and plasmid DNA with *VEGF* gene that was approved by Russian Ministry of Health in July, 2016.

However, large bone defects still remains a big challenge and hardly could be treated with standardized gene-activated bone substitute either in granular or block forms because even being effective in trivial indications such materials are not really eligible for precise and complete replacement of a bone defect with large size and very complex geometry. In attempt to solve this problem we have developed the original technology that allows to create the personalized blocks of gene-activated matrices consisting of any solid scaffold and gene constructs using different 3D-printing approaches. Based on computed tomography data we made the gene-activated implants using OCP as a scaffold that exactly fitted to replace the complex defects of the pig's tibia (model with loading) and mandible (model without loading). All personalized implants were fixed in a proper position using standard plates and screws for reconstruction. The results will be collected to the date of the conference and presented.

Thus, despite the fact that neither standardized nor personalized gene-activated bone substitutes have been yet approved for clinical use, there is a strong background for their successful clinical translation that could expand the set of treatment tools and significantly improve the clinical outcomes. Acknowledgments: This work was supported by the Russian Science Foundation (project No. 15-13-00108).

0269 MicroRNA therapeutics for improved bone repair: Effective collagen-nanohydroxyapatite scaffold mediated delivery to mesenchymal stem cells and rat calvarial defects

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INTRODUCTION: The influence of microRNAs (short, non-protein coding RNAs) on stem cell regenerative capacity offers vast potential for Tissue Engineering; in addition to their gene cohort targeting potential, two opposed modalities - miR-mimics & antagomiRs - may be utilised. We recently introduced a highly effective delivery platform, combining collagen-nanohydroxyapatite (coll-nHA) scaffolds & nHA particles as non-viral vectors (1). Building on this system, the present study focussed on assessing, both *in vitro* and *in vivo,* the osteo-therapeutic potential of: miR-133a targeting Runx2, miR-210 targeting EFNA3 & AcvR1b, and miR-16 targeting VEGF & Smad5. We hypothesised that delivery of antagomiR-133a, miR-210 mimic or antagomiR-16 using the above scaffold system may enhance *in vitro* osteogenesis and, following cell-free implantation in a rat calvarial defect model, support localised microRNA uptake and ultimately promote bone repair *in vivo*.

MATERIALS & METHODS: *nHA particles* combined with 20nM custom Dy547 antagomiR-133a, miR-210 mimic, or antagomiR-16 (Dharmacon), were loaded drop-wise onto freeze-dryed *coll-nHA scaffolds*. *Mesenchymal stem cell (MSCs)* were seeded on miR-coll-nHA scaffolds to undergo 28 days osteogenic culture & qPCR, ELISA (VEGF), DNA content, ALP activity & calcium deposition analysis (n≥3), with 2-way ANOVA plus Tukey post-hoc *statis*. *Rat calvarial defects* (Ø=7mm) were created under HPRA license B100/4416 & ethical approval REC1205. *Uptake & biodistribution in vivo* was assessed 1 week post-op (n=3) by resecting calvaria sections, brain, lung, liver and kidney, followed by Dy547 fluorescence microscopy & quantification. *Bone repair* was examined 4 weeks post-op (n=8) by *microCT scanning* of resected calvaria specimens & *histological analysis*.

RESULTS: This study showed that all miRNA therapeutic candidates enhanced *in vitro* MSC osteogenesis, where interestingly a miR-210/16 dual formulation demonstrated a synergistic increase in VEGF secretion in comparison with the single treatments. Overall, antagomiR-133a and the dual miR-210/16 formulation rendered the most pronounced osteogenic response; thus these two groups were assessed *in vivo*, using empty defect and blank coll-nHA scaffold groups as controls. Initially, host cell infiltration and localized transfection (Dy547-uptake) were demonstrated *in vivo* at 1 week post-surgery, with the tagged miRNA effectively retained within the implant area as indicated by the negligible biodistribution to all organs analysed. Finally, and most importantly, microCT analysis indicated that both nanoantagomiR-133a loaded scaffolds & miR-210/16 dual scaffolds significantly improved bone healing just 4 weeks post-op (12.2±12.1 & 11.1±7.7% BV/TV vs blank scaffolds 5.1±4.4%, **Fig.1**).

DISCUSSION: The application of miRNAs as osteo-therapeutics has focused on viral or lipid-based miRNA delivery; moreover, combinatorial miRNA delivery is a very new concept in the field (2). This study used highly biocompatible nHA particles and demonstrated their significant ability to deliver miR-mimics & antagomiRs, singly & combined, to MSCs. More importantly, we showcased successful *in situ* transfection of host cells, in addition to enhanced bone repair, with coll-nHA scaffold mediated delivery of antagomiR-133a or the miR-210/16 dual formulation, in rat calvarial defects. Collectively, these results underlined the potential to address the repair of other tissue types introducing non-viral miRNA therapeutics to collagen-based scaffolds.

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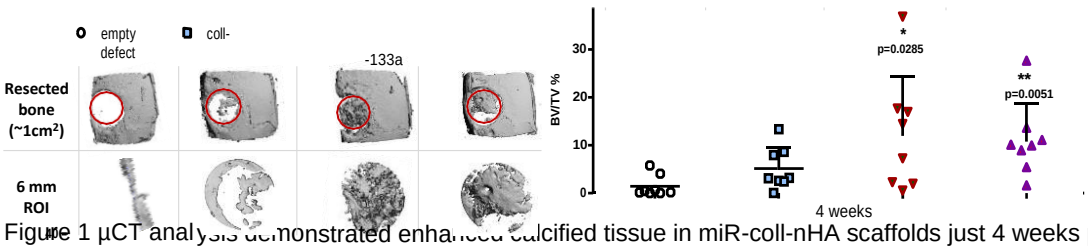

Figure 1 µCT analysis demonstrated enhanced calcified tissue in miR-coll-nHA scaffolds just 4 weeks post-op.

0270 Gene delivery to stem cells enhances their therapeutic potential to obtain a better cell therapy for tissue repair

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Application of gene delivery techniques for modification of cells used in therapeutic methods is a new field that allows to enhance their paracrine and regenerative potential for increased efficacy and better safety. Mostly, these methods utilize the crucial role of paracrine stimuli generated by stem cells during tissue repair or regeneration.

Over last years we have been elaborating to develop strategies using viral vectors to increase production of growth factors and “tune-up” the cells pro-regenerative capacity.

Using adeno-associated viruses (AAV) and baculovirus we managed express growth factors in mesenchymal (MSC) and cardiac stem cells (CSC). Developed methods of viral delivery to express VEGF165 allowed to increase pro-angiogenic potential of cells and induce effective angiogenesis in ischemic tissue of experimental animals rendering effect that was significantly higher compared to GFP-treated or un-modified cells. Indeed, using nude mice to evaluate human MSC impact on recovery of blood flow, we found that VEGF-expressing MSCs had better survival and resulted in higher perfusion and blood vessel counts at experiment’s endpoint.

Another approach we widely use is generation of cell sheets – minimal tissue-engineered constructs that consist of viable cells and extracellular matrix proteins forming a solid multilayered structure. Their expressed therapeutic potential relies on better survival after delivery compared to injection of dispersed cells. However, viral modification of constructs resulted in even better functional outcome in animal models of ischemia compared to untreated cells or injected dispersed. Furthermore, a large subset of data was obtained indicating graft/host interactions, vascularization of implanted construct and limited cell proliferation within the tissue layer.

Mechanisms of gene delivery also remain an issue for better understanding of expression control so we set out to generate efficient methods for viral modification of stem cells to generate next-level cellular therapeutics.

0271 Long non-coding RNAs: novel therapeutic tools in osteogenic differentiation

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Bone regeneration is a dynamic, complex and well-orchestrated process which can be impaired by large defects, delayed unions and non-unions, or by several diseases such as osteoporosis, osteogenesis imperfecta and tumors. Bone repair encompasses distinct biological mechanisms, including cellular proliferation and differentiation. The differentiation of Mesenchymal Stem/Stromal Cells into osteoblasts is crucial for the bone regenerative process. Non-coding RNAs (ncRNAs) do not translate into protein and include small ncRNAs (eg. microRNAs, which are master regulators of gene expression) and long ncRNAs (eg. transcribed ultra-conserved regions (UCR), which do not change throughout evolution). Although some ncRNA are currently being used in clinical trials to treat human diseases, the vast majority remains unexplored. In this study, we aim to identify long ncRNAs (lncRNAs) involved in the osteogenic differentiation process. In the long term, our goal is to use lncRNA molecules as novel tools to promote bone regeneration.

Osteogenic differentiation was induced in MC3T3 cells (mouse preosteoblast cell lines) with dexamethasone, β-glycerophosphate and ascorbic acid. RNA was isolated at day 3 and 7 of differentiation. RNA purity was assessed by ratio of absorbance and RNA integrity was assessed by gel electrophoresis and RNA QC. LncRNA Expression Profiling was evaluated using Mouse LncRNA MicroArray v3.0 from ArrayStar, which comprises intergenic lncRNAs (lincRNAs), UCR and other lncRNAs from public transcriptome databases. Experiments were performed in duplicate. Bioinformatics tools were used to evaluate the degree of conservation along evolution. Results were validated by qRT-PCR. Northern blot was used for detection of UCR transcripts.

Microarrays results show that several lncRNAs are differently expressed after 3 and 7 days of differentiation compared with day 0 and thus are involved in osteogenic differentiation. Importantly, a large number of the differently expressed lncRNA identified is conserved between mouse and human. The majority of UCR analyzed are overexpressed at both time points. Moreover, we identified uc.64+, an intron sense transcript overlapping with EHBP1 gene, as consistently overexpressed during osteogenic differentiation in independent experiments by RT-qPCR. Northern blot results show that this transcript is larger than 200 nucleotides. Furthermore, UCR can potentially act as competing endogenous RNAs since bioinformatic tools show predicted binding sites for multiple microRNAs. In particular, uc.64+ potentially binds to miR-125a-3p, which is a known inhibitor of osteogenic differentiation.

In conclusion, lncRNAs, including UCR, are involved in osteogenic differentiation and might be used as potential novel therapeutics tools to promote cell differentiation into osteoblasts.

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0272 Functional assessment of tissue-engineered vessels for the blood and lymphatic systems

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The vessels of the arterial, venous and lymphatic systems are integral to the circulatory system as a whole. The primary functions of blood vessels are to deliver oxygen and nutrients to organ systems and tissues, and to remove waste products resulting from metabolism. The vessels of the lymphatic system, on the other hand, serve to collect interstitial fluid from the tissues of the body and return it to the circulation via a network of collecting vessels, lymphangions and lymph nodes, which together play an important role in maintaining fluid homeostasis and combating infection. Under physiological conditions, the cellular components present in the vessel walls and fluid phases are continually subjected to shear stresses arising from the pressure gradients imposed on those vessels, and the resulting flow of blood and lymph. The stresses to which cells present in the vessel wall are exposed is known to play a role in their structural development and function; likewise, the stresses to which cells present in the walls of lymphangions are is known to affect their contractility, and consequent ability to transport lymph.

The impact on tissue perfusion of cardiovascular disease, and the removal of lymph nodes and vessels following surgical treatment for cancer, for example, can have a profound effect on the viability of the distal circulation, and lead to fluid imbalances and oedema, which are debilitating for the patients affected; and whereas many groups are active in developing novel strategies for blood vessel replacement, less attention has been paid to research to develop protocols and devices that are able to replicate the functional characteristics of intact blood and lymphatic vessels. Tissue Engineering offers the means to generate *in-vitro* models of blood and lymphatic vessels for drug testing in the short term, and produce wholly biological vessels that surgeons may use to bypass or replace vessels in patients. In order to achieve these goals, however, the engineered vessels must recapitulate the structure and function of the intact vessel wall, yet be able to resist the remodelling pathologies to which venous and synthetic grafts are prone. The purpose of this talk is to review the state-of-the-art in vascular and lymphatic vessel tissue engineering, and show how research of this kind may suggest ways in which engineered vessels may be used to treat conditions associated with inadequate tissue perfusion, oedema, and disorders of the vascular and lymphatic systems. in general.

0273 Evaluating the effect of strain and fluid flow shear stress on vascular endothelial cells

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Cyclic strain and fluid flow shear stress are well known to affect cell behaviour. This is especially true for vascular endothelial cells that are subjected to these mechanical signals in their physiological natural environment. While in-vivo cells experience varying degrees of anisotropy (d.o.a.), in-vitro anisotropic strain studies have mostly focused on uniaxial strains. In order to create a better understanding of cellular behaviour under physiological strain conditions, the response of endothelial cells to strains with varying d.o.a. should be investigated. This is especially important if one wants to use these mechanical signals to optimize the organization and functionality of vascular networks within engineered tissues.

Given the combination of multiple parameters (fluid flow velocity, strain magnitude, strain d.o.a., angle between principal strain and fluid flow) that are subjected to cells simultaneously, we have developed an enhanced throughput device to determine and optimize the mechanical stimulation needed to elicit physiological cellular responses.

Human umbilical vein endothelial cells were found to become elongated and align along the minimum principal strain direction when only strain was applied. An increase in d.o.a. resulted in increased cell alignment. Cells aligned along the flow direction when only flow was applied. When flow and strain were combined, alignment was predominantly in the direction of the flow, but an offset towards the minimum principal strain direction was detected compared to flow alone

The variations in response of cells highlight the need to study the effects of strains of varying d.o.a. on cells. Our device permits such experiments with an increased throughput, which makes it an important tool to better understand these mechanobiological principles.

0274 Assessment of the aggregation capacity of tissue engineering human blood vessel under shear stress

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Previously we have developed a 3D tissue-engineered human blood vessel construct which we hope to use in ex vivo human platelet studies to improve their physiological relevance. Our studies have shown that a construct consisting of a replica of the Tunica Intima and Tunica Media, could replicate the anti- and pro-aggregatory effects of blood vessel on human blood vessels under conditions of magnetic stirring (Musa *et al*., 2016). However *in vivo* platelets come interact with the blood vessel under conditions of shear stress elicited by the flowing blood. In this study, we aimed to investigate whether the pro- and anti-aggregatory effects could be replicated by our tissue-engineered blood vessel construct under physiological flow conditions.

The tissue engineered blood vessel (TEBV) construct was made by a layer-by-layer method in which a tissue-engineered medial layer (TEML) of our blood vessel construct was made by embedding human coronary artery smooth muscle cells (HCASMCs) in a type I collagen hydrogel scaffold, and the tissue-engineered intima layer (TEIL) comprises human umbilical vein endothelial cells (HUVECs) seeded on aligned nanofiber meshes. Platelet aggregation upon the constructs was assessed by fluorescent imaging. The constructs were perfused with washed suspensions of $DiOC_6$-labeled platelets in a ProFlow parallel plate flow chamber (Warner Instruments) at physiological arterial shear stresses of 15 dynes/cm^2.

No platelet aggregation was observed under physiological flow conditions of the full TEBV construct, in line with the ability of the HUVEC mononlayer to prevent interaction with the pro-aggregatory TEML. Similarly no aggregation as observed on collagen hydrogels in which no HCASMCs were seeded, in line with our previous findings that neo-collagen secreted by the HCASMCs was responsible for the pro-aggregatory properties of the construct (Musa *et al*. 2016). In contrast, the TEML construct lacking the TEIL layer was found to support platelet aggregation over 77% of its surface area, consistent with the collagen in this layer being able to support functional thrombus formation. When the construct was damaged by FeCl3 exposure in line with many intravital microscopy experiments, platelet aggregation could be seen across 44% of the construct.

Evaluation of the tissue engineering blood vessel constructs under dynamic shear stress demonstrated that the constructs lacking an intima layer support significant platelets aggregation on their surface, 77% platelets aggregation in terms of the total luminal surface area when comparing with the construct containing a functional intima layer of endothelial cells on top where no aggregation was triggered. This anti-aggregation capacity of the endothelial layer was diminished when the construct was damage by FeCl3, resulting in a 44% aggregation compared to the initial absence of platelets adhesion.

The quantitative and imaging data demonstrate that our TEBV construct contains both the anti- and pro-aggregatory properties of the native blood vessel under physiological flow conditions, such that is could be used to replicate platelet activation in ex vivo conditions.

0275 Lymphangiogenesis in the arteriovenous loop model using mesenchymal stem cells and lymphatic endothelial cells

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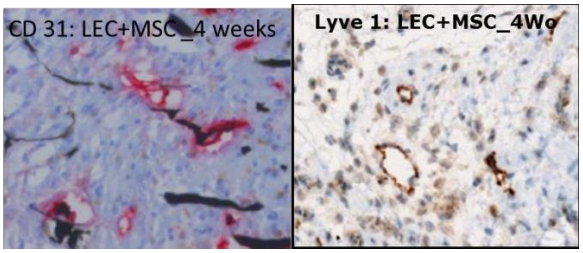
During the last years microsurgical approaches on the lymphatic system came more and more into the focus. Lymphatic vessel transplantation, lymphatic venous anastomoses and lymph node transplantation from an unharmed area from the body are increasingly performed in microsurgical centres all over the world. Using lymphatic vessel transplantation or lymph node transplantation techniques a healthy part of the body has to be used as donor site possibly causing donor site effects like infection, wound healing disorders or even secondary lymphedema on the harvesting side. Until now tissue engineering approaches addressing the lymphatic system are still in the fledgling stages. The present study addresses the interaction between mesenchymal stem cells (MSC) and lymphatic endothelial cells (LEC) and their application in the rat arteriovenous (AV) loop model.

Human LEC were stimulated with MSC conditioned medium (CM) or by co-cultivation with human MSC. LEC proliferation was assessed using an MTT assay. Migration capability was tested by a horizontal and a transmigration assay. To evaluate tube formation capacity LEC were plated on Matrigel™. Cell sprouting was investigated in a 3D-assay. LEC and MSC alone and in combination were implanted in the rat AV loop model for up to 4 weeks and analysed immunohistochemically and on RNA level with real-time PCR.

LEC proliferation and migration could be stimulated by MSC CM due to secretion of LEC stimulating factors in a higher extent than by control medium or growth factors like VEGF-C and bFGF. MSC secreted factors furthermore enhanced LEC tube formation and sprouting. Immunohistochemical stainings against rat and human podoplanin, CD31 and lyve 1 revealed ongoing lymphangiogenesis in the AV loop model. Rat and human lymphatic vessels could be mainly detected in groups with LEC and LEC + MSC. Real-time PCR results showed expression of rat and human lymphatic vessel markers most notably in the LEC + MSC group with and without VEGF- C / bFGF stimulation.

The MSC-derived pro-lymphangiogenic activity could be demonstrated and provide the basis for in vivo experiments. A lymphatic endothelial cell network in the nude rat AV loop model was established. This model will allow deeper insights into lymphangiogenesis and subsequently be used for tissue engineering and lymphangiogenic as well as anti-lymphangiogenesis purposes. Ongoing experiments are focusing on loss-of-function and gain-of-function studies and aiming at identifying the unknown stem cell derived lymphangiogenic activity.



0276 3D bioprinted scaffold-free blood vessels for clinical and research applications

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Tissue engineering and fabrication using 3D bioprinting is an emerging and rapidly expanding field with many exciting applications.

The aims of this work are to produce artificial blood vessels by scaffold-free 3D bioprinting that can be used for medical therapy, as well as research.

We use a novel scaffold-free bio 3D printing method developed in our lab to produce artificial multi-layered blood vessels from spheroids containing human cells. Primary vascular smooth muscle cells, endothelial cells and fibroblasts are used to make tubular vascular structures. We are also currently testing mesenchymal stem cells and vascular progenitor cells. Once the spheroids are assembled on the needle arrays, they mature so that the constructs can fuse and remodel. Cell identity and location are detected by immunohistochemistry. Anti-CD31 and CD34 antibodies identified endothelial cells and smooth muscle cells were detected by an anti-aSMA antibody. Hematoxylin/eosin stain revealed the anatomy of the printed structures. The presence of collagen was evaluated by Mason trichrome staining and elastica Van Gieson stain was employed to assess elastin production, amount and distribution. Mechanical strength was assessed by a tissue puller. We use two pre-clinical models to test properties such as patency, integration, remodelling and endothelialisation: nude rat aorta and arteriovenous shunt in mini-pig.

Cell size, type and percentage determine spheroid size and total cell numbers. Fibroblasts are the cell type capable of making more compact and round spheroids and smooth muscle cells produce bigger spheroids. Tropoelastin was observed, as well as collagen. Flow produced an asymmetrical cell distribution compared to the static control, which shows the importance of sheer stress in cell patterning. There was more angiogenesis in areas of hypoxia, as denoted by CD31 and CD34 staining. Furthermore, the expression of these two markers does not always overlap, showing younger and slightly more mature vessels. Mechanical strength and extracellular matrix production increase at each week of maturation. The artificial vessels are able to maintain patency *in vivo* and endothelialisation was observed.

The printed constructs show similar features to real blood vessels, in terms of cell distribution and response to shear stress and hypoxia, as well as behaviour *in vivo*. Although further improvements can be done, our data show promise for clinical applications, as well as development of in vitro models for cardiovascular diseases and drug testing.

0277 Prevascularization of tissue constructs in collagen hydrogel improves their early perfusion

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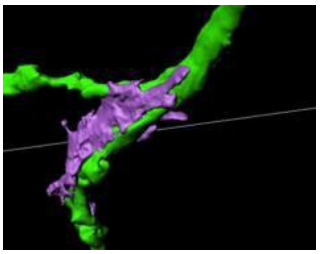
A major challenge in tissue engineering is the promotion of rapid vascularization for survival of engrafted cells in order to repair and regenerate injured tissues. One of the strategies consists in *in vitro* pre-vascularizing the tissue constructs, before implantation. Early blood perfusion of the engineered tissue thus relies on inosculation of the host vasculature with the network generated *in vitro*, rather than on sprouting angiogenesis only. Endothelial capillaries alone are however not sufficient, and a prerequisite for efficient perfusion is the recruitment of perivascular cells that are responsible for the control of blood perfusion and vascular permeability. In this context, we have generated cellularized tissue constructs consisting in collagen hydrogels containing dental pulp stem cells (DPSC). We have recently characterized the angiogenic potential of DPSC and demonstrated the role of FGF2 in the control of HGF and VEGF, two major stimulators of angiogenesis. We then demonstrated that FGF2 treatment was efficient for *in vitro* priming DPSC prior to implantation, in order to increase their angiogenic potential *in vivo* (Gorin et al, Stem Cell Trans Med 2016).

We have now taken advantage of this *in vitro* angiogenic potential and generated tissue constructs co-seeded with DPSC and human endothelial cells. Using light sheet microscopy for rapid and large scale (mm^3) analysis of such constructs, we could detect the engagement of more than 85% of endothelial cells in capillary networks in the hydrogels. We have also characterized culture conditions that control the perivascular recruitment of DPSC on endothelial capillaries. Some DPSC migrate and spread along capillaries, acquiring a typical perivascular cell morphology (purple cell on the green capillary in figure). We are currently further characterizing this subpopulation of DPSC that is recruited by endothelial cells.



We have also implanted these hydrogels subcutaneously in SCID mice in order to analyze the early perfusion of the constructs post-implantation. Using uCT analysis of blood perfusion, we could show that there is a more than two-fold increase in perfusion of the pre-vascularized tissue constructs compared to those seeded only with DPSC pre-conditioned with FGF2. These data provide the proof of concept that pre-vascularizing tissue constructs *in vitro* using endothelial cells and stem cells does allow for their rapid perfusion post-implantation.

0278 Preparation of tissue development-mimicking ECM scaffolds for stem cell culture

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Extracellular matrices (ECM) interact with cells and provide various signals for controlling cell functions. ECM are not only tissue-specific but are also dynamically remodeled and balanced during the development and aging process to maintain the metabolism of each tissue and organ. To mimic the dynamic change of ECM composition, we have developed a method to prepare development-mimicking ECM by using cultured cells. By the method, we have prepared “stepwise osteogenesis-mimicking matrices”, “stepwise adipogenesis-mimicking matrices” and “stepwise osteogenesis-co-adipogenesis-mimicking matrices” that respectively replicated the dynamically changing ECM secreted at different stages of osteogenesis or adipogenesis or osteogenesis-co-adipogenesis [1-3]. In this study, ECM porous scaffold that mimicked the dynamic ECM change during stepwise chondrogenesis were prepared and used to investigate their effects on differentiation of human bone marrow-derived mesenchymal stem cells (MSCs).

MSCs were cultured in a PLGA mesh (template) in growth medium. After 12 h of culture, the medium was changed to chondrogenic medium. The chondrogenic induction was continued for 3 weeks. The MSCs were also cultured in the PLGA mesh template in DMEM medium (growth medium) for 1 week to obtain undifferentiated MSCs as a control. To confirm the different stage of chondrogenesis, content change of sulfated glycosaminoglycans (sGAG) was measured. Gene expression of *COL1A2* (collagen I), *COL2A1* (collagen II), *ACAN* (aggrecan) and *SOX9* was analyzed by Real time-PCR. The results suggested that the chondrogenic differentiation of MSCs was progressed gradually. MSCs cultured in chondrogenic medium for 1 and 3 w were defined as the early stage (CE) and late stage (CL) of chondrogenesis, respectively. MSCs cultured in growth medium was defined as stem cell stage (SC). MSCs at stem cell stage, early stage and late stage of chondrogenesis were used to prepare stem cell ECM scaffold (SC-ECM scaffold), early stage chondrogenesis-mimicking ECM scaffold (CE-ECM scaffold) and late stage chondrogenesis-mimicking ECM scaffold (CL-ECM scaffold), respectively. Cellular components and PLGA template were removed after cell culture to obtain the ECM scaffolds. The SC-ECM, CE-ECM and CL-ECM scaffolds had different composition. SC-ECM scaffold was rich in collagen I and biglycan. CE-ECM scaffold had moderate amount of collagen II and aggrecan while CL-ECM scaffold was rich in collagen II and aggrecan. The ECM scaffolds showed different effects on chondrogenesis of MSCs. The CE-ECM scaffold facilitated chondrogenesis, however the CL-ECM scaffolds remarkably inhibited chondrogenesis of MSCs. The matrices should be useful to investigate the interaction between ECM and stem cells and the role of ECM on stem cell differentiation.

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0279 Facilitating pluripotent stem cell growth and differentiation by using 2D substrates and 3D printable materials

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A stem cell-niche is a microenvironment rich in extracellular matrix (ECM) and growth factors, wherein these cells reside in vivo. The niche saves stem cells from depletion, while protecting the host from over-exuberant stem-cell proliferation. When activated by exogenous stimuli they release the cells to differentiate and repair or regenerate diseased tissue. Therefore the stem cell niche plays not only a structural but also a functional role in maintaining pluripotency and guiding stem cell fate in vivo. So targeting the stem cell niche could have immense therapeutic benefits in a number of strategies aimed at manipulating stem cells for regenerative medicine. In an attempt to mimic the stem cell niche in vitro we employed 2D substrates. In the first example, we utilized biofabrication technique to surface immobilize and pattern Hyaluronic Acid (HA) and Leukemia inhibitory factor (LIF) to maintain the pluripotency of embryonic stem (ES) or induced pluripotent stem (iPS) cells in vitro. In another study, we prepared a chemically-fixed feeder cell derived substrate to mimic a niche for culturing and maintaining the pluripotency of human iPS cells.

Currently we are investigating the utility of 3D printable biomaterials for culturing pluripotent stem cells, including human mesenchymal stem (MSC) and iPS cells. To this end, polyvinyl alcohol (PVA) scaffolds were fabricated using a commercially available MakerBot 3D printer. Various scaffold designs were made including a lattice, mesh and wood pile structure. These scaffolds had different porosities, although similar degradation rates and other material properties when analysed. This lead to their difference in cell adhesion, culture and proliferation when seeded with human MSC cells. Currently work is underway to elucidate and optimize a scaffold design that will promote maximum osteoblastic or chondrogenic differentiation when cultured with human MSC.

In another separate study, we are utilizing the commercially available BioBot 3D printer to mimic the complex architectural patterns of biological tissues and matrices utilizing printable gel-like materials such as Pluronic F-127 (BioKey), visible light induced cross-linkable gelatin (furfuryl-gelatin) and porcine gelatin (controls). The BioBot allows printing via co-deposition of cells and gels with 2 nozzles that work simultaneously. Therefore in our current experiments we are testing the ability of all of the above mentioned gel-like materials for their flexibility and adaptation to 3D printing; and in a second part we will be co-printing patterns of cells in gels to mimic blood vessels, bones and also cardiac tissues in vitro. Ultimately we will be infusing nanostructures such as carbon nanotubes (CNT) to reinforce hydrogels utilizing bioprinting to mimic the rigidity, texture and electrical properties of biological tissues in vivo.

0280 Continuous harvest and culture of stem cells via partial detachment on thermoresponsive nanobrush surfaces

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Stem cells, including human mesenchymal stem cells (hMSCs), human embryonic stem cells (hESCs), and induced pluripotent stem cells (hiPSCs), are attractive reagents for regenerative medicine, translational medicine, and drug discovery. However, stem cell culture is typically based on batch-type culture, which is laborious and expensive. Here, we proposed a continuous harvest method for stem cells cultured on thermoresponsive nanobrush surfaces. In this method, stem cells are partially detached from the nanobrush surface by reducing the temperature of the culture medium below the critical solution temperature needed for thermoresponse. The detached stem cells are harvested by exchange into fresh culture medium. Following this, the remaining cells are continuously cultured by expansion in fresh culture medium at 37 ˚C (Fig. 1A). Thermoresponsive nanobrush surfaces were prepared by coating block copolymers containing polystyrene (for hydrophobic anchoring onto culture dishes) with three types of polymers: (a) polyacrylic acid with cell-binding oligopeptides, (b) thermoresponsive poly-N-isopropylacrylamide, and (c) hydrophilic poly(ethyleneglycol)methacrylate (Fig. 1B). The optimal coating durations and compositions for these copolymers to facilitate adequate attachment and detachment of human adipose-derived stem cells (hADSCs) and embryonic stem cells (hESCs) were determined. hADSCs and hESCs were continuously harvested for 5 and 12 cycles, respectively, via the partial detachment of cells from thermoresponsive nanobrush surfaces (Fig. 1C). We also evaluated hESC pluripotency following 12 partial detachment cycles by immunostaining proteins associated with pluripotency. After 12 cycles of partial detachment, hESCs were found to express pluripotent Oct4 and Sox2 proteins. Furthermore, hESCs retained the ability to differentiate into cells derived from all three germ layers following partial detachment from a the thermoresponsive nanobrush surface.

We successfully demonstrated that culture on a thermoresponsive nanobrush surface enabled hADSCs and hESCs to be continuously harvested for 5 and 12 cycles, respectively, when using a partial detachment protocol. Such continuous harvest of stem cells should downsize the equipment requirements for stem cell culture and simplify the culture process. These improvements should decrease the costs of therapies using hESCs and hiPSC. As cost is the current bottleneck for clinical therapies using hESCs and hiPSCs, the methodology presented herein should expand treatment options.

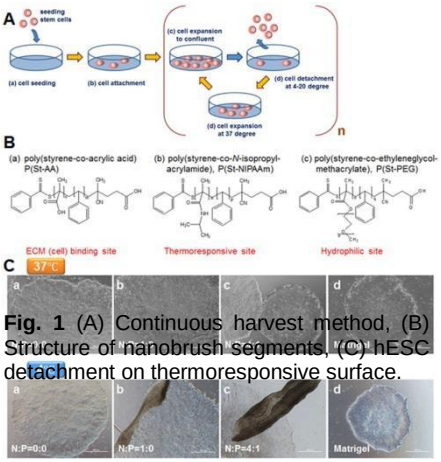

Fig. 1 (A) Continuous harvest method, (B) Structure of nanobrush segments, (C) hESC detachment on thermoresponsive surface.

0281 Liver tissue engineering: differentiation of iPSCs on acellular scaffolds under dynamic culture

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For liver failure pathologies, transplantation remains the main treatment but is limited by donor shortage. Whole organ tissue engineering using decellularised scaffolds may provide potential autologous substitutes that preserve the complex native anatomy of the organ and the biochemical cues to promote cell growth and differentiation. Those features could help promoting a more mature phenotype of liver cells derived from induced Pluripotent Stem cells (iPSc).

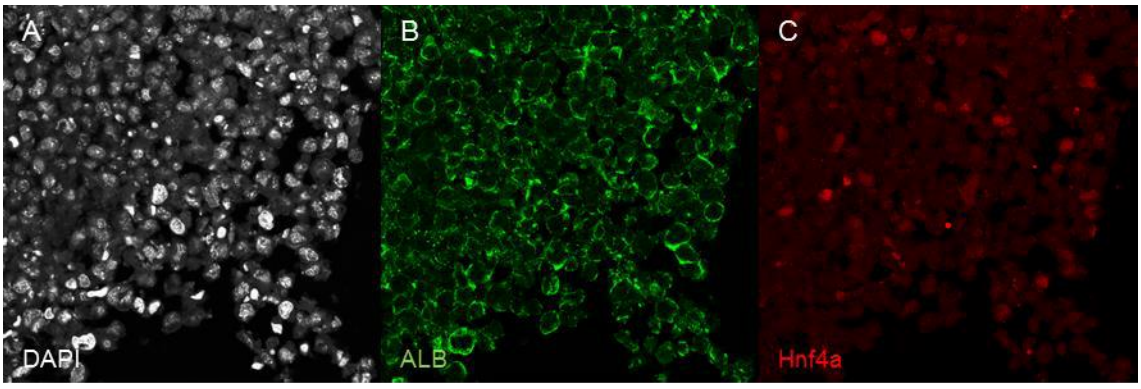
The aim of this study is to investigate the differentiation and growth of iPSCs towards hepatocyte phenotype cultured on decellularised livers inside a custom made bioreactor.

Mice livers were decellularised using a previously developed detergent protocol able to maintain the hepatic extracellular matrix structure. Human iPSCs were differentiated into hepatic endoderm according to a previously published method. Cells were harvested at the end of the definitive endoderm stage and injection-seeded into the decellularised scaffold lobes and cultured in the bioreactor for 14 days. Cells cultured in static on decellularised liver lobes and Matrigel-coated plates were used as control.

Results showed a faster differentiation of iPSCs with higher expression of mature hepatocyte markers (albumin) and lower expression of fetal markers (AFP) in the decellularised scaffold compared to 2D culture. Moreover, the bioreactor cultured scaffold showed an increased distribution and spreading of the seeded cells throughout the whole liver in comparison to static culture (Figure 1).

To date, differentiation of iPSCs toward hepatocytes resulted in phenotype closer to foetal one. Here we demonstrated how a 3D culture on extracellular matrix scaffold inside a bioreactor is able to reach a more mature phenotype. These crucial findings pave the way to the engineering of whole livers for grafting and for complex in-vitro drug response models.

Figure 1



0282 Directed-nanopatterning promotes earlier liver-specific maturation and outperforms metabolic function of differentiated human hepatic HepaRG progenitor cells on standard tissue culture plastic

Katie Morgan[1], Anna Bryans[1], Filip Brzeszczynski[1], Paul Reynolds[2], Michael Mabbott[1], Nicole Martucci[1], Graham Anderson[1], Steve Morley[1], John Plevris[1], Peter Hayes[1], Nikolaj Gadegaard[2], Leonard J Nelson[1]

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INTRODUCTION: Nanotopography has been increasingly used in the assessment of cellular adhesion, proliferation and function, with the differentiation potential of stem cells typically used for their plasticity. We previously demonstrated mesenchymal progenitor cells cultured on nano-displaced topography, significantly increased osteospecific differentiation[1]. Having demonstrated significantly earlier differentiation of HepaRG progenitor cells on prototype 2D-nanopatterned polymer substrates NPS1 and NPS2 (with nano-displaced topography), we now show cells cultured on these patterns show better metabolic functionality and expression of a panel of mature hepatic markers on nanopattern surfaces compared with Corning industry standard culture plastic.

METHODS: HepaRG101 progenitor cells [Biopredic Inc: BPI] were seeded (>95% viable; 30000 cm^2) and cultured for ≤28 days [BPI Growth Medium] on prototype 2D-nanopatterned polymer substrates (NPS: 2 nanopatterns [each well 0.32cm^2]; including 1x planar control; fabricated using high-resolution electron beam lithography); or standard Corning plastic culture dishes (SCPs). Phenotype and metabolic competence (ATP; Prestoblue assays) of cells were assessed in parallel with phenotypic profiling immunocytoflourecence staining (ICS) using liver-specific/ maturation-differentiation markers (Transferrin; Alpha fetal protein; HNF4a; Sox9 and CYP3A4). End point and qRTPCR were used to validate and quantify maturation-differentiation markers.

RESULTS: We previously identified displaced nano-topographies NPS1 & NPS2 which show significantly enhanced liver-like morphological features (granular hepatocytes; with round nuclei), which appeared earlier (d6 *vs* d14 on SCPs), as well as d6 planar or SCP controls. Following on from this study, we can now confirm fully functional cells on day 7 expressing higher viability (PB and total ATP), metabolic function (CYP3A4 ICS) transport of iron (Transferrin ICS), and tight junction protein ZO1, which confirms polarity of functional hepatocytes. Using end point PCR and qRTPCR we also confirm presence of CYP3A4, and reciprocal Sox9/AFP to HNF4a which correlates to previous staining and further validates a mature hepatic culture on day 7.

CONCLUSIONS: Previously we reported HepaRG progenitor cells grown on NPS1 and NPS2 showed earlier commitment to a hepatocyte lineage even in the absence of liver differentiation supplements (DMSO induction) vs Corning or other nanopatterns tested. Herein, we show that these cells express differentiated functional markers CYP3A4, Transferrin and HNF4a, and are capable of higher metabolic activity ~7-10 days earlier than those grown on industry standard Corning. Quantification of genes of interest using qRTPCR confirms HepaRG progenitors grown on NPS1 and NPS2 out perform those grown on Corning by statistically significant fold increases. This prototype nanopattern platform may provide novel substrates for efficient differentiation of hepatic progenitor cells – and a time saving, cost effective and efficient model for *in vitro* drug toxicity testing.

0283 Effect of shear stress on adipose-derived mesenchymal stem cells in tissue-engineered vascular grafts

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Introduction: Adipose-derived mesenchymal stem cells (ADMSCs) have emerged as cell source for vascular tissue engineering because of the possibility of obtaining an initially high number of cells in a minimally invasive way by lipoaspiration. In the attempt to derive endothelial cells from ADMSCs, generally the stem cells are subjected to growth factors in culture flasks for weeks before being exposed to shear stress. In this project, we investigated the effect of shear stress on the differentiation of ADMSCs towards ECs without any precultivation in differentiating medium.

Methods: Small-caliber vascular composite grafts (inner diameter = 3mm) were fabricated using a fibrin scaffold reinforced with a polyvinylidene fluoride warp-knitted macroporous mesh. The grafts were seeded with ADMSCs on their luminal surface. The constructs were then transferred to bioreactor systems containing EGM-2 (Lonza) and were kept under low flow perfusion overnight. Subsequently, the flow was gradually increased to reach 5, 10 or 15 dynes/cm² and a pressure of 80/120 mmHg. The tissue-engineered vascular grafts (TEVGs) were conditioned in the bioreactors for 14 days. TEVGs exposed only to low flow perfusion for 14 days were used as control. Evaluation included immunohistochemistry, quantitative real-time polymerase chain reaction (RT-PCR), nitric oxide (NO) quantification and a thrombogenicity assay.

Results: The cells were aligned along the flow direction in all the TEVGs exposed to shear stress as confirmed by actin filament staining (Fig.1). RT-PCR showed up-regulation of CD31, vWF, VE-Cadherin, and KLF-2, and a down-regulation of α-SMA in the mechanically stimulated grafts compared to the controls. The NO level in the medium increased with increasing shear stress. The thrombogenicity test showed no platelet activation in any of the conditioned samples,

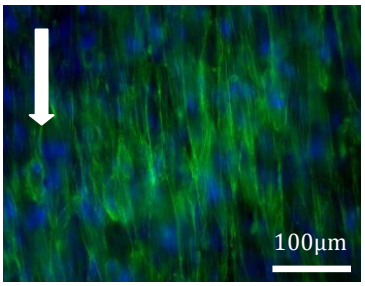

Figure 1. Actin filament alignment in graft exposed to shear stress. Arrow indicates flow direction.

Discussion and conclusion: The alignment in flow direction and the NO synthesis is a characteristic response of endothelial cells to shear stress; these findings along with the increased expression of endothelial specific genes in the mechanically stimulated cells suggest an induction of the ADMSCs towards ECs by shear stress exposure. In addition, the up-regulation of KLF-2 and the results of the thrombogenicity assay indicate the presence of a non-thrombogenic cell layer. The fact that the results obtained were more pronounced in the shear stressed samples indicates that the mechanical stimulation has an accelerating effect on the differentiation process of the ADMSCs.

0284 IMPRESS Network Symposium: Autologous skeletal muscle cell therapy for anal incontinence

Andrea Frudinger

Medical University of Graz, Graz, Austria

Incontinence significantly reduces the quality of life for millions of people and places a massive financial burden on healthcare services. To date, very little research exists that explores the use of regenerative medicine for the prevention and treatment of incontinence.

The IMPRESS Network keynote talk will provide the audience with an understanding of the aetiology, physiology, anatomy, biomechanics, biology and biochemistry of incontinence, together with the impact on quality of life and how this is quantified.

The talk will then discuss the use of regenerative medicine research that has investigated the feasibility and efficacy of delivering autologous myoblasts to treat anal incontinence (AI) caused by obstetric external anal sphincter disruption. This will focus on the author's published data from observational pilot studies conducted in women with AI refractory to conventional non-surgical therapy, which showed that injection of autologous myoblasts was technically feasible, well tolerated and improved symptoms of anal incontinence. A recently published follow-up at 5 years has shown sustained improvement of incontinence symptoms with no adverse events.

Finally the talk will discuss the challenges that have been identified along the pathway for clinical translation. This will highlight what has been learnt from the author's pioneering studies, including new high-impact opportunities for the tissue engineering and regenerative medicine research communities, such as the mode of cell application and the use of bespoke ultrasound sound technology for targeted delivery.

The intended outcome from this keynote talk is for the TERMIS audience to be educated in the wide range of new opportunities that exist in the field of incontinence research.

0285 IMPRESS Network Symposium: Delivery of myoblasts in an anchored state for sphincter muscle regeneration

Pauline Guhmann[1], Eseelle Hendow[1], Sara Maffioletti[1], Ornella Cappellari[2], Kim Wells[2], Stephen Patrick[1], Dominic Wells[2], Francesco Saverio Tedesco[1], Richard Day[1]

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Background: Obstetric trauma is a common cause of incontinence in women as a result of volumetric muscle loss (VML) and functional impairment of the sphincter muscle. The use of autologous myoblasts in suspension for cell-based incontinence therapy has led to mixed results. Outcomes might be improved if myoblasts are delivered anchored to a substrate, more closely resembling cells in their natural state. Biodegradable microcarriers could offer a novel approach for localized delivery of adherent cells, such as myoblasts, into localized sites of VML such as that associated with obstetric trauma.

Aim: The aim of this study was to investigate the attachment of human myoblasts to the surface of highly porous TIPS microcarriers and assess their ability to rescue a functional defect created in skeletal muscle *in vivo*.

Methods: eGFP-Luciferase transfected human skeletal muscle myoblasts (HSMM) were attached to the surface of poly-DL(lactide-co-glycolide) TIPS microcarriers under static-dynamic incubation. Phenotypic analysis of HSMM attached to TIPS microcarriers under conditions promoting proliferation or differentiation was examined by immunostaining and pre-clinical potency tested by transplantation into a punch biopsy wound created in the tibialis anterior of SCID mice. Control groups included cells delivered as a suspension. Retention of cells at the implant site was determined by bioluminescence and functional rescue measured via tetanic force generation.

Results: HSMM attached readily to the surface of TIPS microcarriers. Once attached to the surface of TIPS microcarriers, myoblasts proliferated when maintained in suspension culture. Attachment of HSMM to TIPS microcarriers did not impair their ability to migrate, proliferate and form skeletal muscle myosin heavy chain-positive myotubes. Bioluminescence indicated myoblasts transplanted *in vivo* on the surface of TIPS microcarriers were retained at the delivery site and remained viable as the microcarriers biodegrade. Tetanic force in the injured muscle treated with HSMM attached to TIPS microcarriers was restored to a greater extent and more consistently compared with muscle treated with HSMM in suspension.

Conclusion: The model used in the study simulates obstetric trauma and damage to skeletal muscle in the external anal sphincter muscle that results in reduced force generation and incontinence. The results indicate it is technically feasible to use specific formulations of TIPS microcarriers to deliver human myoblasts for functional rescue in a SCID murine model of volumetric skeletal muscle loss.

0286 IMPRESS Network Symposium: Development of smart injectable bulking agent formulations for the treatment of urinary incontinence

Elif Vardar[1], Hans Mattias Larsson[1], Eva-Maria Engelhardt[1], Ganesh Vythilingam[1,2], Jeffrey Alan Hubbell[1,3], Matthias Lutolf[1], Peter Frey[1]

[1]EPFL, Institute of Bioengineering, Lausanne, Switzerland, [2]University Malaya, Kuala Lumpur, Malaysia, [3]University of Chicago, Institute for Molecular Engineering, Chicago, USA

Urinary incontinence is a devastating medical condition where over 200 million patients suffer from a deficient sphincter/bladder function, resulting in involuntarily urine leakage (1). The use of urethral-vaginal slings and injectable bulking agents are two of the common treatment modalities for this condition. They help re-establish the urethral outlet-resistance. However, due to risk of urethral infection, irritation or inflammation, and urine retention, the performance of sling operations is still under discussion (2). The use of urethral bulking agents is preferred due to its less invasive nature. The ideal bulking agent should be easily injectable, should result in persisting bulking effect, and regenerate functional tissue without causing scaring, as seen in the use of clinically available urethral bulking agents. Additionally, it should have a good biocompatibility and low immunogenicity. In this project a new generation of injectable bulking formulation, which will provide permanent healing by de novo regeneration of smooth muscle, was developed. This injectable bulking agent consists of fibrin micro-beads, which are delivering covalently linked growth factors in a controlled and sustainable manner at the injection site, and which are incorporated within homogenized cross-linked collagen, acting as a bead carrier material. The fibrin micro-beads containing recombinant human insulin like growth factor-1 (rIGF-1), previously produced in our lab (3), were fabricated using a microfluidic platform. Scanning electron microscopy (SEM) was performed to reveal the size and morphology of fibrin micro-beads. They were homogeneous in terms of size and porosity, showing highly dense fiber bundling. rIGF-1 binding efficiency to fibrin micro-beads was assessed using ELISA. After an intensive wash of the fibrin micro-beads, over 86% of the initially loaded amount of rIGF-1 was still present within the fibrin micro-beads. Migration of GFP-labeled human smooth muscle cells (SMCs) was monitored with a Cell IQ imaging system. GFP-SMCs penetration into homogenized collagen matrices was evaluated using inverted confocal microscopy. A significantly greater number of GFP-expressing SMCs had migrated towards rIGF-1 factor loaded fibrin micro-beads compared to the number of SMCs that had migrated towards fibrin micro-beads without growth factor. Fibrin micro-beads were then embedded in homogenized collagen gels and their mechanical stability investigated to evaluate its potential as a bulking agent. The resulting bulking formulation had shown an improved mechanical properties compared to Deflux®, a commercially available urinary bulking agent. Moreover, it has been successfully injected into the rat bladder wall and under the dorsal skin of rats. Further, retention time of subcutaneously injected homogenized cross-linked collagen, containing fibrin micro-beads, will be evaluated *in vivo*.

0287 IMPRESS Network Symposium: Current bottlenecks in stem cell treatment of urinary incontinence

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Stress Urinary Incontinence (SUI) is a disease affecting over 200 million people worldwide (over 37 million people in Europe) and is twice as common in women as in men. Childbirth and menopause are major reasons of the increased prevalence of incontinence in women, whereas prostatectomy is one of the main causes in men. The quality of life of patients suffering from urinary incontinence is markedly decreased due to limited daily activities, unpleasant sensation, odour and infections caused by wet diapers.

Current treatment options for SUI include mainly non-surgical therapy (including behavioural therapy e.g. bladder training, fluid/dietary modification), drug therapy and surgical therapy. These therapies only offer short-term relief and the overall success is often limited by complications (invasiveness of surgery, damage to surrounding tissues, leading to increased urinary infection rates) or side-effects (drugs, tissue damage by non-degradable biomaterials).

The significantly reduced quality of life due to incontinence; high healthcare costs and complication rates, with rather limited success of the available therapies; and the constantly aging population are just some of the main factors, showing the urgent clinical need for novel treatment modalities.

Advances in cell therapy approaches to treat urinary incontinence show promising results towards correcting the underlying etiology using the patient's own cells. The quality of the regenerated tissue is of crucial importance for its proper function and thus, many efforts have been applied to improve in vivo tissue development and cell survival.

Early clinical trials using stem, or progenitor cells for the treatment of SUI in both male and female patients have promising functional results with minimal adverse effects. However, as simple as the concept seems to be, the precise identification, isolation and transplantation of these cells could be more complex than initially thought. Some points seem to be fundamental for the long-term success of this therapy: functional distinction between stem cells and their progenitor cells; the transition of the stem cell between quiescent and active stages; control of the microenvironment and stem cell niche; provision of additional factors to the cellular component for improving engraftment and survival/differentiation.

Although many challenges remain to be addressed for the optimal clinical implementation of this technology, novel stem-cell-based multisystem-therapies are an exciting potential therapy for voiding dysfunction.

0288 Mechanotherapies for optimizing skeletal muscle regeneration

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Muscle regeneration is predominantly dictated by the action of muscle stem, or “satellite”, cells (MuSCs), a reserve cell population that demonstrates considerable dysfunction with disease and increasing age. According to the stem cell niche concept, stem cell responses are significantly influenced by biophysical and biochemical cues that emanate from the surrounding microenvironment. An improved understanding of how disease and aging drive defects in tissue regeneration will help optimize the development of targeted and specific rehabilitation protocols to maximize physical functioning for individuals across the lifespan.

This presentation will highlight work investigating mechanisms underlying the decline in muscle regenerative capacity over time, and the potential for the application of targeted mechanical stimulation protocols to rejuvenate skeletal muscle healing. More broadly, the goal is to stimulate reflection and debate into the emerging role of regenerative medicine and regenerative biology in physical therapy practice.

0289 Tenogenic phenotype maintenance using macromolecular crowding and low oxygen tension

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Cellular therapies play an important role in tendon tissue engineering and regenerative medicine with tenocytes being described as the most prominent cell population for these applications if available in large numbers. However, this is difficult to achieve, because *in vitro* expansion of tenocytes leads to phenotypic drift and loss of function. Recent work suggests that maintenance of tenogenic phenotype *in vitro* can be achieved by recapitulating different aspects of the native tendon microenvironment. One approach used to modulate *in vitro* microenvironment and enhance extracellular matrix (ECM) deposition is macromolecular crowding (MMC). MMC is based on the addition of inert macromolecules to the culture media to mimic the dense extracellular matrix and accelerate the production of ECM-rich substitutes. In addition, as tendon has been described to be a relatively avascular and hypoxic tissue and low oxygen tension can stimulate collagen synthesis and cross-linking through the activation of hypoxia-inducible factor 1-alpha (HIF1-α), we venture to assess the synergistic effect of MMC and low oxygen tension on human tenocyte phenotype maintenance by enhancing deposition of tissue-specific synthesis and deposition.

SDS-PAGE and immunocytochemistry analysis, demonstrated that human tenocytes treated with the optimal MMC concentration at 2% oxygen tension showed increased collagen type I synthesis and deposition after 7 days (Figure 1). Moreover, immunocytochemistry for collagen type III and fibronectin illustrated enhanced deposition when cells were treated with MMC at 2% oxygen tension. In addition, it was shown that low oxygen tension and MMC did not affect the spindle-shape morphology, metabolic activity, proliferation and viability of human tenocytes Collectively, these results suggest that the synergistic effect of optimal macromolecular crowding concentration and low oxygen tension (2%) can accelerate the formation of ECM-rich substitutes, which may stimulate tenogenic phenotype maintenance. Further gene and protein analysis for tendon specific markers should be performed to validate our promising results.

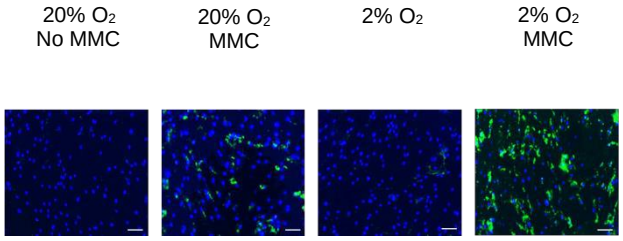

Figure 1: Immunocytochemistry for collagen type I deposition by tenocytes cultured at 20% and 2% O_2.

0290 Nanotopographical features influence derivation and selection of reference genes for progenitor cell differentiation protocols

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INTRODUCTION: Mechanotransduction pathways convert mechanical stimuli such as substrate rigidity, which can lead to changes in gene expression profiles [1]. Similarly, responses to different nanopattern surface-mediated biophysical cues may alter the stability of reference genes (RefG) (eg GAPDH, B-actin) widely used in normalization for quantitative Real Time PCR experiments (qRT-PCR). Adherence to MIQE guidelines are now essential for publication of meaningful quantitiative gene expression data. This includes use of RefGs that are stable across all experimental conditions. Here we describe an approach to determining the most appropriate RefGs across different nano-topographies, utilizing human progenitor cells. Our aim was to provide a rational approach for determination of the most appropriate RefG, under different culture conditions (cell differentiation/ surface engineering) from a set of tested candidate RefGs using qRT-PCR.

METHODS: HepaRG101 bipotential progenitor cells [Biopredic Inc: BPI] were seeded (>95% viable; 30,000 cm^2) and cultured for ≤28 days [BPI Growth Medium] under 4 conditions: On prototype 2D-nanopatterned polymer substrates (NPS: 2 nanopatterns [each well 0.32cm^2]; including planar control; fabricated using high-resolution electron beam lithography); or standard Corning plastic culture dishes. RNA extraction was undertaken using Life Technologies RNAqueous kit and RNA purity/quantity was measured using Thermo Fisher nanodrop and Agilent chip technology. cDNA was created using PrimerDesign nanoScript to RT kit and qRTPCR was performed using PrimerDesign-validated primers and master mix. 12 candidate genes were assessed using geNorm. **RESULTS:** Total RNA samples showed both high quality (260/280 ratios: 1.8-2.0) and RNA integrity (RIN 9-10). geNorm analysis of the initial 6 candidate genes did not provide a suitable RefG for data normalization (M>1 indicated low expression stability; where M = expression stability). Next, we assessed the expression stability levels of 12 candidate genes' and identified an optimal single RefG (*CYC1*) as the most suitable RefG with the lowest M value for HepaRG transcriptional profiling across all conditions. These data sets revealed that the widely used RefGs, GAPDH, 18s and β-Actin, lack expression stability as ranked by the geNorm algorithm, and that expression profiles of RefGs can both vary across different experimental treatment/ conditions. **CONCLUSIONS:** Adherence to MIQE guidelines is crucial when selecting reference genes to ensure results for TERM applications are reliable and reproducible. Our validation of RefGs for HepaRG progenitor cells concurs with recent studies [1] in which application of standard RefGs (GAPDH/18s/β-Actin) were sub-optimal for transcriptional profiling of HepaRG cells. Thus reliance on coventional RefGs, often under diverse test conditions, introduces potentially high variability of quantitative transciptomic expression data. In conclusion, stringent, objective validation of RefG(s), that are stable across all test conditions including cell type and biophysical substrate, is critical for meaningful, biologically relevant quantative gene expression results.

0291 Biological and biophysical stimulation on implant osteolysis and aseptic loosening: effects of pulsed electromagnetic fields and platelet derivatives

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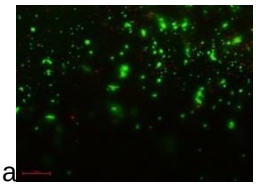
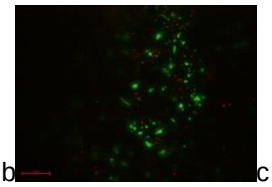
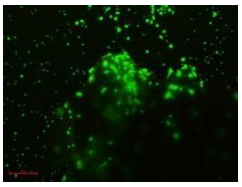
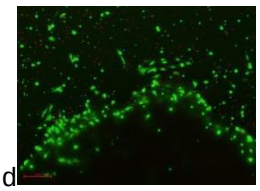
Rizzoli Orthopedic Institute, Bologna, Italy

Wear-particle osteolysis affects prosthesis survival leading to implant loosening up to 70% of revisions: particles generated from metal, ceramic or plastic materials, as polyethylene (PE) and accumulate in the peri-prosthetic tissue, are quickly swallowed up by monocytes, leading to the release of proteolytic enzymes and pro-inflammatory cytokines. The prolonged macrophage activation leads to their differentiation into osteoclasts (OCs) and to the onset of an osteolysis phenomenon.

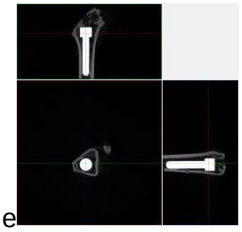
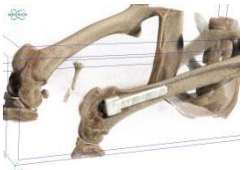
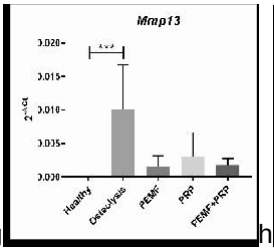
The aim of the study was to evaluate whether Platelet Rich Plasma (PRP), biophysical stimulations, Pulsed Electromagnetic Field (PEMF), or their combination might impact on *in vitro* and *in vivo* models of osteolysis induced by PE.

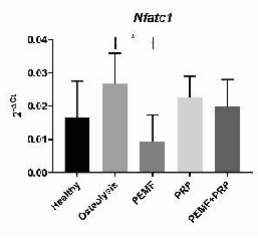
Monocytes, cultured in presence of PE particles (Fig.a), were treated with PEMF (Fig.b), PRP (Fig.c) or their combination (Fig.d) and their effects were investigated. PEMF and PEMF+PRP increased cell viability more than PRP; all treatments were able to reduce the expression of Catepsin K, a marker of osteoclastogenesis, and the inflammatory response, in terms of IL1β production. PEMF was associated with an increased TGFβ1 expression.

a  b  c  d 

An *in vivo* study of peri-implant osteolysis was performed in rats: intra-articular knee injections of PE were used to induce bone resorption around knee titanium implants, as evidenced by MicroCT (Fig.e, f). Then treatments were given: PEMF stimulation (6 hrs/day for 60 days), three PRP intra-articular injections, the association of both or no treatment.

Preliminary results suggested that, at the synovium level, osteolysis caused an increase in Catepsin K, Tnfα, IL1β, IL6 and MMP13 (Fig.g) in comparison to healthy rats. Treatments were able to modulate catabolic enzymes expression (Fig.h).

e  f  g 

h 

The authors would like to thank Programma Programma di Ricerca Regione-Università 2010-2012, Area 1-“Bando Giovani Ricercatori” for providing the financial support to BIO.BIO.ST.IM.O.LO project and Rizzoli Orthopedic Institute (Ricerca Corrente).

0292 Human central nervous system stem cell intramedullary transplantation in thoracic spinal cord injury: findings of a phase I/II study

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Objective: Demonstration of comprehensive safety and preliminary efficacy of human neural stem cells (HuCNS-SC®) is critical for the overall consideration of applicability in spinal cord injury (SCI). Final data from the Phase I/II study involving intramedullary transplantation of allogeneic HuCNS-SC in subjects with sub-acute/chronic thoracic (T2-T11) SCI will be presented including clinical (motor and sensory function) and corresponding neurophysiological data.

Design: Prospective open-label, multicentre, multinational study of HuCNS-SC transplantation in subjects with complete and incomplete thoracic SCI (ASIA Impairment Scale [AIS] A and B). An independent Data Monitoring Committee reviewed on-going safety data throughout the study, including the transition of enrolment from AIS A to AIS B subjects.

Results: In total, 12 subjects (mean age 33 years; 11 males) were transplanted with HuCNS-SC at a mean of 11 months post-injury. The 1 year follow-up data, to date, for all subjects demonstrated safety (e.g., no post-surgical deterioration of spinal neurological function). Segmental and below level sensory improvements were observed in 7/12 subjects.

Conclusion: The data from the completed 1 year Phase I/II study involving the first investigation of HuCNS-SC transplantation in spinal cord injury will be presented. Safety and preliminary efficacy findings demonstrating the feasibility and potential of neural stem cell transplantation in human SCI will be summarized.

Support: This study is sponsored by StemCells, Inc. (www.stemcellsinc.com).

0293 Combining adipose tissue-derived stem cells and olfactory ensheathing cells for spinal cord injury recovery

Eduardo Gomes[1,2], Sofia Mendes[1,2], Rita Assunção-Silva[1,2], Fábio Teixeira[1,2], Hugo Leite-Almeida[1,2], Jeffrey Gimble[3], Nuno Sousa[1,2], Nuno Silva[1,2], António Salgado[1,2]

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Patients suffering from spinal cord injuries (SCI) still have a dismal prognosis. Despite all the efforts developed in this area, currently there are no effective treatments. Therefore, cell therapies have been proposed as a viable alternative to the current treatments employed. Adipose tissue-derived Stem Cells (ASCs) and Olfactory Ensheathing Cells (OECs) have been used with promising results in different models of SCI, namely due to the regenerative properties of the secretome of the first, and the guidance capability of the second. Using an *in vitro* model of axonal growth, the Dorsal Root Ganglia explants (DRGs), we demonstrated that OECs induce neurite outgrowth mainly through cell-cell interactions, while ASCs' effects are strongly mediated by the release of paracrine factors. Then, the co-transplantation of ASCs and OECs showed to improve motor deficits of SCI-rats. Particular parameters of movement such as stepping, coordination and toe clearance were improved in rats that received the transplant of cells, in comparison to non-treated rats. A histological analysis of the spinal cord tissues was done in order to assess levels of axonal regeneration (neurofilament and tyrosine hydroxylase), astrogliosis (glial fibrillary acidic protein) and inflammation (cluster of differentiation 11b/c). Results revealed that transplantation of ASCs and OECs had a major effect on the reduction of inflammatory cells close the lesion site. A slight reduction of astrogliosis was also evident. Overall the results obtained with the present work indicate that the co-transplantation of ASCs and OECs brings important functional benefits to the injured spinal cord.

0294 Delivery of dental mesenchymal stem cells from apical papilla via an extracellular matrix hydrogel for spinal cord repair

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Introduction: Inflammation and predominant pro-inflammatory macrophage phenotype are characteristics of spinal cord injury, creating an unsuitable environment for regeneration (1). This study determines whether bone and spinal cord extracellular matrix hydrogels (bECM and scECM) can be used as a vehicle for local delivery to spinal cord lesion, and whether they can be combined with stem cells from apical papilla (SCAP) as a strategy to modulate the microenvironment.

Materials and methods: ECM hydrogels were formed by neutralising decellularised, digested material and by raising the temperature to 37°C (2,3). SCAP viability in hydrogels, $1 \cdot 10^6$ cells/ml of gel, was determined using a metabolic assay (PrestoBlue) (n=3, N=3). Ability of the different hydrogels to gel *in vivo* was tested in a rat brain biopsy model.

Results and discussion: SCAP viability was high in both bECM and scECM, but decreased significantly with increased bECM concentration (10mg/ml) (B10) (Fig. 1). scECM hydrogels at the highest concentration (10mg/ml) were heterogeneous, and therefore inadequate as potential active substance or cell delivery vehicle. bECM in both concentrations could form *in vivo*, while 8mg/ml scECM (SC8) could not. Nonetheless, spinal cord 8mg/ml was selected for further investigation based on previous results with homologous tissue derived hydrogels (3,4). Combination of SC8 and B10 in different proportions (75:25, 50:50, 25:75 v/v) allowed gelation *in vivo*, possibly due to the higher solid-like properties of B10 (4). bECM concentration in the mixes did not have significant influence on SCAP viability, compared to SC8 ($p<0.05$).

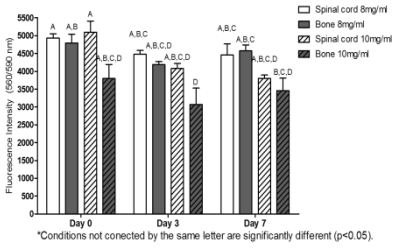

Fig. 1. Viability of $1 \cdot 10^6$ SCAPs/ml in spinal cord and bone ECM derived hydrogels of two different concentrations.

It is likely that a fast gelling material may be needed to overcome the flow of blood and cerebrospinal fluid *in vivo* and to prevent the gel from dissolving in spinal cord and brain lesions (5). Therefore, SC8 was mixed with fibrin (fibrinogen 50 mg/ml and thrombin 50 IU/ml, v/v=1) in 75:25 and 50:50 v/v. However, SCAP viability in SC8/fibrin mixes was lower by 30% on day 0, compared to the viability in SC8 on day 0. Viability in SC8/fibrin 75:25 mix was higher than in 50:50 mix, on days 3 and 7. After 7 days, 62-80% of SCAP survived in SC8 mixes with either B10 or fibrin, compared to the viability in SC8 on day 0.

Conclusion: Homologous tissue derived hydrogels demonstrated several advantages with high SCAP viability observed in a 75:25 v/v mix of SC8 and B10 whereas fast gelation occurred in a mix of SC8 with 25% fibrin. Although the non homologous bECM gelled more slowly than the 75:25 SC/fibrin hydrogel, it may be a potential candidate for delivery due to the high SCAP viability and the simplicity of its making.

0295 In situ delivery of locked nucleic acid-based antisense oligonucleotides aiming at spinal cord injury therapy

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Introduction

In order to achieve nerve regeneration in the context of spinal cord injuries new molecular therapeutic strategies that block the growth inhibitory signals coming from the extracellular environment at the injury site and increase the intrinsic regenerative capacity of neurons are warranted.

The potential of antisense oligonucleotides (AONs) to modify gene expression at different levels, achieving regulation of cell survival and cell function, together with the availability of chemically modified nucleic acids makes them an attractive tool for novel SCI therapy developments.

In this work, we aimed at exploring the potential of locked nucleic acids (LNA)-modified single stranded AONs in combination with a fibrin hydrogel matrix to induce gene expression changes in situ at a SCI lesion site.

Materials and Methods

Antisense LNA-DNA gapmer oligonucleotides (LNA-AONs) against RhoA, GSK3β and GFP (control) were used.

Fibrin (Fib) gels were prepared with or without AONs. The Fib gel structure was analysed by confocal microscopy (CLSM) using fluorescently-labelled fibrinogen (FITC-Fib) and Cy5-AONs.

Dorsal root ganglion (DRG) explants were cultured embedded in Fib gels containing either Cy5-AONs, to analyse the distribution of AONs inside the DRG, or LNA-AONs to analyse gene down-regulation activity.

For in vivo experiments a spinal cord hemisection was performed in rats. The lesion site was filled with Fib gel containing AONs. Additionally, a polymerized gel patch also with AONs was placed covering the previously filled lesion. Distribution of Cy5-AONs in and around the lesion area was analysed 3 days post lesion by CLSM. Gene expression levels were analysed after extracting RNA from lesion site 5 days post-lesion.

Results and Discussion

LNA-AONs were designed against two promising gene targets aiming at neuronal regeneration, RhoA and GSK3beta AONs were successfully loaded in fibrin hydrogels with close association between the AONs and fibrin fibers being observed.

This fibrin-based AON delivery system was successful in mediating potent RNA knockdown in vitro in a DRG explant culture system and in vivo at a SCI lesion site, achieving 80% down-regulation 5 days after gel implantation.

With this dual antisense-hydrogel system we expect to achieve a combinatorial type of approach able to provide a more permissive microenvironment for neuronal regeneration.

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0296 Does an experienced adaptive immunity hinder the healing process in bone?

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Aims: Understanding cascades of endogenous regeneration in bone (leading to restitutio ad integrum) may serve as blueprint even for tissues currently leading to scar formation. The multistage regenerative cascade is initiated in response to injury and vessel disruption with a well-orchestrated inflammatory response. Specific immune cell subsets of the adaptive immune system influence the regenerative healing capacity after fracture. The impact of the immune cells on the quality of the newly formed, hierarchically organized bone tissue however, have so for not been fully understood.

Methods: In vitro systems to investigate the influence of immune cytokines on the osteogenic differentiation of mesenchymal stem cells were used to investigate the impact of the experience of the immune system on the bone formation capacity. In vivo mouse osteotomy models were used to investigate the influence of immune modulation on the bone healing and to determine the influence of immune cell activity on the newly formed bone (µCT, histology, immune histology, FACS).

Results: Both, in vitro and in vivo results, confirmed the essential influence of the immune cell composition on the bone formation process. An aged immune system with an experienced adaptive immune system reduced the osteogenic capacity of mesenchymal stem cells. The bone healing in animals with an unfavourable ratio of effector T cells and regulatory T cells showed a significantly reduced healing capacity while a low CD8 T effector – regulatory T cell ratio showed a significantly enhanced bone healing in our model system.

Impact: The aging population in developed countries makes personalized therapies a must: Patients with an aged and experienced immune system require different treatment strategies compared to those with a less developed immunity leading to different speeds of successful restoration of tissue integrity.

0297 Inhibiting STAT signaling pathways in macrophages to improve tissue regeneration

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Introduction*:* Macrophages, as specialized cells of the immune system, are present in many different tissues. Macrophages have different phenotypes, ranging from pro-inflammatory to anti- inflammatory/ repair depending on the environmental cues they receive. The different macrophage subtypes can either positively or negatively affect surrounding tissues and influence regenerative processes. The current study aims to examine which intracellular STAT signaling pathway is activated in which macrophage subset and to examine the effect of STAT modulation on the behaviour of macrophage phenotypes. **Methods***:* Human monocytes were isolated from buffy coats using magnetic CD14+ selection. After seeding in 500.000 cells per cm^2, cells were cultured in X-Vivo culture medium with 20% fetal calf serum (FCS) in the presence of either interferon (IFN) γ and tumor necrosis factor (TNF) α, Interleukin (IL) 4, or IL10 (all in 10 ng/ml) to obtain the macrophage phenotypes M(IFNγ/TNFα), M(IL4), or M(IL10). Synovial tissue was obtained from osteoarthritic knees and directly analysed or cultured for 24 hours in low glucose Dulbecco's modified Eagles medium (DMEM-LG), 10% FCS with 50 μM STAT-1 inhibitor NSC-118218 and 100 μM STAT-3 inhibitor S3I-201. STAT-1, STAT-3 and STAT-6 activation in the macrophage subsets and synovial explants was determined with Western blot. Cells, tissue and medium were also harvested to determine chemokine ligand 18 (CCL18), IL6 and soluble cluster of differentiation (sCD) 163 protein production and gene expression of *CCL18,* cluster of differentiation *(CD)* 163*,* Mannose Receptor, C type 1 *(MRC1), IL6* and *TNFA* was analysed. **Results:** STAT-1 was specifically phosphorylated in M(IFNγ/TNFα), STAT-6 in M(IL4) and STAT-3 in M(IL10). Macrophage polarization was confirmed as M(IFNγ/TNFα) typically had high expression levels of *IL6* and *TNFA* and high IL6 protein production. M(IL4) expressed high levels of *CCL18* and *MRC1*, and high CCL18 production, whereas M(IL10) had high expression of *MRC1* and high sCD163 production. STAT- 1, STAT-3 and STAT-6 could be detected in osteoarthritic synovium when analysed immediately after surgery, After 24 hours in culture however, only STAT-1 and STAT-3 could be detected. Treatment of synovial explants with NSC-118218 specifically decreased STAT-1 phosphorylation and downregulated *CD163* expression without affecting *TNFA, CCL18, MRC1* and *IL6* expression. Treatment with S3I-201 decreased STAT-3 phosphorylation, whereas it increased *TNFA* expression and decreased *IL6* and *CD163* expression, without affecting *CCL18* and *MRC1* expression*.* **Conclusions:** Macrophage subsets obtained in this study have a specific pSTAT-1, pSTAT-3 or pSTAT-6 pattern. Inhibiting STAT-1 activation with NSC-118218 and STAT-3 activation with S3I-201, affected specific gene expression patterns of synovium. Based on these results, targeting STAT signaling pathways seem promising to modulate macrophage behaviour. This in turn might provide a better environment allowing tissue regeneration of other joint tissues.

0298 Viral interleukin 10-expressing mouse mesenchymal stem cells exert anti-inflammatory effects on activated immune cells in vitro

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Background and aim: Osteoarthritis (OA) is a degenerative disease of the whole joint, with inflammation a pivotal cause of disease progression [1, 2]. The synovium of the joint harbours macrophages and T cells that secrete inflammatory cytokines, stimulating chondrocytes to release cartilage-degrading proteinases during the osteoarthritic process [3, 4]. As a prelude to testing in an OA mouse model, we have used the tetracycline system (Tet) to modify mouse mesenchymal stem cells (mMSCs) to over-express viral interleukin 10 (vIL10). This anti-inflammatory cytokine will be assessed for modulation of the osteoarthritic environment and disease development. The study aims to induce vIL10 release by adenoviral (Ad) transduction of mMSCs using Tet and demonstrate immune modulation of activated macrophages and T cells.

Methods: MSCs were isolated from the marrow of C57BL6/J mice and transduced with Ad vectors carrying CMVIL10 and TetON as test groups, and untransduced, AdNull and TetOFF as negative controls. The cells were tested for their growth potential, differentiation capacity (osteogenesis, adipogenesis and chondrogenesis), expression of MSC markers ($CD90.2^+$, $SCA\text{-}1^+$, $CD105^+$, $CD140a^+$, $CD34^-$, $CD45^-$ and $CD11b^-$) by flow cytometry (FCM) and production of vIL10 by ELISA. Bone marrow-derived macrophages (BMDMs) were characterised for expression of CD45, F4/80 and CD11b by FCM, followed by subsequent activation by lipopolysaccharide in the presence or absence of various MSC conditioned media (CM). ELISA quantified the release of pro- and anti-inflammatory cytokines. Co-culture of stimulated macrophages and T cells with MSCs was also performed, followed by detection of macrophage polarisation status by quantifying MHC-II and CD206 expression. T cell proliferation status in terms of percentage of CD4+ and CD8+cells was quantified by FCM.

Results: Transduced MSCs maintained viability above 80% and growth potential over 3 doublings. Efficient and tightly controlled vIL10 production was demonstrated by CMVIL10 and TetON MSCs. The cells expressed positive markers that define mouse MSCs and differentiated into all three mesenchymal lineages. Co-incubation of vIL10 MSC CM with activated macrophages resulted in reduction of TNF-α and IL-6 levels and elevated production of IL-10. Co-culture of splenocytes with MSCs resulted in inhibition of both CD4+ and CD8+ T cell proliferation with maximal reduction seen with TetON MSCs. Macrophage polarisation from M1 to M2 populations was demonstrated by activated macrophages, co-cultured with TetON MSCs.

Conclusion: vIL10 MSCs proved to be immuno-regulatory on both macrophages and T cells. The biscistronic TetON transduced MSCs proved to be most immuno-suppressive proving the hypothesis. TetON MSCs are therefore feasible as an efficient anti-inflammatory therapy that can utilised in vivo.

0299 Immunomodulatory response mechanisms to cardiovascular matrices are affected by the cryopreservation strategy applied

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Introduction: Cryopreservation of cardiovascular matrices is commonly used in transplant medicine. However, the durability of conventional frozen cryopreserved (CFC) transplants is limited, due to the induction of inflammatory processes, calcification and tissue structure damage. The alternative method of ice-free cryopreservation (IFC) has recently been demonstrated to better preserve matrix structures, to reduce immune cell infiltration *in vivo* and to lower adaptive T cell responses *in vitro*.

Objectives: To explore the underlying mechanisms of the beneficial immune mediated IFC effects, the objective of this study was to investigate modulation of human immune responses to ice-free cryopreserved cardiovascular matrices including macrophage activation and polarization, induction of regulatory T cells (Treg) and immune cell migration.

Materials & Methods: Human peripheral blood mononuclear cells (PBMC) were separated from buffy coats. Monocytes (CD14+) and T cells (CD3+) were isolated and CD14+ cells were differentiated to macrophages by M-CSF. In co-cultures with macrophages or PBMCs and human CFC or IFC treated human aortic tissue (obtained with ethics approval), macrophage activation and induction of FoxP3+ T cell subsets (Treg) was investigated by flow cytometry. Migration behavior of monocytes and T cells towards cryopreserved tissue was examined in transwell settings using a Calcein based assay. Additionally, cytokines and chemokines released by the tissue after cryopreservation were analyzed by ELISA.

Results: In our recently developed *in vitro* macrophage polarization assay, macrophages did not upregulate CD80 or HLA-DR (M1 markers) in both types of tissue co-culture. Interestingly, IFC significantly increased the Fcγ-Receptor CD16 expression on macrophages compared to CFC co-cultures. Investigations of soluble factors released by the tissue treated with the respective cryopreservation strategies revealed a significantly lower release of cytokines (e.g. IL-6, MCP-1) from IFC than from CFC tissue. In accordance with this lower release of cytokines, we detected in our migration studies less T cell and monocyte migration towards IFC tissue conditioned media than to CFC media. However, IFC tissue released more active TGF-β than CFC. Analyzing T cells subsets in the tissue co-culture system, consequently a higher proportion of Treg was induced with IFC tissue than with CFC.

Conclusion: IFC treatment of cardiovascular tissues modulates immune cell responses by reducing immune cell infiltration, particularly by reducing tissue cytokine release. Furthermore, IFC treated tissue is able to modulate macrophage activation and to induce a regulatory subset of T cells. Therefore, IFC treatment creates new possibilities for tissue transplant preservation leading to improved *in vivo* performance.

0300 Augmenting tendon repair using stem cells

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Tendons can be injured through over-strain at a number of different sites. When injured outside a synovial cavity (extra-thecal), injuries frequently mount a fibrotic repair but this tissue is functionally deficient compared to normal tendon. Regenerative medicine offers the prospect of restoring normal structure and function to an injured organ and thereby resulting in a successful restoration of activity without the risk of re-injury.

Naturally-occurring equine superficial digital flexor tendon (SDFT) overstrain injuries provide a relevant (for human Achilles tendon) large animal model for evaluating regenerative strategies. The lesion is contained thereby enabling simple intra-tendinous injection and, by the time of stem cell implantation, is filled with granulation tissue which acts in the role of a vascularized scaffold. An anabolic drive is provided by the cytokine and mechanical environment within the tendon and the suspension of mesenchymal stem cells (MSCs) in bone marrow supernatant, which we have shown to have significant anabolic effects in vitro. We have hypothesised that autologous implanted bone marrow-derived cultured MSCs would induce normalization of the tendon matrix which would be reflected in a clinically-relevant reduced re-injury rate. To test this, a controlled experimental study of naturally-occurring SDFT injuries (n=12) has been performed. MSC treatment appeared to 'normalise' the tissue parameters so that they were closer to the contralateral, relatively normal, and untreated tendons than saline-injected controls, in spite of labelling experiments showing the majority of cells being lost within 24 hours. A second adequately powered and independently analysed study evaluated the clinical outcome of naturally occurring SDFT injuries treated using this technique (n=113) which showed a significantly reduced re-injury rate (approximately one half). Efficacy was improved with shorter interval between injury and implantation and with greater numbers of cells implanted. The inclkusion of a scaffold may help improve cell retention for greater efficacy.

Inside a synovial cavity (intra-thecal), the tendon disruption usually communicates with the synovial cavity where the synovial environment is particularly challenging for successful repair. As examples of these injuries, equine (naturally-occurring) and ovine (induced) deep digital flexor tendon (DDFT) tears provide an analogous injury to human rotator cuff tears. However, MSCs administered intra-synovially failed to improve healing in either of these models. Labelling of the implanted cells showed them to lodge within the synovium with no cells present in the tendon defect. In addition, tenocytes, or MSCs, fail to survive in tendon matrix *in vitro* when exposed to synovial fluid, indicating that inclusion of a protective scaffold is likely to be essential for efficacy in this location.

0301 Biphasic silk fibroin scaffolds for tendon/ligament-to-bone tissue engineering

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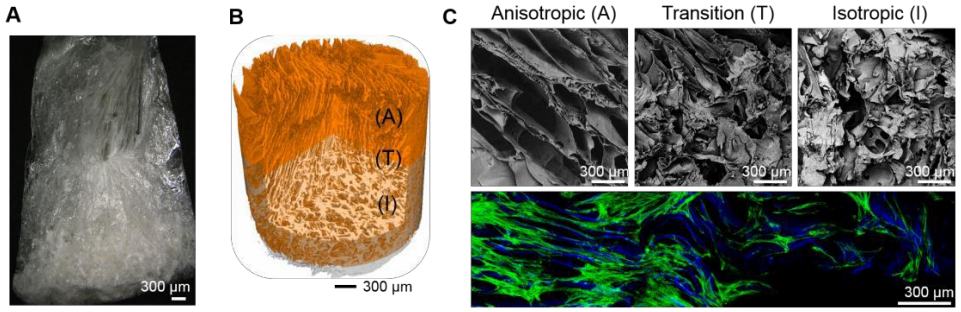
The tendon/ligament-to-bone interface (enthesis) ensures a smooth mechanical stress transfer between tendons/ligament and bone. The composition of the native enthesis is complex and heterogeneous. Moving from the tendon/ligament to the bone the enthesis is characterized by a gradual decrease in collagen organization and a change in ECM composition and cell phenotype. These structural features are essential for enthesis function but are not regenerated after injury, increasing the risk of rupture recurrence.

Tissue engineering is an attractive strategy to promote enthesis regeneration. In this study, biphasic silk fibroin scaffolds were fabricated combining freeze-drying and salt leaching techniques. The scaffolds were designed to mimic the change in collagen alignment of the native interface and presented two different pore architectures: anisotropic pores at the tendon/ligament side and isotropic at the bone side (Figure 1). The two types of porosity were integrated in a single construct, with a continuous and smooth transition between the two parts. Scaffold morphology and pore alignment were characterized by optical microscopy (Figure 1A), micro-computed tomography (Figure 1B) and scanning electron microscopy (Figure 1C, top). Total porosity ranged from 50 to 80% and 85% of the pores were <100 up to 300 µm. Mechanical properties were evaluated by tensile testing. Scaffold integrity was maintained up to 15% strain and Young's modulus was 1.3 ± 0.3 MPa.

The impact of pore morphology on cell proliferation and gene expression was evaluated *in vitro* with human adipose-derived mesenchymal stem cells. Biphasic scaffolds supported cell attachment and influenced cytoskeleton organization and gene expression. Cell organization and alignment were influenced by scaffold anisotropy, as shown in the confocal microscopy images of figure 1C, down panel (actin cytoskeleton shown in green and scaffold matrix in blue). In addition, the expression of tendon/ligament (scleraxis, collagen III), enthesis (aggrecan, collagen III) and cartilage (sox9, aggrecan) markers significantly changed depending on pore alignment in each region of the scaffolds. In conclusion, the biphasic scaffolds fabricated in this study show promising features for tendon/ligament-to-bone tissue engineering. Acknowledgements: this research work and the position of the first author were supported by the Zeidler Research Foundation.

Figure 1



0302 Investigating hierarchical collagen fiber formation for functional meniscus and tendon tissue engineering

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Introduction: Collagen fibers are the primary source of strength in orthopaedic tissues, particular menisci and tendons. Injuries that disrupt collagen fibers in these tissues result in over 1,350,000 surgeries a year in the US.[1,2] Engineers are striving to create replacement tissues; however these attempts often lack organized collagen, essential to long-term mechanical success. Recently, aligned 30 µm diameter collagen fibers were developed in engineered menisci using high density collagen gels;[3,4] however the tensile properties were inferior, suggesting the need for further development. The objective of this study was to create a simplified system to further drive collagen fiber formation in meniscus and tendon constructs and to compare collagen development at the fibril, fiber and tissue level in an effort to engineer functional musculoskeletal tissue with native organization and strength.

Methods: Cell-seeded high density 20 mg/ml collagen gels were cultured in a device with boundary constraints to harness cellular contraction and drive formation of aligned collagen fibers. These clamped samples were cultured up to 6 weeks and compared to unclamped controls. Tenocyte-seeded and meniscal fibrochondrocyte-seeded gels were evaluated for collagen fiber development, collagen accumulation, and tensile mechanical development.

Results/Discussion: Confocal reflectance revealed clamped constructs (meniscal and tendon) develop aligned collagen fibrils by 2 weeks which grew to large fibers 20-50 µm in diameter by 6 weeks. Unclamped samples had no organization. Confocal 3D reconstructions of clamped samples revealed clearly defined collagen bundles and early hierarchal fiber organization. Image analysis revealed both types of clamped scaffolds matched native alignment by 2 weeks and native fiber diameters by 4 weeks. Interestingly meniscal constructs developed significantly larger collagen fibers than tendon constructs by 6 weeks, matching the larger fibers measured in native meniscal tissue at this magnification. This difference in fiber sizes suggests cell types are capable of producing intrinsic fiber sizes.[3,5] Preliminary AFM analysis suggests fibrils organize similar to native tissues with an average band length of 54.5±12 nm. SEM, SAX and Raman microscopy techniques are currently underway to further evaluate organization and packing.

Collagen content was largely unchanged through 4 weeks. By 6 weeks meniscal clamped scaffolds had significantly higher collagen content than all other scaffolds, mirroring the larger fiber diameters. Further, meniscal and tendon clamped constructs had a 20-30 fold increase in tensile properties to ~1 MPa by 6 weeks, more than 8-fold higher than previous studies[3,4] and approaching native neonatal properties.[3] This increase compared to the minimal change in collagen content supports the theory that organization is more important to construct maturation than matrix accumulation,[3,4,6] and stresses the importance of developing well organized collagen in engineered tissues. This study provides new insight into how different cell types develop collagen fibers and produces the largest, most organized collagen fibers to date for both meniscal and tendon tissues.

0303 3D hierarchical bionanocomposite nanofiber scaffolds for tendon and ligament tissue engineering

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Aims: The morphology and function of tendons and ligaments (T/L) are intrinsically related with their unique hierarchical and anisotropically organized extracellular matrix (ECM) fibrous structure. The natural healing capacity of these tissues is however limited and Tissue Engineering (TE) offers the most promising prospect of achieving regeneration of damaged tissue. We have previously developed mechanically competent and aligned nanocomposite nanofibrous 2D membranes-like scaffolds for T/L TE based on polycaprolactone/chitosan blends reinforced with cellulose nanocrystals (PCL/CHT/CNC), which shown to provide the necessary topographical cues to maintain the phenotype of seeded human tendon derived cells (hTDCs). In this study we propose a new process to produce continuous and aligned nanofiber threads, which can then be incrementally assembled into higher hierarchical structures through textile techniques. We aim thus to mimic the ECM structure of native T/L, meeting their non-linear biomechanical behavior, while providing the topographical cues to either maintain cells hTDCs phenotype and/or to induce the tenogenic differentiation of stem cells.

Methods: An adapted electrospinning setup was applied to produce continuous aligned nanofiber threads. Textile techniques were incrementally applied by first twisting the threads into yarns that were then braided or waved into 3D scaffolds. The mechanical properties of all the incremental units were assed and optimized. hTDCs and human adipose stem cells (hASCs) were seeded and cultured onto waived scaffolds and their biological behaviour (metabolic activity, morphological and immunocytochemistry assays) was evaluated along the culture period.

Results: Both braided and waived scaffolds presented tensile properties in T/L range. Nonetheless, only the waived structure shows an initial toe region, a hallmark of T/L, thus simultaneously mimicking their non-linear biomechanical behaviour and nano- to -macro hierarchical structure. After 28 days of culture, both cell types remain metabolically active (no significant differences after day 3). The topography of the nanofiber threads induces a significant cell cytoskeleton elongation and alignment along the nanofibers aligned axis. The expression of Col I, Col III and TNC was evident as early as day 7 for both cell types and after 21 days, an increased matrix deposition was observed for both cell types which seemed to be deposited in an aligned manner.

Impact on the investigation: The 3D waived scaffold structure mimics the hierarchical and anisotropically aligned organization of collagen fibrils in T/L, meeting their mechanical requirements and biomechanical behavior. hTDS phenotype was maintained and hASCs present a similar behaviour to hTDCs, suggesting their differentiation towards a tenogenic-like phenotype. Altogether, the structural properties of the 3D scaffold provide therefore key features that can be advantageously explored in acellular and cellular T/L TE strategies.

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0304 Regulation of epidermal homeostasis by intercellular communication

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Stem cell behaviour is controlled by intrinsic mechanisms and by external signals from the local microenvironment or niche. My lab is using genetically modified mice and cultures of primary human epidermal stem cells to investigate the interplay between specific intrinsic and extrinsic signals in regulating exit from the stem cell compartment. In my talk I will discuss progress that we have made in defining soluble factors, cell surface ligands, extracellular matrix components and topographical features that control the onset of terminal differentiation and how we are mapping the signal transduction pathways involved.

0305 3D microenvironments to uncover therapeutic niches for skin diseases and disorders

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Skin diseases and disorders are among the most common health problems worldwide and are associated with a considerable burden. It encompasses psychological, social and financial consequences not only on the patients, but also on their families and on society. Despite the longest history of application and the highest record of marketing, skin tissue engineered substitutes still lead skin repair rather than promote tissue regeneration, creating additional dysfunction. Similarly, effective therapeutics for skin diseases are limited and in many cases poorly explored due to low incidence of the disease.

Under this context we have been focusing on unravelling new approaches that are capable of improve the interaction/response of mesenchymal stem cells (MSCs) to the injured microenvironment, particularly addressing the need to promote cell survival rates and engraftment after transplantation. Moreover, we have been transposing the concepts of tissue engineering to generate different 3D microenvironments that allow posing different questions regarding skin diseases and disorders ultimately helping to uncover associated "therapeutic niches". This has been achieved by exploring cell-sheet engineering and innovative hydrogel-like matrices which allow to accurately recreating cell-cell and -cell-ECM interactions specific of those microenvironments. Additionally we have been able to modulate cellular crosstalk by targeting specific signalling pathways in order to more closely reproduce the biochemical milieu of the microenvironments, often hallmarks of skin diseases.

Ultimately, we envision taking advantage of the generated knowledge to propose tissue engineered substitutes with improved functionality, as well as to advance the development of new or improved therapies for skin diseases and disorders.

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0306 Biofunctionalized gellan gum hydrogels as tailored platforms for skin regenerative purposes

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Gellan gum (GG) hydrogels have been progressively explored for different tissue engineering and regenerative medicine applications but skin regeneration. In this work we aimed to biofunctionalize GG tailoring the specificities of GG hydrogels according to the skin cells requirements and the nature of the ECM of the numerous microenvironments that compose this complex organ. Native GG was chemically functionalized with divinyl sulfone (DVS) and then T1 or Hep III cell adhesive thiol peptide sequences to promote respectively endothelial cells and keratinocytes adhesion. In order to mimic the sulphated character of the inductive niche of the hair follicle, the dermal papilla, acid-treated GG was sulfated by an SO_3-pyridine complex. Different hydrogels were formed either by a Michael type addition of a dithiol peptide crosslinker sensitive to metalloproteinase-1 (MMP-1) or by ionic crosslinking. Chemical modification was followed by FTIR, ^{1}H-NMR, GPC and protein/sulfate quantification analysis prior hydrogel formation, which were then characterized regarding viscoelasticity, swelling and MMP-1-driven degradation. Human skin keratinocytes, fibroblasts, endothelial and dermal papilla cells were encapsulated within GG-based hydrogels and then cell response was evaluated. The modification of the GG was confirmed by the new peaks at 6.1, 6.2 and 6.9 ppm of the ^{1}H-NMR spectra (GG-DVS), at 1070 and 1260 ppm in the FTIR spectra (GG-SO_3), as well as by an increase of the molecular weight detected in the GPC analysis. By changing the polymer amount and crosslinking strength we were able to tailor the storage modulus of the hydrogels. Moreover, we confirmed that MMP-1 driven degradation was proportional to the amount of enzyme and peptide crosslinker. Keratinocytes were able to adhere and keep their immature phenotype within the GG-HEPIII hydrogels. Endothelial cells behavior was dependent on the concentration of T1 peptide while a partial recovery of the inductive phenotype of dermal papilla cells was attained in the GG-SO_3 hydrogels, depending on the amount of SO_3. Biofunctionalized GG-based hydrogels were tailored according to the skin tissue specificities representing suitable 3D platforms for skin regenerative purposes.

0307 Enhancing vascularisation using gene therapy: Development of gene-activated scaffolds for skin tissue regeneration

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INTRODUCTION: Skin grafts are required for the treatment of full or partial thickness skin defects caused by burns, chronic wounds, infection or following melanoma removal. An estimated 11 million people sought medical attention for burn injuries in 2004 and a further 20 million people currently suffer from chronic wounds costing an estimated $25 billion in the U.S alone[1]. Current skin grafting materials, including autografts, donor allografts or biomaterial-based skin grafts, often fail to integrate with the host tissue at the defect site due to a lack of neo-vascularisation[2]. The delivery of genes within a skin-mimicking biomaterial might induce the formation of a stable, functional vascular network. Using gene-activated scaffold technology previously developed within our lab[3-5], the aim of this study was to develop a gene-activated collagen-glycosaminoglycan scaffold capable of promoting angiogenesis for enhanced skin repair.

MATERIALS AND METHODS: The efficient encapsulation and delivery of the genetic cargo, or transfection efficiency, depends on the ratio of gene delivery polymer, polyethyleneimine (PEI) to DNA (N/P ratio). Therefore a screen of conditions was tested using reporter genes green fluorescent protein (GFP) and *Gaussia* luciferase. The optimal PEI-pDNA nanoparticle formulation was then used to deliver plasmid DNA (pDNA) encoding for vascular endothelial growth factor (VEGF) or stromal cell derived factor-1α (SDF-1α) to human mesenchymal stem cells (MSCs). VEGF and SDF-1α production by the cells was quantified using ELISA. Functionality of the protein produced in enhancing endothelial cell vessel formation was determined using Matrigel™ assay. Finally, PEI-pVEGF or PEI-pSDF-1α nanoparticles were loaded into collagen-glycosaminoglycan scaffolds, previously developed for skin repair, and seeded with hMSCs and endothelial cells and new vessel formation was assessed using immunohistochemistry.

RESULTS: PEI-pGFP nanoparticles at N/P 7 carrying a 2 µg dose of pDNA had a transfection efficiency of 45-60%. Following transfection of MSCs with PEI-pVEGF or PEI-SDF-1α, the concentration of each protein released into the media was approximately 50-70 ng/mL over 7 days. This growth factor 'conditioned' media was used to feed endothelial cells seeded in Matrigel™ and induced microtubule formation, indicating that the proteins were functional. When PEI-pVEGF nanocomplexes were incorporated into collagen-glycosaminoglycan scaffolds, there was sustained expression of each protein for 14 days, peaking at day 7 post-transfection. This functionality of this gene-activated scaffold to induce new vessel formation is now being tested *in vitro* by seeding both MSCs and endothelial cells and *in vivo* in a subcutaneous rat model.

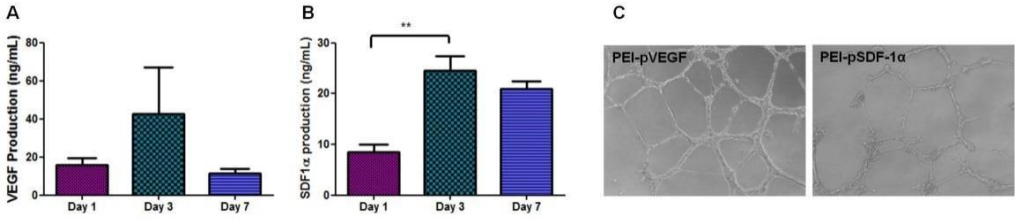

Figure 1: VEGF (**A**) and SDF-1α (**B**) production by MSCs following transfection was quantified using ELISA. Functionality of each protein was tested by growing endothelial cells on Matrigel™ in media conditioned by transfected MSCs (**C**)

DISCUSSION: This study has shown that the gene-activated scaffold technology, previously developed for orthopaedic applications, can also be translated for use in skin repair and wound healing by potentially enhancing vascularisation post-implantation which may in turn enhance skin wound healing.

ACKNOWLEDGEMENTS: EU Horizon 2020 **"**Gene2Skin" and Science Foundation Ireland

0308 Enhanced imaging techniques and their role in regenerative medicine

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Regenerative medicine offers unique opportunities for developing new, potentially personalized, therapeutic approaches to treat and ultimately prevent life-threatening diseases. This includes strategies for the replacement, repair, and regeneration of tissues and organs damaged by disease and/or traumatic injury. It is the fabrication of replacement tissues and organs that here is called tissue engineering. This represents a rapidly growing interdisciplinary field within regenerative medicine involving biology, chemistry, physics, engineering and medical sciences. A major focus of tissue engineering is the creation of ex vivo manufactured tissues and organs, even multi-organ systems, in order to explore fundamental questions of (stem) cell, extracellular matrix and developmental biology. These in vitro manufactured systems can also be used as sophisticated tissue and organ test systems to either reduce or even replace the need to use test animals. The monitoring of tissue-engineered constructs during their in vitro maturation or post-implantation in vivo is highly relevant for test system or graft quality evaluation. While traditional methods for studying (stem) cell and extracellular matrix components in engineered tissues and organs such as histology, immunohistochemistry or biochemistry require invasive tissue processing, resulting in the need to sacrifice the in vitro-engineered structures, multiphoton imaging and Raman microspectroscopy allow the non-invasive, marker-free monitoring. Moreover, Raman spectroscopy can also be used to generate biochemical profiles of living cells. Different cell phenotypes and cell fate decisions can be therefore assessed based on their Raman spectroscopic signature. My presentation will present an overview of our work on the utilization of marker-free monitoring modalities for basic and applied biomedical research and disease diagnosis.

0309 Droplet-based microfluidics: towards massively parallel chemistry and biology

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The past 25 years have seen considerable progress in the development of microfabricated systems for use in the chemical and biological sciences. Interest in microfluidic technology has driven by concomitant advances in the areas of genomics, proteomics, drug discovery, high-throughput screening and diagnostics, with a clearly defined need to perform rapid measurements on small sample volumes. At a basic level, microfluidic activities have been stimulated by the fact that physical processes can be more easily controlled when instrumental dimensions are reduced to the micron scale[1]. The relevance of such technology is significant and characterized by a range of features that accompany system miniaturization. Such features include the ability to process small volumes of fluid, enhanced analytical performance, reduced instrumental footprints, low unit costs, facile integration of functional components within monolithic substrates and the capacity to exploit atypical fluid behaviour to control chemical and biological entities in both time and space. My lecture will discuss why we have been motivated to use microfluidic systems for chemical and biological experimentation and will focus particularly on recent studies that exploit the spontaneous formation of droplets in microfluidic systems to perform a variety of analytical processes.

Droplet-based microfluidic systems allow the generation and manipulation of discrete droplets contained within an immiscible continuous phase[2]. They leverage immiscibility to create discrete volumes that reside and move within a continuous flow. Significantly, such segmented-flows allow for the production of monodisperse droplets at rates in excess of tens of KHz and independent control of each droplet in terms of size, position and chemical makeup. Moreover, the use of droplets in complex chemical and biological processing relies on the ability to perform a range of integrated, unit operations in high-throughput. Such operations include droplet generation, droplet merging/fusion, droplet sorting, droplet splitting, droplet dilution, droplet storage and droplet sampling[3-4]. I will provide examples of how droplet-based microfluidic systems can be used to perform a range of experiments including nanomaterial synthesis[5], cell-based assays[6] and DNA amplification[7]. In addition, I will describe recent studies focused on the development of novel imaging flow cytometry platform that leverages the integration of inertial microfluidics with stroboscopic illumination[8] to allow for high-resolution imaging of cells at throughputs approaching 10^5 cells/second.

0310 Skin-on-chip for next generation immune-competent in vitro skin models

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Background: To study skin disease e.g. skin-cancer, fibrosis and allergy, an immune competent human skin model is necessary. Existing models, like animal models or 2D in vitro cultures are inadequate because they lack the complexity relevant for human skin. The exchange of immune cells between microvasculature and (epi)dermal layers is a critical aspect for next generation skin models. To realize this, the first step is to integrate a perfused microvasculature beneath a full thickness skin equivalent.

Aim: To create a full thickness skin equivalent in an endothelium-lined microfluidic device that mimics microvasculature.

Methods: A microfluidic device is employed having a microchannel below a culture chamber. The flow conditions are designed to mimic microvasculature flow. The chamber and channel are separated by a micro-porous Transwell-like membrane. Primary human cells are employed. The skin equivalent cultured on top of the membrane consists of a fibroblast filled collagen-based gel onto which keratinocytes are cultured. The endothelial layer is cultured on the bottom of the membrane. This complete equivalent is exposed to 1-week perfusion flow in a microfluidic setup. Characterization of structure is subsequently performed through immunohistology of relevant markers (Vimentin, α-SMA, CD31, cytokeratin). Viability is characterized with LDH measurements during the flow while MTT-assay is used for end-point analysis.

Results: We show that 1) a construct can be cultured having an endothelial barrier below a full thickness skin equivalent and 2) after complete growth of this construct it is viable after 1-week perfusion.

Conclusion: We created a full thickness skin equivalent augmented with an endothelial-lined microchannel.

Importance: this model will enable flowing immune cells to be introduced in the future to realize a fully immune-competent skin-on-chip. It also enables connection to other perfused tissue models like gut-on-chip or liver-on-chip to study complex interdependent responses of organs to therapeutics, chemicals and substances. This will provide an optimal drug development and risk assessment platform, representing healthy and diseased organs, while potentially replacing animal use.

0311 Microfluidic-based fabrication of microgels to engineer multiscale hierarchical tissues

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Multiscale modular designs are an intrinsic and essential characteristic of native tissues. Cells and matrix are spatially organized into complex functional building blocks, containing 3D structured biochemical and mechanical functionalization as well as functional domains such as stem cell niches. This modular design is key to obtain the multifunctionality that is necessary for proper tissue functioning. Integrating such modularity into engineered tissues is expected to improve their functionality on multiple scales without imposing on existing optimized construct properties. To this end, we are incorporating micromaterials in the design of biomaterials to enable this desired multi-scale complexity. Microfluidic technologies are uniquely suited to produce such micromaterials. We have developed microfluidic droplet generation platforms for the production of single cell microgels that were mere micrometers larger than the single cell they encapsulated. Innovatively, we used enzymatically crosslinkable hydrogel for microgel formation, which supported survival, entrapment, and function of the microencapsulated cells for at least one month. More specifically, we have developed a unique chip design that centers the cells in the middle of the microgel, which prevents the otherwise occurring cell escape. Using this platform, we engineered 3D single stem cell microniches with on-demand tunable stiffness that were able to controllably program stem cell differentiation along chosen lineages, even in the absence of growth factors. Specifically, we could temporally tune the microgels' Young's modulus accurately from 2 to 50 kPa. Single cell analysis revealed that softer microgels stimulated adipogenesis, while stiffer microgels induced osteogenesis. Temporally stiffening the microgels revealed that the first three days of differentiation where of key importance for stiffness-induced stem cell fate decisions. We then combined our microgels with distinct biomaterials to create advanced bioinks, which effectively uncoupled the implants' micro- and macro-environment. This allowed for an unprecedented control over the design and behavior of the implant. As an example, by loading fibrin hydrogel with single stem cell microgels of polyethylene glycol-tyramine we engineered a tissues that was both angiogenic and immunoprotective and thus could endow our tissues with two properties that would otherwise be conflicting. Lastly, to endow our tissues with macroscale properties such as tissue shape, we combined our bioinks with various biofabrication techniques such as injection molding, photolithography, and 3D printing to engineered multiscale hierarchical living implants. In short, here we present several microfluidic microgel-based concepts that are focused to advance the engineering of multiscale hierarchical tissues constructs.

0312 Microengineered 3D alveolar tissue analogues on chip

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MERLN Institute, Maastricht, The Netherlands

Introduction: Currently, the only option for end-stage lung disease is organ transplantation, which is limited by donor availability and post-transplant chronic allograft dysfunction. The urgency for novel strategies for treatment and repair of damaged lung tissue is obvious. Realistic in vitro models to study lung (patho-) physiology can help in this. Current chip-type lung (disease) models, despite having fluid and air flow and/or mechanical stretch, are still based on 2D cell culture which does not reflect the curved microanatomy of the alveoli. We hypothesize that curvature has an impact on the correct physiological representation of such lung models. To address this, we describe an advanced dyamic 3D lung (disease) model to study different approaches supportive of regeneration. For this purpose, we recreated the 3D alveolar structure by integrating hemispherical microwells in a membrane, as opposing to the standard 2D membrane platforms. The membrane will finally be incorporated in a microfluidic chip, allowing for a comprehensive recapitulation of the alveolar physiology.

Methodology: Arrays of curved microwells were fabricated by thermoforming dense and porous, ion track-etched polycarbonate (PC) films. The curvature radii ranged from 158 µm to 113 µm (similar to human alveoli). Lung epithelial cells were seeded on the concave side and microvascular cells on the opposite side of the membranes (Fig. 1a). Cell behaviour was characterized by immunostaining for cell-material interaction, cell-cell contact, cell morphology and polarization to reveal the effect of curvature (Fig. 1b, 1c). A microfluidic chip assembly has been designed to accommodate the alveolar mimetic 3D air-liquid interface.

Results and Discussion: We have shown that consistent arrays of porous PC microwells could be successfully fabricated with the above dimensions. These structures allow the organotypic, 3D culture of cells, namely lung epithelial cell lines and microvasculature cells. Cells were cultured for up to one week. Thereby, cells were able to line the curved thin walls of the microwells, creating confluent monolayers. Tight junctions, as a pre-requisite for a functional barrier, could be observed between epithelial cells. The expression of CD31 indicated that microvascular cells preserved their phenotype over time.

Conclusions: We expect that the above described microengineered 3D alveolar tissue analogue will provide a valuable tool for compound screening and as an advanced study model, also for recapitulating regenerative strategies or therapies.

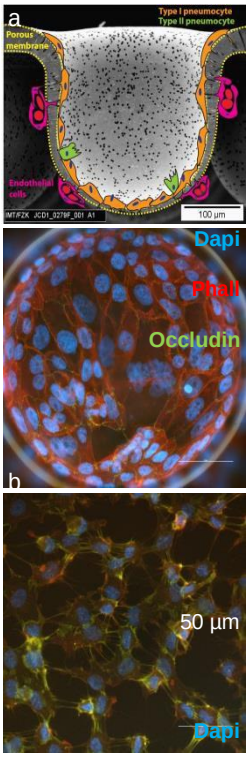

Figure 3 (a) Concept of bioengineered alveolar membrane. (b) Epithelial cells and (c) endothelial cells seeded on curved PC

0313 Electrosprayed capsules co-encapsulating cells and microparticles as minimally invasive active cell microfactories

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The main drawback of the existing bioencapsulation approaches is the diffusion efficiency of essential molecules for cell survival. In order to overcome this limitation, we developed a liquified bioencapsulation system. However, liquefying the core to achieve an excellent diffusion of essential molecules will, on the other hand, compromise cell survival, since most cells are anchorage-dependent. For that, we propose the co-encapsulation of cells with surface-modified microparticles to provide adhesion sites required for cellular functions. Cells and microparticles are surrounded by a flexible, semipermeable, and multilayered membrane produced by layer-by-layer (LbL). We tested liquified capsules for the encapsulation of stem cells to regenerate cartilage [1], or combined with co-cultured endothelial cells for bone tissue engineering [2]. Results show the successful development of microtissues inside the compartmentalized and controlled environment of capsules, ensuring the required diffusion of nutrients and cytokines even at the inner region of the bioencapsulation system. Additionally, the liquified environment allowed spatial freedom for cell communication and self-organization. Here, we propose a second-generation of liquified capsules, giving the importance of developing hybrid devices implantable by minimally invasive procedures for Tissue Engineering and Regenerative Medicine (TERM) applications. Accordingly, we combined the electrospraying (ES) and microfluidics (MF) techniques. ES and MF have been extensively studied for biomedical and pharmaceutical applications over the past years. The flexibility of ES and MF techniques as well as their efficiency to produce tailored objects at the micro- to nanoscale make them one of the most promising approaches to produce biomaterials. Nevertheless, some issues still remain with respect to low throughput as well as the complex interplay between a great number of processing and formulation factors. Hydrogel microspheres are particularly challenging to produce, commonly presenting a tear-like shape. We demonstrate the optimized production of such spherical alginate microgels by varying different parameters of the ES process, namely the alginate concentration, needle diameter, flow rate, needle-collector distance, and the voltage applied. Additionally, MF allowed to produce tailored, monodisperse, and surface-modified poly(L-lactic acid) microparticles that could be encapsulated within the microgels. The loaded microgels with cells and microparticles are then used as templates to produce liquified microcapsules. The templates are coated with multilayers of poly(L-lysine), alginate, and chitosan nano-assembled through LbL. The last layer is then functionalized with alginate-RGD to allow cellular adhesion surrounding the capsules. The developed second-generation of liquified capsules is able to provide a controlled microenvironment allowing cellular processes (adhesion, proliferation and/or differentiation) within the inner microenvironment, while also acting as a cellular supportive system on the outside. We intend to use the proposed system to produce co-cultured hierarchical and complex systems for TERM applications.

0314 Macrovascular channels in a tissue engineered construct with an integrated perfusion system

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Introduction

Mass flow allows increased viability and extended culture of tissue engineered constructs. Such mass flow is typically generated using spinner flasks or, for greater physiological relevance, using an external perfusion pump. Organ-on-a-chip models have been developed creating specific channels between homologues of various tissues in co-culture. Integrating the pump into the culture construct may simplify the model, make it more portable and increase physiological relevance.

Aims and Methods

The goal of this project is to study the molecular diffusion characteristics, cell viability and phenotypic expression of cells grown in a tissue engineered construct with an integrated perfusion system. A multimodal polar bio-printer is used to manage a hydrogel based construct with macrovascular channels (smallest channel diameter is 1.5mm) within an ordinary untreated culture flask with a magnetically driven centrifugal impeller embedded within it. The network is designed with consideration to Starling's law with the smallest total cross-sectional diameter in the capillary bed to promote extra-vascular movement of fluid from the osmotically active perfusing medium. The channels are seeded with HUVEC cells, and after 24 hours, interstitial spaces with the construct are seeded with human MSCs. Video analysis of the movement of acellular spheroids is used to estimate mass flow rates in the vascular channels. Extravascular movement of methylene blue, oligopeptides, glucose in perfused and non-perfused areas of the construct are estimated and cell viability assays are performed using the sampling mode of the printer.

Results

The integrated perfusion system is able to create mass flow in the engineered vascular channels within the hydrogel construct. As expected, molecular movements are significantly greater in perfused areas of the construct compared with non perfused areas. Perfusion extends the period for which MSCs can be cultured in this model.

Conclusions

It is recognised that overcoming the diffusion limit by perfusion extends the culture of cells in 3D tissue engineered constructs. The majority of these perfusion systems rely on an external device to provide mass fluid flow. This study shows that it is possible for the perfusion system to be integrated within the construct itself. Bidirectional mass flow into and out of the vascular space is possible. This may yield a system of organ culture amenable to modular deployment in a larger scale than currently possible. More studies are required to assess the physiological relevance of this construct and the ability to maintain and direct phenotypic expression of seeded cells in co-culture.

0315 Chitosan-based scaffolds for repairing peripheral nerve lesions with substance loss

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Peripheral nerve lesions with substance loss require a conduit to bridge the gap between the two nerve ends.

In this presentation, I will describe the potential use of chitosan as bio-active material for developing bioengineered scaffolds for bridging peripheral nerve gaps. I will summarize a body of preclinical research which showed the great potentialities of this biomaterial for application in peripheral nerve reconstruction in different regions of the body. Yet, I will present some promising clinical results on the application of chitosan based conduits for nerve reconstruction in patients.

Taken together, our results showed that chitosan-based medical devices combine several requisites for a successful clinical acceptance for peripheral nerve regeneration in various anatomical districts.

0316 Interpenetrating polymer network of collagen, chondroitin sulfate and hyaluronic acids - a novel scaffold for tissue engineering for the brain

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Tissue engineering for the brain emerged as a promising therapy for treating stroke and traumatic brain injuries, as the brain lack the ability to regenerate lost tissue. Implantation of stem cells growing in a scaffold would create a beneficial environment for the cells to reconstruct tissue, thereby filling the injured sites and potentially restoring functions. We have developed an interpenetrating polymer network (IPN), consisting of collagen, hyaluronic acids and chondroitin sulfate, for a potential scaffold. Materials characterisations proved the incorporation of hyaluronic acids and chondroitin sulfate in collagen. This study further investigated the proliferation, differentiation, migration and survival of neural stem cells from the subventricular zone of postnatal mouse brains on our engineered scaffolds.

Differentiation studies indicated the neural stem cells primarily differentiated into astrocytes. After one week, the IPNs were shown to have higher astrocytic differentiation in chondroitin sulfate-rich scaffolds, compared to pure collagen, which showed more cells remained undifferentiated. The IPNs also showed enhanced level of proliferation and similar cell viability compared to collagen. Furthermore, migration studies using neuroblast spheroids also indicated enhanced level of migration on the IPN scaffolds. The overall results indicated a promising use of IPN consisting of collagen, hyaluronic acids and chondroitin sulfate for further in vivo studies.

0317 Bioengineered scaffolds and cell transplantation for brain repair in an animal model of focal ischemia

Ting-Yi Wang[1], Fahad Somaa[1], Jonathan Niclis[1], Kiara Bruggeman[2], JA Kauhausen[1], H Guo[1], Stuart McDougall[1], RJ Williams[3], David Nisbet[2], Lachlan Thompson[1], CL Paris[1]

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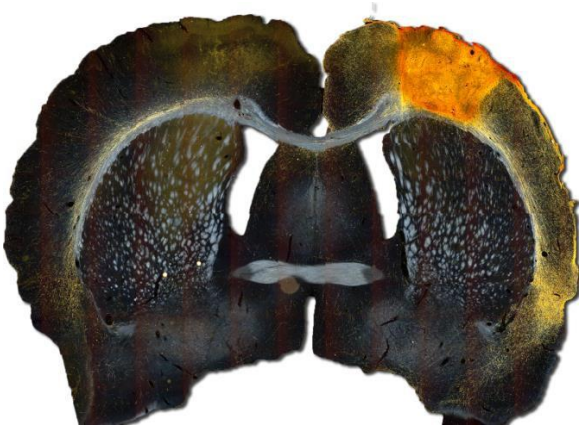
Background: The majority of strokes result from loss of blood flow to parts of the brain - the consequence of which is the death of brain cells in these areas. Additionally, the surrounding brain tissue can also become vunerable and die. However, current therapies for stroke are limited – providing only modest efforts to protect against the ongoing cell death, whilst failing to address the initial cell loss [1].

Experimental design: The bio-engineered scaffolds have been developed as a new therapy for brain repair, which offeres both physical and biological supports to re-build the lost tissue architecture. These scaffolds are gels made from proteins normally present in the brain's extracellular matrix, and are thereby able to fill tissue voids and provide tissue support [2]. Added to this, the scaffolds can incorporate cell transplantation into these gels to replace those lost neurons during the injury. These scaffolds were implanted with cell transplantation in an animal model of stroke for 9 months to assess the repair ability.

Results: The bio-engineered scaffolds have been shown to significantly descrease the atrophy caused by stroke, which show the protection to host neurons against ongoing degeneration in the injured brain. Additionally, the bio-engineered scaffolds can promote both survival and graft volume of hESC-derived cortical progenitors in the comparision to cell transplantation alone. The study also shows the functional recovery in the injured animals under the scaffolds treatment after 9 months.

Conclusion: These scaffolds were shown to restore the lost tissue mass, as well as providing structural support and protein delivery to promote the survival and integration of neural transplants in stroke rats. While the focus of this study is on the ability to repair the injured brain following ischemic trauma, it will also yield important knowledge for the ability to exploit such conduits for other neural injuries.



0318 Development of novel electroactive biomaterials based on poly(aniline) nanotubes and poly(ε-caprolactone)

Ozge Lalegul, A. Eser Elcin, Y. Murat Elcin

Ankara University Faculty of Science, and Ankara University Stem Cell Institute, Tissue Engineering, Biomaterials and Nanobiotechnology Laboratory, Ankara, Turkey

Electroactive biomaterials have aroused considerable interest in biomedical applications due to their unique structures and multifunctional properties. Electrical, chemical and mechanical stimulations can be delivered directly to cells by the utilization of electroactive biomaterials. Intrinsically conductive polymers (ICPs) and their composite systems are promising for regenerative medicine owing to their outstanding features. Among the ICPs, poly(aniline) (PANI) allows the delivery of electrical stimulation to cells and provides efficient control of the electrical stimulation which can influence stem cell survival and differentiation. Highly ordered PANI nanotubes (PANI-NT) have many advantages related to their large surface area and enriched electrical properties. The aim of this study was to develop novel electroactive nanocomposites based on PANI-NT loaded poly(ε-caprolactone) (PCL) blends and also evaluate their potential for regenerative medicine. For this purpose, PANI-NTs were prepared through template-free oxidation polymerization of aniline using ammonium persulfate as the oxidant. Different amounts of PANI-NTs were dispersed ultrasonically to obtain a homogeneous dispersion in various PCL-based matrices. The novel electroactive nanofiber membrane was prepared by using the electrospinning technique. Bone marrow mesenchymal stem cells (MSCs) isolated from femurs of adult Wistar rats were seeded on the electroactive PANI-NT/PCL membranes and cultured for upto 21 days at 5% CO_2-95% air and 37^oC. The morphology, electrical conductivity and composition of electroactive PANI-NT/PCL nanocomposites were characterized by SEM-EDX, TEM, XRD, FPP and FTIR techniques. The mechanical properties of the nanocomposites were also investigated. Additionally, the viability of MSCs seeded on electroactive PANI-NT/PCL nanocomposite membranes was determined by using the colorimetric MTT assay. Results showed that electroactive PANI-NT/PCL nanocomposite membranes have in-vitro cellular biocompatibility, with good mechanical and electrical properties. Our findings demonstrate that, PANI-NT/PCL nanocomposite membranes have potential as a novel biomaterial for in-vitro cellular assays, and for regenerative medicine applications. Further studies are currently being performed to determine the effect of electroactive PANI-NT/PCL nanocomposites on stem cell differentiation with or without external electrical stimulation.

0319 MARCKS peptide functionalized intraluminal collagen fibre-based conduit regulates functional recovery in a critical gap model of peripheral nerve injury

Secil Demir, William Daly, Sahana Ganesh, Abbah Akogwu Sunny, Kyriakos Spanoudes, Isma Liza Mohd Isa, Dimitrios Zeugolis, Leo Quinlan, Abhay Pandit

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Identifying key molecules that increase regeneration has been a long-term goal of tissue engineering. Our detailed proteomic analysis of the material-based regenerative response showed that Myristoylated alanine-rich protein kinase C substrate protein (MARCKS) was upregulated in autologous graft sciatic nerve injury repair but not in biomaterial graft implants. This study compared the efficacy of the functionalized intraluminal fibre collagen conduit with autograft (the current “gold standard”) in regeneration of sciatic nerve critical gap injury.

ELISA was used to compare upregulation of MARCKS in both non-critical (10 mm) and critical gap (15 mm) injuries. Effect of MARCKS functionalized conduits was assessed using hooks attached to the Extensor digitorum longus (EDL) and Tibialis anterior (TA) muscles and connected to the 5N and 20N load cells, respectively, for the purpose of measuring force production evoked upon electrical stimulation. Sixty rats with critical gap injury were used for force measurements; groups as follows: (a) autograft, (b) hollow collagen conduit, (c) fiber conduit, (d) MARCKS funtionalized conduit (low dose, 0.05 mg/ml), (e) MARCKS functionalized conduit (high dose, 0.2 mg/ml).

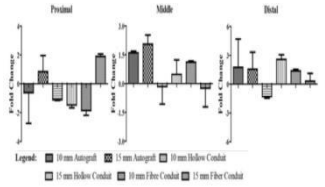

Figure 1: ELISA data for treatment groups based on the gap distance treated. All data was analysed by one-way ANOVA followed by a Newman Keuls *post-hoc* test for comparison of groups at different gap lengths and compared to the 10 mm ($) and 15 mm (*) autograft respectively (a *p*-value < 0.05 was deemed significant).

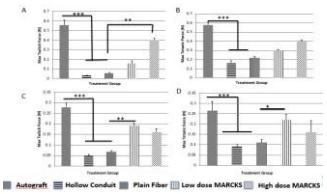

Figure 2: Force measurement analysis of the TA muscle and EDL muscle 12 weeks post implantation. (A) Maximum twitch force in TA muscle, (B) Maximum tetanic force in TA muscle (C) Maximum twitch force in EDL muscle (D) Maximum tetanic force in TA muscle. (**p*-value < 0.05, one-way ANOVA, n=8 per group for all measurements).

ELISA results combined with the proteomics data suggest that expression of MARCKS can significantly upregulated during peripheral nerve regeneration (Figure 1). Assessment of functional recovery in TA and EDL muscles showed that recovery in autograft group was significantly greater than that of the hollow conduit and plain fiber conduit. In terms of maximum twitch and tetanic force, MARCKS peptide treated groups showed significant differences in EDL groups (Figure 2). Results showed that conduits functionalized with MARCKS peptide enhanced functional recovery following critical gap nerve injury.

0320 Cells, growth factors and engineered nerve conduits for modulating the adult axonal growth capacity

Lucas Degrugillier[1], Daniel Kablbermatten[1], Bruno Gander[2], Srinivas Madduri[2,3]

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Peripheral nerve reconstruction is critical and challenging due to lack of potential apporaches to create a complex, multifactorial and dynamic microenvironment required for axonal path finding. Here, we report on bioengineered nerve conduits (NCs) orchestrated with important biological functions for supporting complex requirements of axonal regeneration. Firstly, an efficient and tropism modified adenovirus for generating genetically modified Schwann cells (GMSCs) was identified and engineered for single or co-expression of synergistic growth factors GDNF and NGF. Resulting recombinant virus, within GMSCs, proved to be safe and efficacious both *in vitro* and *in vivo* a in nerve crush injury model. Subsequently, collagen or silk fibroin nerve conduits (NCs) possessing aligned nano-topography were fabricated using a novel technique and orchestrated with GMSCs or multiple biological functionalities. Resulting eight different bioengineered NCs varying in structural and functional features were implanted in a 10 mm nerve gap model in rats. The anatomical, behavioural and electrophysiological outcomes differed significantly between the treatment groups. The virus neutralization assay indicated absence of undesired immune response against the recombinant adenovirus used for genetic modification of the Schwann cells. Importantly, bioengineered collagen NCs carrying most important biological functions, in contrast to silk fibroin NCs, showed potential to overcome the present hurdles of nerve regeneration and matched well with autograft performance. This study not only revealed the importance of new approaches for orchestrating complex biological functions for effective axonal regeneration, but also demonstrated the impact of biomaterial composition in mounting effective tissue response.

0321 Journey of traditional suture to the realm of biomaterials

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Conventionally, we have a tendency to exploit our natural resources for human use. Silk is universally known as the ancient suture. It is now proven to be much more than just a thread. Over the decades, extensive works on silk proteins (fibroin and sericin isolated from silk cocoon, peduncle or larvae) have recognised and validated their beneficial characteristics, which are suitable for biomedical use. Silk is established as one of the most promising natural biomaterial for the applications in tissue engineering and regenerative medicine. The silk proteins fibroin and sericin are individually utilised in fabrication and evaluation of various forms of matrices (thin membranes, porous scaffolds, hydrogels, microcapsules, nanoparticles, micro/nano architectures, composites and electrospun nanofibrous mats) for their potential benefits in different tissue engineering areas (bone, cartilage, skin, cardiac, vascular, alveolar, auricular, lung, corneal, and neural). Modifications to augment their existing properties or compliment with an additional feature are requisite to employ these matrices in the specific target or application including the delivery of bioactive molecules (drug, growth factor, antibiotic or gene). This enhances their prospective to reconstitute the loss or damaged tissue structurally and functionally. The focus of the presentation will be on the recent trend based on different varieties of silkworm silks and their success story in various fields of biomedical applications (supported by Department of Biotechnology (BT/PR10941/MED/32/333/2014), Indian Council of Medical Research (5/13/12/2010/NCD-III), Govt. of India and EC funded FoReCaST - project grant number 668983).

0322 Differentiation of human mesenchymal stem cell differentiation towards intervertebral disc-like cells on engineered *Bombyx mori* silk scaffolds for disc repair

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Introduction: Low back pain is an increasing problem for a high number of people. It is often caused by trauma and intervertebral disc (IVD) degeneration. Yet, no treatment option is available that is capable of restoring the biological function of the IVD. In this study, we aim to investigate the feasibility of using engineered *B. mori* silk containing growth and differentiation factor 6 (GDF6) for IVD repair by differentiating human mesenchymal stem cells (MSC) towards a "disc-like" phenotype.

Material and Methods: Silk fleece-membrane composites were produced from *B. mori* larvae infected with baculovirus to produce silk protein functionalized with either GDF6 or TGFβ3 (Spintec Engineering GmbH) [1]. Unmodified silk was used as a control without growth factors (cSilk). MSCs of five human donors were used to test differentiation potential of functionalized silk composites. Cells were seeded at a density of 120,000 cells per 5x5mm^2 scaffold and cultured for 21 days in high glucose DMEM. cSilk ± exogenous GDF6 (exGDF6, 100ng/ml) were used as controls. On day 1, 7, 14, and 21 mitochondrial activity, glycosaminoglycan (GAG) and DNA content were determined. Furthermore, live/dead stain was performed using confocal laser scanning microscopy. Additionally, relative gene expression of IVD marker genes was assessed on day 21 relative to day 0.

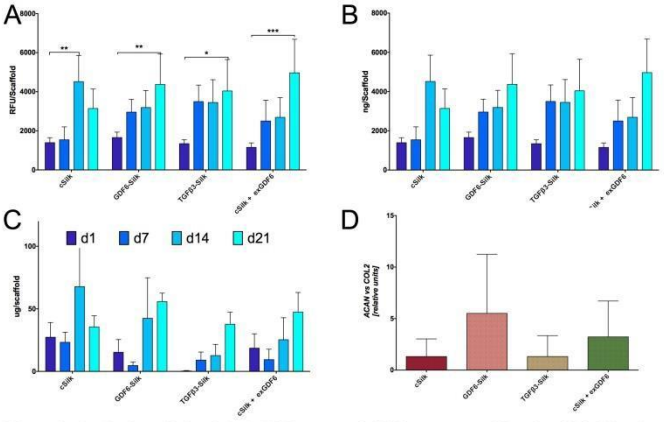

***Figure 1: A** mitochondrial activity **B** DNA content **C** GAG content and **D** ratio of ACAN and Col2 gene expression. All 2-way ANOVA, Bonferroni's multiple comparisons test ±SEM of n= 5 donors; p-values: *= 0.01, **<0.01, ***=0.0002.*

Results: Good cytocompatibility of silk scaffolds could be confirmed by live/dead stain as cells adhered well to the scaffold and completely covered the silk fibers. Moreover, cells were active on all three silk scaffolds, Fig 1 A. Further, DNA and GAG content were increasing over culture period except GAG content on cSilk, Fig 1 B-C. qPCR showed a aggrecan/collagen type 2 ratio that indicates differentiation towards disc-like cells for GDF6 silk [2], Fig 1D.

Discussion: Engineered silk containing GDF6 is a promising biomaterial for IVD repair. It might be part of a future treatment option that not only reduces pain but restores the biological function of the disc.

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0323 Immunomodulatory bio artificial pancreas for sustained insulin production in diabetic patients

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The increasing prevalence of diabetes throughout the world has led to its consideration as an epidemic of the 21st century. Within the population, type I diabetes represents around 10% of all diabetic patients worldwide. Clinically, this form of diabetes is associated with hyperglycemia due to defects in insulin secretion and production which is associated with eventual failure of several vital organs [1]. Islet transplantation is considered the most effective treatment to restore normoglycemia in diabetic patients. It is considered more successful than insulin injections because the islets may function both as sensors of glucose as well as source of insulin [1,2]. In the current study, we have developed a functional cell-encapsulating 3D immunoisolatory and immunomodulatory silk scaffold system as a bio-artificial pancreas for islet transplantation and sustained insulin production. Scaffolds were fabricated from mulberry silkworm (*Bombyx mori*) using a salt leached technique and cell encapsulation was conducted utilizing alginate and agarose [3]. Scaffolds were characterized for morphology, swelling, density and porosity. Cell-encapsulating scaffolds presented superior characteristics in terms of cell proliferation (~2.5-fold) when evaluated for RIN-5 insulinoma cells in 05 weeks culture. Cells formed 3D islet-like spheroids spontaneously. Also, rat primary islets maintained their function in silk macrocapsules with an enhanced glucose stimulation index (SI) when compared to non-encapsulated islet controls, 1.2 vs. 1.7. RT-qPCR and immunohistochemistry for insulin and glucagon supported the results obtained from the glucose challenge assay [1,2]. As a proof of concept, anti-inflammatory cytokine interleukin-4 (IL-4) and dexamethasone were effective in reducing local foreign body response and immunosuppression. The released IL-4 was biologically active and polarized M0 macrophages to the M2 phenotype, advocating immunosuppressive function [4]. *In vivo* subcutaneous implantation of scaffolds in mice showed minimal fibrosis and inflammation after 28 days. In conclusion, the scaffolds provided a pancreas-like niche to re-establish the islet microenvironment lost during isolation. The goal is to advance this direction toward viable “Bio-Artificial Pancreas” development for the treatment of diabetes.

Keywords: *Biomaterials; Tissue Engineering; Silk; Macroencapsulation; Bio-artificial pancreas, Immunoisolation*

0324 Combinatory approach for developing silk fibroin-based scaffolds seeded with human adipose-derived stem cells for a cartilage tissue engineering applications

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Several processing technologies have been combined to create scaffolds for different tissue engineering (TE) applications. Hydrogels have been extensively used for cartilage TE applications, presenting several structural similarities to the natural extracellular matrix of cartilage tissue environment[1]. From the different biodegradable materials proposed as matrices for cartilage scaffolding[2], silk fibroin (SF) presents high versatility, processability and tailored mechanical properties, which make this protein attractive for the development of innovative matrices for cartilage TE purposes[3]. In a previous study, we proposed fast formed SF hydrogels produced through a horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2) crosslinking reaction, taking advantage of the presence of tyrosine groups[4]. In this work, macro-/micro-porous SF scaffolds derived from enzymatically cross-linked SF hydrogels by a HRP/H_2O_2 complex were produced in combination with salt-leaching and freeze-drying methodologies. The scaffolds morphology, mechanical properties and chemical characterization were assessed by mean of different characterization techniques (SEM, micro-CT, Instron, FTIR and XRD). The scaffolds structural integrity was evaluated by swelling ratio and degradation profile studies. The *in vitro* ability to support the adhesion, proliferation and differentiation into the chondrogenic lineage was tested using human adipose-derived stem cells (hASCs) cultured over 28 days in basal and chondrogenic conditions. Cell behaviour in the presence of the SF scaffolds was evaluated through different quantitative (GAGs/DNA and RT-PCR) and qualitative (live/dead, SEM, histology and immunocytochemistry) assays. The *in vivo* biocompatibility of the SF-based scaffolds was also assessed by subcutaneous implantation in mice for 2 and 4 weeks and analysed by means of hematoxylin & eosin (H&E) staining and immunohistochemical analysis of CD31 angiogenic marker. The results showed highly porous and interconnected SF structures that allowed cell adhesion and infiltration into the scaffolds. *In vitro* cell viability and proliferation were also observed over the 28 days of culturing in basal conditions and a significant increase of GAGs content was detected on constructs cultured in chondrogenic differentiation medium. *In vivo* results showed that the implanted scaffolds allowed tissue ingrowth's and blood vessels formation/infiltration. The obtained results demonstrated that the innovative approach of combining enzymatically cross-linked SF hydrogels with the salt-leaching and freeze-drying methodologies allowed to produce more versatile scaffold architectures with appropriate mechanical properties and large swelling ability. The positive influence over *in vitro* chondrogenic differentiation and *in vivo* response, revealed by the new tissue formation and angiogenesis within the porous scaffolds, validates the proposed macro-/micro-porous SF scaffolds for being used in cartilage TE applications. Moreover, the versatility of these combinatory approach can allow for further applications in other musculoskeletal TE strategies.

Acknowledgments:

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0325 Bioactivation of spider silk with basic fibroblast growth factor (bFGF) and its use for cell culture and tissue engineering applications

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Introduction: Growth factors are considered to be essential for cell culture and tissue engineering applications. However, they are rapidly degraded in physiological conditions. Immobilization of growth factors can extend their *in vivo* half-life (Masters KS, *et al*, 2011). The partial spider silk protein (4RepCT) (Stark M. *et al*, 2007) is produced in bacteria and purified using chromatography. Recently, 4RepCT has been functionalized with cell binding (Widhe M. *et al*, 2013), affinity (Jansson R. *et al*, 2014) and catalytic (Jansson R. *et al*, 2015) properties. In this study, basic fibroblast growth factor (bFGF) was conjugated with 4RepCT using recombinant DNA technology. Obtained silk-bFGF fusion proteins were characterised in terms of ability to self-assemble onto surfaces, subsequent binding to cognate FGF receptor 2, and finally by cell culture studies.

Methods: The silk-bFGF fusion protein was produced in *Escherichia coli* and purified using IMAC chromatography under non-denaturing conditions with subsequent removal of endotoxins. Quartz Crystal Microbalance with dissipation (QCM-D) was performed to study the self-assembly of silk-bFGF onto a hydrophobic surface. In order to assess the bioactivity of immobilized silk-bFGF, endothelial cells (HUVECs) were cultured in culture medium depleted from growth factors (MII). Alamar blue and live/dead viability assays were performed to determine the viability of seeded cells.

Results: The purified silk-bFGF fusion protein could spontaneously self-assemble to silk-like fibers (Figure 1a) despite fusion with bFGF (17.2 kDa). QCM-D studies revealed adsorption and subsequent self-assembly of silk-bFGF proteins into stable surface coatings (Figure 1b). Culture of endothelial cells in MII medium on silk-bFGF coatings resulted in significantly (*$p<0.05$) higher cell proliferation when compared to coatings of non-functionalized silk (Figure 2a), and also more live cells (Figure 2b). Taken together, the results verify the bioactivity of bFGF conjugated to silk.

Conclusions: The results from the present study showed that silk-bFGF materials can promote proliferation of endothelial cells also in media without supplemented growth factors. Therefore, we envision future use of silk-bFGF materials for *in vitro* cell culture studies as well as for promotion of endothelialisation of vascular grafts.

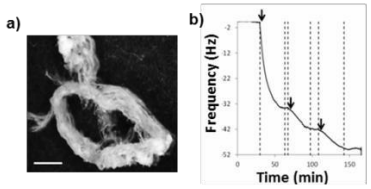

Figure 1: a) Light microscopy image of a macroscopic silk-bFGF fiber. Scale bar: 1 mm. b) QCM-D analysis of silk-bFGF fusion proteins adsorption on a surface. Arrows indicate injection of silk-bFGF proteins. Vertical dashed lines indicate switching of tubes between protein solutions and sample buffer.

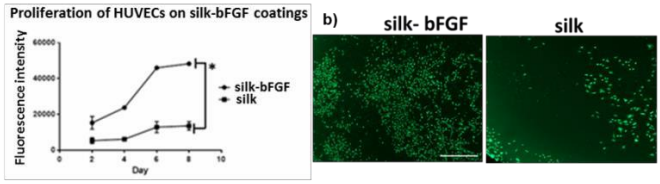

Figure 2: a) Alamar blue viability assay of HUVECs on silk-FGF and silk coatings. b) Representative images from Live/dead staining of HUVECs after 8 days of culturing on silk-bFGF and silk coatings. Scale bar: 500 µm.

0326 A highly integrated layered scaffold of silk knitted fabric - plastic compressed collagen for bladder tissue engineering

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Introduction: The uninary bladder wall consists of four main layers being the mucous (urothelium), the submucosal connective tissue layer, the muscular layer, and the serosal layer covering the external surface [1,2]. To have a successful bladder wall regeneration, replicating the layered structure of bladder wall (urothelial cells (UC) making a multilayered specialized epithelium covering the innermost layer, and smooth muscle cells making up around 60%–70% of the wall tissue of normal bladder in the middle layers) is crucial. To reach this goal, layered cell seeding is routinely performed into/onto the 3D scaffolds. Although there are several reports about successful regeneration of urothelium, incomplete regeneration of smooth muscle cells, especially in large area defects has remained a challenge in bladder regeneration[3,4]. Moreover, in such situations that different layers of biomaterials make up the scaffold, optimizations or surface treatments are needed to avoid delamination between adjacent layers[5,6].

Methods: In this study we have shown that combination of silk knitted fabric with plastic compressed collagen leads to a highly integrated construct in which collagen penetrates through the knitted fabric. To build up the seeded scaffold, a weft-knitted silk fibroin fabric with high porosity was placed on a semi-gel collagen inside a mold. Minced detrusor particles from porcine bladder were distributed on top of knitted fabric and the construct was covered by a second collagen hydrogel. Finally, minced mucosa of porcine bladder was seeded on top. The whole construct underwent plastic compression (PC), and was incubated for 2 and 6 weeks *in vitro* for electron microscopy, histology and immunoassay studies.

Results and discussion: Our SEM and histology imaging at 2 and 6 weeks showed that the collagen hydrogel not only covered the silk fabric, but also infiltrated the pores of the silk knitted fabric so that the collagens originating from both top and bottom met each other in the middle. It led to an integrated structure in which the gradual decrease in collagen concentration from both ends towards the middle was obvious. Cell studies showed that a multilayered urothelium is achieved within 2 weeks. We also observed that viable spindle shaped smooth muscle cells migrated from the minced detrusor and formed groups of Smooth muscle cells in a 3D distribution inside the scaffold. We believe such a hybrid construct of silk knitted fabric – PC collagen has a great potential of application in similar tissue engineering fields where layered cell/tissue seeding is needed.

Acknowledgements: This work was supported by Danish Research Council Foundation (DFF–4093-00282), the Freemason Foundation for Children's Welfare, the Stockholm City Council and the Swedish Society of Medicine.

0327 Aging mechanisms relevant for cartilage degeneration and regeneration

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Tissue-specific regenerative cells are required for tissue replacement throughout human lifespan. Characterized by a self-renewing capacity and a multilineage differentiation potential, mesenchymal progenitor cells can yield on demand the different cells of a tissue. Mesenchymal stroma cells (MSC) are thought to be largely retained in a quiescent stage over prolonged periods of dormancy in the body, but can be stimulated to enter the cell cycle to produce different effector cells through subsequent rounds of proliferation. A hierarchical differentiation permits the production of large numbers of differentiated cells from a single MSC and balances the need for high cell numbers with the protection of cells from mutagenesis and replicative exhaustion.

Evident aspects of human ageing like chronic degeneration of articular cartilage and intervertebral disc tissue, progressive decrease in bone mass, replacement of bone marrow by fat marrow, and delayed fracture healing may result from an age-associated decline in either the number or the replicative function of MSC. In addition, activation of regenerative cells from dormancy into the cell cycle in response to extracellular cues and their lineage choice to undergo differentiation in a repair situation may change with increasing age. This leads to the question whether the proper physiological function of MSC will be maintained throughout life. In this context the question arises whether donor age might be one factor influencing the frequency and characteristics of MSC in a way that MSC-based therapies should be restricted to a certain age group. This talk will summarize age-related aspects of cartilage degeneration and regeneration with a special focus on MSC-based tissue regeneration.

0328 Stem cell therapy in orthogeriatrics: the effect of aging on stem cell functions

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Aging of musculoskeletal tissues and the associated functional deficits are major concerns in the growing field of orthogeatrics. Several studies dealing with human aging and age-associated diseases such as sarcopenia, anemia or impaired wound healing have indicated that the reduced regenerative potential of adult tissues is linked to a decline in their stem cell pools. Thus, it is now assumed that aging is partly driven by an age-associated shortage in the number, stress-resistance and repair capacity of tissue-specific adult stem cells.

Although, the link between altered stem cell properties and tissue aging has been recognized, the molecular and cellular process of tendon aging has not been elucidated.

Tendon matrix is predominantly composed of collagen type I fibrils organized in great hierarchical manner that is unique for this tissue. Collagen I provides the mechanical strength of tendons, but also environment for the endogenous tendon stem/progenitor cells (TSPC). This cell type is strongly suggested as a key cellular player in tendon maintenance and repair.

TSPC establish interactions with the surrounding collagen I matrix mainly through integrin receptors, which via complex downstream signalling regulate critical cell functions, such as cell adhesion, spreading, survival and differentiation. Previously, we have shown that tendon aging results in profound loss of self-renewability, premature and augmented senescence as well as distorted actin cytoskeletal dynamics. Microarray and PCR data pointed out a dysregulation of TSPC integrin receptor profile. We speculated that this phenomenon might be the foundation of abnormal cell-to-collagen I interactions. By cultivating TSCP from young and aged donors in three-dimensional collagen gels, we observed clear differences in the kinetics of collagen I adhesion and remodelling. Next, by implementing step-by-step follow up on the integrin signalling pathway we identified a novel integrin-dependent mechanism that is critical for the collagenous matrix remodelling in tendons and is malfunctioning during tendon aging.

Hence, we suggest that some of the mechanisms of aging such as replication, senescence and stress response are inevitable in order to prevent tumour formation, but others may vary between cell types due to different niche environments, cell fates and regulatory networks.

0329 The balance of transient receptor potential channel TRPC6 to TRPC1 determines ageing and mechanotransduction in intervertebral disc cells

Alfredo Franco-Obregón[1], Hiroshi Kobayashi[2,3], Helen Greutert[3], Timon Wernas[4], Marcel Egli[4], Elena Cambria[3], Miho Sekiguchi[2], Norbert Boos[5], Oliver Hausmann[6], Stephen Ferguson[3], Karin Wuertz-Kozak[3,7]

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Objective: Transient receptor potential channels TRPC and TRPV transduce mechanical signals into biochemical responses with developmental and regenerative consequences in a variety of tissue types, but few data exists on TRP channels in the intervertebral disc (IVD). Therefore, the objective of this study was to analyze whether age-related changes in TRP channel expression are associated with altered IVD mechanotransduction.

Methods: Human IVD cells were expanded up to low passages (LP) or high passages (HP). The ageing phenotype (proliferation, senescence, matrix expression, TRP channel expression) was assessed. Subsequently, LP and HP cells were exposed to mechanical unloading (simulated microgravity) or pharmaceutical TRP channel inhibition (SKF-96365) and analyzed for cell growth, cell cycle progression, senescence and TRP channel expression.

Results: Cell quiescence after serial passaging was strongly associated with an upregulation of TRPC6 relative to TRPC1 (Fig. 1) and this was accompanied by an increase in SA-β-galactosidase-positive cells, reduced cell proliferation rate and a shift in the coll-1/coll-2 expression pattern. SKF-96365 slowed cell proliferation in a dose-dependent manner, exhibiting enhanced antagonism in less quiescent LP cells as well as at lower cell densities. In addition, SKF-96365 recapitulated the accumulation of cells into the G2/M phase. Mechanical unloading of IVD cells also slowed cell proliferation, produced G2/M accumulation and increased the incidence of quiescent cells, however reduced the ratio of TRPC6 to TRPC1.

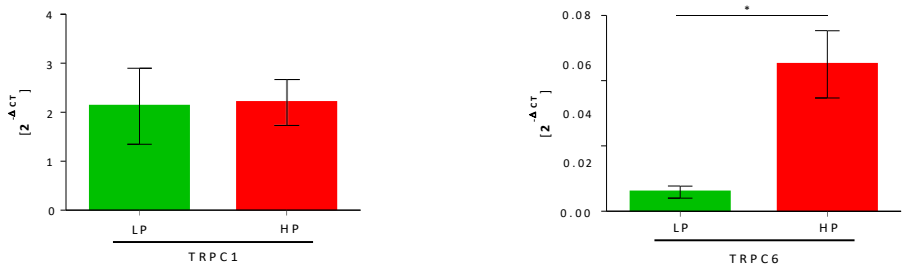

Figure 1: Ageing-related changes in TRP channel expression. Gene expression of members of the canonical TRP family (TRPC1, TPRC6) in LP and HP, as assessed by qPCR and displayed as $2^{-\Delta Ct}$ (n=6). Data is shown as mean ± SEM. LP = low passage (green); HP = high passage (red).

Conclusion: Our study reveals a feedback mechanism whereby mechanical input influences TRPC expression that, in turn, influences mechanoresponsiveness of IVD cells, hence providing supportive evidence for age-related functional balancing of TRPC6 against TRPC1.

0330 GDF6-induced differentiation of adipose-derived stem cells to nucleus pulposus cells is enhanced by culture in a hypoxic and loaded microenvironment

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Intervertebral disc (IVD) degeneration represents the leading cause of chronic low back pain (LBP). The increasing prevalence of LBP and lack of successful therapies highlights the growing need for novel biologic and/or cell-based therapies aimed at regenerating the central nucleus pulposus (NP) region through generation of functional tissue. We have previously demonstrated that adipose-derived mesenchymal stem cells (AD-MSCs) can be differentiated to NP cells though culture in 3D in the presence of 100ng/ml growth differentiation factor 6 (GDF6). However, the NP of the IVD is a harsh microenvironment with cells being exposed to hypoxia and high levels of mechanical load. Thus this study aimed to establish how hypoxia and load affected the differentiation of AD-MSCs to NP cells.

Human AD-MSCs (N=3) were cultured in type I collagen gels for 14 days in the presence of GDF6 under either normoxic (20% O_2) or hypoxic (2% O_2) conditions with or without dynamic compression applied using a Flexcell system (0.04MPa, 1Hz, 1 hour per day). After 14 days differentiation was assessed using qPCR for expression of NP marker genes. Glycosaminoglycan production and fibrillar collagen content was investigated through DMMB assays and picrosirius red staining. Construct mechanical properties were assessed using atomic force microscopy (AFM).

NP marker gene expression was responsive to hypoxia and load. SOX9 and COL2A1 were significantly upregulated under load or hypoxia with combined conditions further elevating expression. ACAN was more responsive to hypoxia than load, with highest upregulation under combined conditions. Loading upregulated keratin 8, 18, 19 and FOXF1 expression, while hypoxia upregulated CA12 and keratin 8. Combined conditions increased keratin 8, 18, 19, FOXF1 and CA12 expression. Total sGAG content was highest in constructs cultured under combined conditions, though fibrillar collagen was not significantly altered. AFM analysis showed a significant decrease in construct reduced modulus under combined conditions.

This study demonstrates that GDF-6 induced differentiation of AD-MSCs to NP cells is enhanced by culture under hypoxic and loaded conditions, with a combination of the two microenvironmental factors eliciting the highest levels of gene expression and most NP-like matrix formation. This highlights the importance of considering microenvironmental conditions when designing tissue engineering strategies and that hypoxic loaded conditions akin to those found within the intervertebral disc are not detrimental to AD-MSC based therapies for IVD degeneration.

0331 Validation of an osteochondral bioreactor applied to study the protective role of sex hormones

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Introduction: Cartilage-bone interactions play a critical role in osteoarthritis (OA). For instance, loss of bone integrity in osteoporosis affects cartilage health, and in OA the osteochondral (OC) junction has been identified as a locus of the disease (1). To study the OC complex we have developed and validated a bioreactor in which OC biopsies can be cultured with separate media conditions for cartilage and bone, while maintaining cartilage-bone contact. Furthermore, the same bioreactor can be used to generate engineered OC constructs to model the OC complex (2). Hence, our 3D osteochondral microphysiological system (MPS) could be used for the screening for OA drugs and compounds (3). We have used our OC MPS to study the effects of female sex hormones on the OC complex. In fact, OA incidence in women is 36-45% higher than in men and postmenopausal women experience twice higher risk of osteoporosis then men over 70. However, standard hormone replacement therapy (HRT), improves bone conditions, but not cartilage. We hypothesized that restoring hormonal exposure mimicking the menstrual cycle would have a protective effect on both bone and cartilage.

Materials and Methods: MPS validation. Fluid dynamic simulations were carried out in ANSYS to assess the extent (if any) of fluid mixing and transport of nutrients within the MPS (4). Each simulation was then compared to experimental tests using similar conditions. Effects of sex hormones. Native human OC biopsy plugs were harvested from postmenopausal female patients undergoing total joint replacement (IRB: University of Washington). Either cartilage or the bone were exposed for 1 month to a sequence of hormonal concentrations simulating that of the menstrual cycle, and the full OC model was then examined to assess bone-cartilage interaction, using histology, immunohistochemistry, microCT and RT-PCR, and ELISA of culture supernatants.

Results and Discussion: Simulation and experimental results for fluid flow rate and mixing, diffusion of molecules (bovine serum albumin, BSA) and nutrient (glucose) consumption matched and confirmed negligible leaking. The OC tissues not exposed to hormones had greater GAG loss (histology) and greater bone volume loss (micro-CT), especially at the OC interface. Both cartilage and bone respond when directly exposed to hormones as well as when the other tissue is exposed. The OC MPS can effectively maintain distinct medium environments while providing effective nutrient supply. The strong osteoprotection by sex hormones is not unexpected, and notably one month of sex hormones cycling prevented bone loss. The high bone loss at the OC interface vs. no bone loss when hormones were administered, suggested a possible effect of cartilage-bone communication and points to signaling pathways responsive to hormones for potential targets for OA prevention.

Conclusion: The effects of cyclic concentrations of sex hormones point to possible improvements in HRT regimes and suggest potential target for OA therapy. The OC MPS is an effective tool for in vitro culture and study of native and engineered OC tissues.

0332 Poly(amido amine) based nanogels for highly efficient intracellular gene delivery

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Gene therapy is considered to have high potential for the treatment or prevention of diseases caused by genetic defects. However, a major hurdle in the progress in clinical applications is the poor availability of safe and efficient delivery systems. Although viral vectors are designed by nature to deliver genes, their application is severely limited due to limited loading capacity, potential toxicity, mutagenicity, and immune response. By using non-viral delivery systems, these limitations can be avoided, but these systems generally suffer from lower efficacy in gene transfection. Here we present a novel generation of multifunctional polymeric nanogels that can be easily loaded with oligo- and polynucleotides in quantitative yield and give excellent transfection properties in a broad variety of cell lines. Non-targeting *in vivo* experiments with siRNA loaded nanogels show effective delivery of the siRNA payload to liver, spleen and kidney, without accumulation in lungs, followed by rapid clearance of the nanogels. The nanogels can be used and stored as a freeze dried powder or in solution. An example of the downregulation of GFP by anti-GFP siRNA is given in Figure 1. Multifunctionality of the nanogels for a variety of applications will be presented.

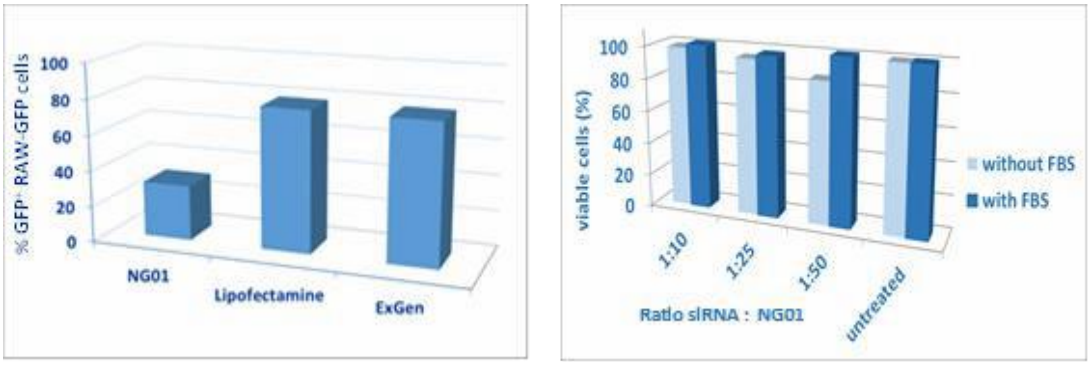

Figure1: GFP knock down (80%) and cytotoxicity (MTT) of anti-GFP siRNA delivered with 20Med nanogel NG01 in RAW cells. One nanogel contains ca 100 siRNA at 1:25 weight ratio siRNA:NG01.

0333 Non-viral gene therapy for musculoskeletal tissue regeneration using advanced gene-activated matrices

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Gene-activated matrices (GAMs) delivering gene-encoded differentiation cues by *in vivo* gene transfer, were introduced to the field as a promising treatment strategy more than a decade ago. Whilst the combination of appropriate genetic cues and biomaterials could indeed revolutionise current treatment strategies, translation has been hampered by lack of effective *in vivo* gene delivery, therapeutic efficacy, and spatio-temporal control. These obstacles to translation are reflected by the small number of clinical trials and will need to be overcome by developing novel approaches beyond the current state of the art. Furthermore, in order to deliver morphogenic cues required for complex tissue regeneration, platform technologies would need to enable the delivery of multiple genes within different areas of the biomaterial.

Recent work of our group in this field has led to the development of two technology platforms, capable of addressing some the challenges associated with effective and controlled non-viral GAM-mediated gene delivery:

- Matrix-assisted sonoporation (MAS™) has been developed as an active, trigger-controlled gene delivery method to cells within biomaterials using sonoporation as translation-friendly, efficacy-enhancing method. Using MAS™, it was possible to demonstrate higher gene delivery efficacy compared to standard GAM-based approaches and to induce complex tissue formation *in vivo* employing inducible and constitutive bone morphogenetic protein expression systems. The combination of the conditional ultrasound trigger for enhanced gene delivery and control of transgene expression on the transcriptional level through a small-molecule inducer, enabled methods for spatio-temporal control of GAM-mediated gene delivery and complex tissue formation *in vivo* and is currently being evaluated for its potential in orthotopic defects.

- The second platform technology developed for the advancement of GAM technology is a cell-free construct in which the distribution of multiple therapeutic DNAs and the synthesis of transfection-enhancing nanoparticles can be precisely controlled using an electric field. Using this novel system, it was possible to demonstrate spatial control over payload distribution within a biomaterial and to simultaneously control the distribution of a calcium-phosphate mineralisation gradient, thereby creating a graded material suitable for tissue interface engineering. *In vitro* and *in vivo* gene delivery has been achieved using this system and its potential for the delivery of complex morphogenic cues is currently being investigated. Furthermore, this approach has been demonstrated to be suitable for the delivery of a multitude of different classes of charged biomolecules, such as extracellular matrix components and recombinant growth factors.

Several issues of translation regarding GAM-technology will be highlighted and put in context with our current work to advance GAM platform technologies. Finally, recent encouraging results will be presented with a vision for future tissue interface engineering approaches *in vivo* using smart biomaterials to deliver complex morphogenic cues via therapeutic gene gradients.

0334 Design of siRNA for efficient delivery

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Introduction. Oligonucleotides for regenerative medicine could have a significant translational impact because these are relatively small robust molecules and can be treated as small molecule drugs well understood by the FDA and pharmaceutical drug companies. The major hurdle for clinical application is, however, delivery.

Nucleic acid therapeutics acts at the molecular level. After the genome of an organism is sequenced, RNAi can be designed to target every gene in the genome and target for specific phenotypes, including genes for which traditional small molecule inhibitors cannot be found.

One of the major challenges in RNAi therapeutics is to achieve the preferential selection/delivery of the desired antisense strand over the sense strand within RNAi machinery to avoid off-target effects. Specific proteins in the RISC selectively bind the thermodynamically less stable 5'-end of siRNA, thereby regulating this selection process.(1) Engineering this type of selection so that the cellular machinery preferentially recognizes and recruits the desired antisense strand would not only improve RNAi efficacy but also reduce dosage and mitigate sense strand–mediated off-target effects. We have developed a novel siRNA design that is capable of inducing thermodynamic asymmetry with unique cellular uptake and endosomal escape characteristics that have not been observed with conventional siRNA.

Experimental. To design siRNA with the desired 5'-end thermodynamic asymmetry that could be applied to any siRNA sequence, we extended the strand length at the 3'-end of the sense strand from two nucleotide (nt) overhangs to five and eight nt. We envisaged that this strand extension (*wagging tail*) at one end (3'-overhang in sense strand) of the siRNA duplex would induce thermodynamic destabilisation at that end, which could facilitate the ATP-dependent selective unwinding and RISC recruitment of the desired strand.

Result & Discussion. Our siRNA melting studies (using temperature-dependent UV spectroscopy) clearly supported our hypothesis. Transfection experiments targeting a housekeeping gene 'glyceraldehyde 3-phosphate dehydrogenase' or GAPDH of these modified siRNAs corroborated our hypothesis, which revealed that an increase in the overhang length indeed improved the gene silencing efficiency using magnetic bead (MATra-assisted) transfection from 60% (with dT_2) to 80% (using dT_5) in MG63 (human osteosarcoma) cells. The gene knockdown efficiency was determined using quantitative RT-PCR experiments.

Serendipitously, when we performed control experiments using these 3' extended sequence without any transfection reagent, we observed a surprising result. These experiments showed that the dT_5-modified sequence gave 80% gene knockdown (similar to MATra-based transfection), whereas dT_2 and dT_8 modified siRNA showed 28% and 50% knockdown, respectively.

Conclusion. This unanticipated result suggests that dT_5-*modified siRNA performs all essential steps for siRNA-based drug design, from cellular uptake and endosomal escape to selective RISC recruitment*. Therefore, we termed this siRNA design with a five nt overhang as cell penetrating siRNA or cpRNA. We have evaluated the versatility of cpRNA in different cells and observed similar exceptionally high gene knockdown efficiency in human cancer cells (MG63 and HCT116), primary cells (HOB), and non-adherent cells (U937). We also demonstrated gene-silencing efficiency by knocking down another model protein called Green fluorescent protein (*GFP*) in MG63 cells.

0335 Inhibition of miR100 overexpression in osteoporotic osteoblasts results in upregulation of RunX2, ALP and Collagen I via BMPR2

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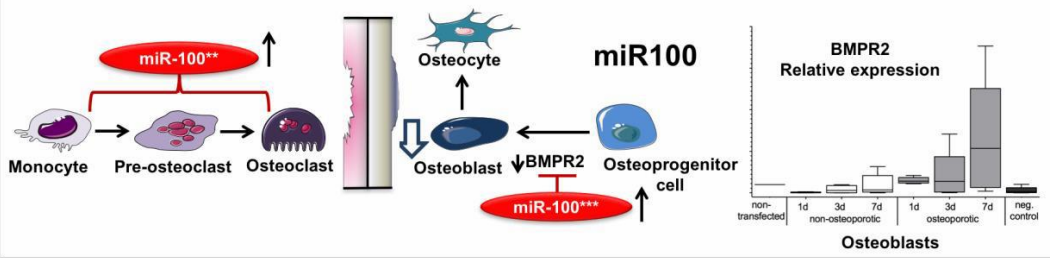
Osteoporosis is a common, systemic skeletal disorder characterized by impaired bone strength and quality with a subsequent increased risk of bone fractures. As a consequence, osteoporotic patients present poor bone healing capacity. Osteoporosis is accompanied by a change in the expression of specific miRNA signatures. Among the several miRNAs associated with osteoporosis, miR100 has been related to bone homeostasis and regeneration. In the present study, we evaluated the expression of miR100 in serum, bone tissue and bone cells from osteoporotic patients. N=28 patients were included in our study and classified in osteoporotic (N=14) and non-osteoporotic (N=14). miR100 was found to be upregulated in serum, bone and simultaneously in osteoblasts and osteoclasts from the osteoporotic group. Interestingly, the expression of this miRNA in serum showed to be gender independent whereas presented a linear correlation (r=0.94) with bone mineral density. These results support the potential role of miR100 in bone formation and remodelling. Thus, we then hypothesized whether a miR100 inhibition may enhance osteogenesis of osteoporotic osteoblasts. For this, osteoblasts were transfected with antagomiR-100 by means of a non-viral lipid transfection enhancer and their osteogenesis was studied in vitro up to 14 days. *In vitro,* miR100 inhibition did not show any adverse effects on osteoblast viability or proliferation. Over 50% suppression of miR100 expression was obtained in osteoporotic osteoblasts as result of the antagomiR-100 transfection. miR100 inhibition resulted in enhancement of *in vitro* osteogenesis of these cells. Alkaline phosphatase (ALP) activity increased when compared to the non-transfected controls. Interestingly, all investigated osteo-related genes, namely RunX2, Collagen type I and ALP displayed a significant upregulation after miR100 inhibition in osteoporotic osteoblasts compared to non-transfected controls. Moreover, in the case of Collagen type I and BMPR2, the expression of these genes was significantly higher even compared to healthy osteoblasts. This upregulation was observed up to 14 days *in vitro*. Finally, matrix mineralization, as assessed by alizarin red staining and quantification, showed enhancement in the antagomiR-100 transfected osteoporotic cells. Altogether, our study indicates that miR100 may play a negative role in osteoblasts osteogenesis possibly acting through targeting BMPR2. Inhibition of miR100 expression may be an interesting approach to enhance osteogenesis in the context of osteoporosis.



0336 BDNF gene delivery vectorized by neuron-targeted nanoparticle is neuroprotective in peripheral nerve injury

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Peripheral neuropathies are common and still lack an effective treatment option. Neuron-targeted gene delivery is a promising strategy to treat peripheral neuropathies. Here we propose the use of polymeric nanoparticles based on thiolated trimethyl chitosan (TMCSH) to mediate targeted gene delivery to peripheral neurons upon a peripheral and minimally invasive intramuscular administration.

Nanoparticles were grafted with the non-toxic carboxylic fragment of the tetanus neurotoxin (HC) to allow neuron targeting and retrograde transport, as confirmed using compartmentalized primary neuron cultures and taking advantage of (quantitative) bioimaging tools.

Subsequently, we explored the delivery of a plasmid DNA encoding for the brain-derived neurotrophic factor (BDNF) in a peripheral nerve injury model. The TMCSH-HC/BDNF nanoparticle treatment promoted the release and significant expression of BDNF in neural tissues, which resulted in an enhanced functional recovery after injury as compared to control treatments (vehicle and non-targeted nanoparticles), associated with an improvement in key pro-regenerative events, namely, the increased expression of neurofilament and growth-associated protein GAP-43 in the injured nerves. Moreover, the targeted nanoparticle treatment was correlated with a significantly higher density of myelinated axons in the distal stump of injured nerves, as well as with preservation of unmyelinated axon density as compared with controls and a protective role in injury-denervated muscles, preventing them from denervation.

These results highlight the potential of TMCSH-HC nanoparticles as non-viral gene carriers to deliver therapeutic genes into the peripheral neurons and thus, pave the way for their use as an effective therapeutic intervention for peripheral neuropathies.

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0337 Spatial deposition of gene activated bioinks for osteochondral tissue engineering

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Engineering complex interface tissues such as the osteochondral unit requires precise spatial control of cellular differentiation and matrix deposition and organization. Additive fabrication techniques such as 3D bioprinting can be used to spatially deposit different materials (bioinks) and bioactive molecules. The incorporation of gene delivery into a bioink offers a unique approach for the controlled release of therapeutic factors in a spatially and temporally defined manner in order to control the differentiation of encapsulated mesenchymal stem cells (MSCs).

The objective of this study was to develop a chondro-inductive and an osteo-inductive gene activated bioink (GAB) for the 3D printing of an osteochondral construct.

For this purpose, two different MSC-laden GABs were developed: (A) a chondro-inductive GAB consisting of the incorporation of MSCs and RALA peptide-pDNA complexes into unmodified alginate, and (B) an osteo-inductive GAB consisting of the incorporation of MSCs and nanohydroxyapatite (nHA)-pDNA complexes into a RGD-modified alginate hydrogel. Green fluorescent protein (GFP) (Fig.1.A) and luciferase analysis showed effective transfection over time of the encapsulated MSCs. Co-printing of GFP and red fluorescent protein (RFP) transfected cells showed spatial distribution of the populations inside the construct (Fig.1.B). Upon therapeutic gene-activation of the chondro-inductive (pTGF-β3 and pBMP2) and osteo-inductive (pBMP2) bioinks, bi-layered hydrogels were printed and cultured *in vitro* for 21 days. Assessment of calcium and sulphated glycosaminoglycan (sGAG) content showed a significant increase in mineralization in the osteo layer and a significant increase of GAG production in the chondro layer (Fig.1.C).

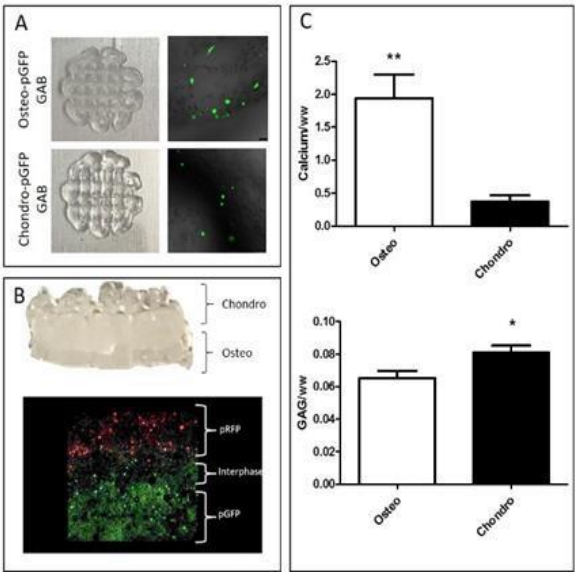

Figure 1. (A) macroscopic images of both 3D printed GABs and GFP positive cells after 3 days of printing. (B) macroscopic appearance of the 3D printed osteochondral construct and spatial distribution of RFP and GFP positive cells in the bilayer. (C) Calcium/ww and GAG/ww content in the osteo-inductive and chondro-inductive layer of the osteochondral construct after 21 days of *in vitro* culture.

This study highlights the potential of the 3D bioprinting of gene-activated materials for the spatial delivery of genes for the local production of growth factors, and for the recapitulation of the biochemical and phenotypical gradients found in native tissues.

0338 GMP for Beginners - Wasted time or new impulses for my scientific career?

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Research in tissue engineering and regenerative medicine is tiddly coupled with the whish and the aim to bring these approaches into new clinical application and ultimately to the patient. Therefore, the topic “usability” in your research grant application is often fulfilled with convincing arguments. However, in real life, the number of advanced therapies involving new implantable or injectable medicinal products or medicinal devices is very low and doesn’t reach the hand of the implanting surgeon nor the patient.

One reason for this discrepancy is that the regulatory requirements on Good Manufacturing Practice (GMP) for these products often represent an insurmountable hurdle in an academic environment. However, it is precisely here where an early addressing of this issue is paying off.

Therefore, this session will introduce you in the way of thinking of Local and National Authorities when they are going to evaluate your process and your product for the intended use in human. This knowledge can help you avoiding major undesirable problems at a very early time point of your experimental strategy. Furthermore, a smart GMP behaviour definitely enhances the quality of your results and your scientific work.