a91677

39-O-1 Direct Reprogramming of Human Dermal Fibroblasts into Osteoblasts using Cell Penetrating Peptide, 30Kc19

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Despite the countless hours and efforts put into drug production, most of them are rendered useless because they are either cytotoxic or unable to sustain their structure *in vivo* and, thus, functions. As a result, different approaches have been taken to overcome these obstacles, one of which is cell penetrating peptide (CPP).

30Kc19 is a type of CPP that has also been noted for its cargo stabilizing abilities. Because it is derived from silk worm (*Bombyx mori*) and induces far less stress than other transfection methods, such as electroporation, it is regarded non-cytotoxic and safe. The matter at hand, like most CPPs, has been its efficacy. However, when it is attached to each of the three Yamanaka factors – Oct 3/4, Sox2, L-myc - via recombinant technology and mass-produced using BL21 *Escherichia coli*, 30Kc19 is able to induce pluripotent stem cells from Human Dermal Fibroblast (HDF). In this study, one of the two key osteogenic transcription factors, Osterix (Osx), is attached to 30Kc19 to promote direct conversion of Human Umbilical Vein Endothelial Cells (HUVEC) and HDF into osteoblasts. Although the other osteogenic transcription factor, Runx2, is considered the master regulator, it requires extensive post-translational modification that *E. coli* has difficulty emulating. Osx, on the other hand, does not have this problem, while having as much influence in osteogenesis – specifically osteoblast maturation, bone calcification, and bone homeostasis. As a result, when cells treated with Osx-30Kc19 are analyzed, calcification and up-regulation of osteoblast-specific genes are observed, which implies that transdifferentiation occurred.

a90082

39-O-2 Application of MRI for Localization of Neocartilage on Human Auricular Chondrocyte-seeded Polymeric Scaffolds

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Introduction

Tissue engineering a human auricle (ear) as an alternative approach to current surgical procedures to address auricular loss or repair is a promising advance in regenerative medicine. The aim of this study was to utilize magnetic resonance imaging (MRI) and histology for identification and localization of neocartilage in implanted tissue-engineered human auricular chondrocyte-seeded ear scaffolds.

Methods

Three-dimensional (3D) printed human ear-shaped polycaprolactone (KLS Martin GmbH, Germany)/Neoveil nano (D15, Gunze, Ltd., Japan) scaffolds (N = 3) were seeded with ~10^8 human auricular chondrocytes initially obtained at surgery and then expanded in culture. Each seeded scaffold was transversely bisected into helix/concha and tragus/lobule regions and implanted subcutaneously in the dorsum of athymic mice for 10 weeks. Chondrocyte/scaffold constructs harvested at that time were fixed in 10% neutral buffered formalin for 7 days prior to MRI scanning. The helix/concha portions of the constructs were immersed in fluorinert (FC-40; 3M, Saint Paul, MN) and placed in a custom-built chamber to provide enhanced contrast during MR scanning. Specimens were imaged with an ICON 1 Tesla small animal MRI (Bruker, Billerica, MA) using standard acquisition protocols (T1, T2 relaxation times). MR images were analyzed using ImageJ and compared with histological sections of the same specimens treated by Safranin-O and Verhoeff staining for cartilage proteoglycans and elastin, respectively.

Results

Correlated cross-sectional MRI as well as Safranin-O and Verhoeff staining showed neocartilage formation in all specimens after 10 weeks of implantation. From histological sections and 3D-rendered and projected MR images of helix/concha portions of the three chondrocyte/scaffold constructs, the volumetric percentage of neocartilage to total helix/concha volume was calculated to be 32.4±9.8%.

Conclusions

A methodology has been developed for utilization of MRI to identify and localize neocartilage on human chondrocyte-seeded 3D-printed ear scaffolds. MRI has also been applied uniquely to calculate volume percentage of neocartilage in such scaffolds. From the data obtained in the present study, MRI techniques may be suggested as capable of tracing non-invasively and quantitatively assessing the progressive formation of cartilage in vivo, including that of auricular neocartilage on polymeric scaffolds for tissue engineering purposes.

a90633

39-O-3 Engineering the chondrocytes derived from cartilaginous graft by platelet-rich fibrin scaffolds for cartilage regeneration

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Cartilage injury is a common problem of clinical practice because cartilage is important for many anatomical structures within human body. Cartilage is an avascular tissue with limited capacity of self-repair, these chondral lesions are prone to continuously progress and result in osteoarthritis. To prevent further degeneration of articular surface, many treatments have been developed to promote cartilage healing. The scaffolds composed of platelet-rich plasma are promising in enhancing the healing process by offering growth factors to nourish cells. It has been applied for recruiting cell populations for cartilage repair process. Platelet-rich fibrin (PRF) scaffold is a second generation of platelet concentrate produced from autologous blood because there is no need of anticoagulant supplement and immediate centrifugation for PRF activation, and accordingly can be used as a fibrin biomaterial containing a high concentration of growth factors. The effects of PRF on cartilage regeneration were evaluated both in vitro and ex vivo by engineering the chondrocytes derived from autologous cartilage graft. It was found that the PRF improved the chemotaxis, proliferation, and viability of the cultured chondrocytes. The gene expression of the chondrogenic markers, including type II collagen and aggrecan, revealed that PRF induced the chondrogenic differentiation of cultured chondrocytes. PRF increased the formation and deposition of the cartilaginous matrix produced by cultured chondrocytes. The efficacy of PRF on cell viability was comparable with that of fetal bovine serum. In animal disease models, morphologic, histological, and objectively quantitative evaluation demonstrated that PRF combined with cartilage granules was feasible in facilitating chondral repair and regeneration. It was concluded that the migration, proliferation, viability, and differentiation of chondrocytes could be enhanced by PRF. The data altogether provide evidence to confirm the feasibility of applying PRF scaffolds to engineer chondrocytes derived from autologous cartilage graft for cartilage repair and regeneration.

a91213

39-O-4 Internalised Phosphatidylserine Liposomes Encourage Osteogenic Differentiation of Human Bone Marrow Stem Cells Through the SMAD Signalling Pathway

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Approximately 5-10% of bone fractures result in impaired healing, which can lead to poor clinical outcomes and additional surgeries being required [1]. Existing treatments have limitations such as high costs, donor site morbidity or immunological rejection [2]. Phosphatidylserine liposomes (PSLs) have shown promise in overcoming these limitations for bone repair applications. Although previous studies have shown PSLs to induce mineralisation [3], the exact cellular mechanism behind this has not been elucidated.

PSLs were cultured with human bone marrow stem cells (hBMSCs) and osteoblast-like (MG63) cells at 0, 10, 100 and 1000 μg/mL in basal and osteogenic media for 3, 9 and 21 days. Cell viability was assessed using MTT and TUNEL assays. Osteogenic differentiation was assessed by qPCR analysis of collagen I, osteocalcin, alkaline phosphatase and RunX2 expression and by alizarin red staining for mineralisation. Cell response was investigated through Western Blots for β-catenin, p-p38, pSMAD and an ELISA for TGF-β1 expression after 30, 60 and 120 minutes treatment. Cellular uptake of the liposomes was quantified using a lipophilic fluorescent marker (DiD) combined with flow cytometry.

PSL concentrations of 1000 μg/mL induced apoptosis and caused a significant reduction in cell viability. In both osteogenic and basal conditions, PSLs increased mineralisation. PSLs did not influence osteogenic differentiation of hBMSCs in basal media, however in osteogenic media PSLs upregulated the expression of all osteogenic markers at 9 days (up to 5 fold). This is linked to an increase in TGF-β1 expression, which increased canonical SMAD signalling. Flow cytometry data demonstrated substantial uptake of PS liposomes by the cells (98.9% positive cells) at 100 μg/mL.

Internalisation of PSLs induced an increase in TGF-β1 expression, canonical SMAD signalling and ultimately an increase in osteogenic differentiation. These results demonstrate a mechanism of action behind the osteogenic effect of PSLs and highlights its potential for use in bone repair applications.

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a92086

39-O-5 Enhancing cartilage synthesis via GDF2 stimulation for aggregated chondroprogenitor cells loaded in melt electrospun writing microfiber meshes

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Articular cartilage-derived progenitor cells (ACPCs), are a promising cell source for cartilage regeneration, as they can be expanded in culture, achieving clinically relevant numbers without losing differentiation capacity[1]. However, culture protocols for ACPCs are not optimized and are based on media used for differentiation of mesenchymal stromal cells. Also, comparatively little is known of their interaction with 3D scaffolding biomaterials. The aim of this study is to fabricate durable, organized cartilage constructs by i) maximizing cartilage production by ACPCs via an optimized growth factor supplementation, and ii) incorporate ACPCs within 3D meshes of organized microfibers to guide the proliferation and eventual condensation of these cells into a cartilage-like tissue.

Adult ACPCs were cultured in 3D pellets of 250.000 cells for 4 weeks, supplementing the media with 100 ng/ml of growth differentiation factor 2 (GDF2), previously tested for immature ACPCs and suggested as potent factor for early chondrogenic differentiation[1]. As a control, pellets were grown with the standard dose of 10 ng/ml of TGFbeta1. To test if an initial boost of GDF2 was sufficient for differentiation, a group was switched from GDF2 to TGFbeta1 after the first week.

GDF2 supplementation significantly improved and hastened cartilage-like matrix deposition. After 1 week in culture, the pellets of ACPCs were 40% larger in size compared to growing in TGFbeta1. Histology showed GAG and collagen II rich matrix (SafraninO and immunohistochemistry). After 4 weeks of culture with GDF2, the ACPCs presented 30x higher GAG production compared to the pellets of ACPCs cultured with TGFbeta1, and 25% higher than the switched group. Next, to promote the formation of a larger cartilage tissue patch, GDF2 grown chondrogenic pellets were combined with polycaprolactone (PCL) meshes, fabricated via melt electrowriting (MEW). Microfibers (20 µm) were printed to form a boxed structure (0-90° laydown, fiber spacing 800 µm). Pellets were individually housed into each printed box, to provide structural stability, mechanical reinforcement[2] and guide the pellet growth and condensation into a larger tissue construct, grown in presence of GDF2. Overall, the combination of an optimized culture condition with the physical support given by the microfibrous scaffold, provides a promising platform for cartilage tissue engineering.

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a90889

39-O-6 Alternative strategies for the treatment of critical size bone defects using naturally occurring growth factors

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For the successful regeneration of large bone defects a vascular network within tissue engineered implants is essential to ensure sufficient nutrient and oxygen supply. In the here proposed project the bone regenerative potential of bioactive factors derived from adipose tissue, platelet rich plasma (PRP) and conditioned medium from hypoxia-treated bone marrow-derived mesenchymal stroma cells (BM-MSCs) were investigated with the aim to develop cost-effective bone substitutes for optimized regeneration of large bone defects.
After approval of the Institutional Review Board adipose tissue was harvested from healthy human donors undergoing reconstructive surgery and adipose tissue extract (ATE) was prepared and pooled. Platelet lysates (PL) were produced by freeze-thaw cycles of PRP and hypoxia-conditioned medium (HCM) was obtained by culturing BM-MSCs for 5 days with 1 % O_2. Beside determination of the total protein content ELISA was performed for selected proteins (angiogenin, VEGF, TGF, bFGF, PDGF, TIMP-1, CXCL1/GROα , and MCP-1) to characterize the growth factor mixtures. Mineralized collagen scaffolds were functionalized with concentrated PL, HCM and ATE (PL: 10fold, HCM: 20fold, ATE: 40fold) and incubated for up to 14 days. Growth factor release was measured by ELISA whereas the chemoattractive potential was tested using a transwell migration assay.
A total protein content of 11.60 ± 0.15 mg/ml in PL, 14.57 ± 5.97 µg/ml in HCM and 4.05 ± 0.08 mg/ml in ATE (mean ± SD) was measured. As determined by angiogenesis protein array HCM and ATE contained high amounts of angiogenin whereas PL contained high amounts of IGFBP-1. High amounts of the cytogenetic protein CCL2/MCP-1 were found in HCM and ATE, but it was not detectable in PL. Growth factor release from PL-/HCM- or ATE-functionalized MC scaffolds was highest during the first 3 days of incubation. Concerning PDGF a delayed release was detectable over a period of 14 days. Analysis of the transwell migration assay revealed a high chemoattractive potential of PL and HCM.
Multiple angiogenic proteins and cytokines were detectable in all 3 tested naturally occurring growth factor mixtures independent of their different total protein content. Via lyophilization growth factor concentrations of more than 10fold are possible which is important for scaffold functionalization. To determine synergistic effects of bioactive growth factors in vivo testing of functionalized scaffolds is pursued.

a92729

39-O-7 Improved Cartilage Formation in a Functionally Graded Scaffold: an Ovine Animal Study

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The treatment of osteochondral (OC) lesions has become a major concern in Orthopaedics because predominantly these defects do not heal spontaneously which make the joints susceptible to "early onset" secondary osteoarthritis [1]. Tissue engineering approaches have emerged for the repair of cartilage defects and damages to the subchondral bones and have shown potential in restoring joint's function. However, tissue engineering scaffolds often fail to satisfactorily regenerate the bone and the native hyaline cartilage and result in the formation of mechanically inferior fibrocartilage [2], affecting the durability of the regenerated tissue. We have developed a functionally graded multi-layered scaffold to address large OC defects, with focus on improving bone ingrowth and cartilage quality. This study investigated the efficacy of this scaffold *in vivo* following implantation in sheep knee.

The scaffold was fabricated using a combination of additive manufacturing and freeze-drying/critical drying techniques. Three layers of Ti matrix, PLA and collagen-PLGA were created to mimic subchondral bone, calcified cartilage and articular cartilage in native tissue, respectively. Multi-layered collagen/hydroxyapatite scaffolds were used as control. Ten sheep were operated on and either the scaffold (n=6) or the control (n=4) was implanted in the left medial condyle. The tissue was retrieved 12 weeks post-operation. Bone ingrowth into the titanium matrix and quality of the cartilage was assessed macroscopically, histologically and with the use of uCT.

It was observed that gross morphological appearance of regenerated cartilage was superior in OC scaffold group compared to the control group. Collagen-II and Safranin-O stainings confirmed formation of a hyaline-like cartilage. The uQCT examination revealed that the BV/TV ratio in the subchondral bone was significantly higher (p=0.01) in the OC scaffold group (~40%) than in the control group (~15%). The bone-scaffold contact analysis revealed the bone-implant contact achieved was 61%. It is believed that the new bone growth into the Ti matrix at bone section provided a stable mechanical fixation and a strong support to the overlying cartilage layer leading to an improved cartilage formation.

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Acknowledgements:
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a92959

39-O-8 Human bone marrow mesenchymal stem cells: Intrinsic sex-linked differences, and markers to predict their bone forming potency *in vivo*

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The success of Bone Marrow Mesenchymal Stem Cell (BMSC) therapy for regenerating bone is hampered by the significant heterogeneity between BMSC donors in terms of their bone forming potency, the cause of which is unknown. The aims of this study were to decipher whether intrinsic sex-linked differences in BMSC exist, and to determine if any in vitro BMSC parameters could predict their bone forming potency in a tissue-engineered in vivo setting.
In this study, BMSC from 12 young, healthy, age-matched human donors were expanded in GMP xeno-free conditions and their in vitro profiles in terms of proliferative capacity, gene expression, cytokine secretion, and osteogenic differentiation potential were measured at passages 0-2. Bone forming potency was assessed in a nude mouse model of bone regeneration using a combination of BMSC and biphasic calcium phosphate biomaterial scaffolds.
BMSC from male donors proliferated at a significantly faster rate compared to those from their female counterparts. As measured by BioPlex cytokine arrays, male BMSC were shown to secrete higher quantities of cytokines (MCP-1,3 and IL-6,8) compared with female BMSC. Similarly, higher osteogenic differentiation was demonstrated by male BMSC compared with female BMSC (higher intra- and extra-cellular ALP expression). No significant difference in the quantity of bone formed *in vivo* was observed between donor MSC sex. However, the quality of bone formed, in terms of the number of osteocytes per bone area, was significantly higher by transplantation of male BMSC. Irrespective of donor sex, significant positive correlations between the quality of bone formed *in vivo* (# osteocytes/mm^2) with 1) cell growth rate, and 2) ALP expression of BMSC, were observed. Furthermore, osteocalcin (both gene expression and cytokine secretion) correlated positively with the quantity of bone formed *in vivo*.
This work represents the first study to compare donor sex or to find a marker of bone formation with BMSC expanded in xeno-free conditions. We demonstrate that the sex of donor BMSC have a marked bearing on the cell functionality. Additionally, we identify important markers that can predict the bone forming capacity of donor BMSC.

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a90109

49-O-1 An in vitro System to Model the Effects of Fibrosis on Liver Development

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Congenital liver disorders (CLD) can culminate in the need for pediatric liver transplant. CLD's often result in a profibrotic environment generated largely by activated hepatic stellate cells (HSC), which produce large amounts of TGF-β and extracellular matrix (ECM) proteins. On the other hand, perturbations in TGF-β signalling can result in ductal plate malformations (DPM) often associated with CLD's. Several 3D cell culture systems are currently available to create liver organoids. In general, these systems display better physiologic and metabolic aspects of intact liver tissue, compared with 2D culture systems. However, none of these reliably mimic human liver development, including parallel formation of hepatocyte and cholangiocyte anatomical structures. We are developing an *in vitro* model capable of recapitulating processes of biliary tubulogenesis. In the 1st model, human fetal liver progenitor cells self-assemble inside acellular liver extracellular matrix (ECM) scaffolds to form 3D liver organoids that recapitulated several aspects of hepato-biliary organogenesis and resulted in concomitant formation of progressively more differentiated hepatocytes and bile duct structures. The duct morphogenesis process was interrupted by inhibiting Notch signaling, attempting to create a liver developmental disease model with a similar phenotype of Alagille syndrome. In the 2nd model, an immortalized liver progenitor cell line, HepaRG, and primary fetal HSCs (pfHSC) or LX-2, an immortalized HSC cell line, were co-cultured in 3D collagen I-based organoids. We used this system to model the effects of the fibrotic environment during development and specifically, if activated HSC-secreted TGF-β could cause DPM. Several cell combinations were tested: pfHSC's or LX2 only, HSC's + HepaRG and LX2 + HepaRG. These combinations were grown on tissue culture plastic dishes and in a Collagen I gel for 7 days. Cells were analyzed for changes in liver developmental pathways and genes using qPCR. Co-cultures of liver progenitors and activated HSC showed increased signs of tissue fibrosis and a variation of gene expression in the liver progenitor cells as compared to progenitors alone. Together, these models provide a unique platform to study mechanisms of hepatic and biliary development and disease modeling for drug development and screening.

a92165

49-O-2 Bioprinted Liver Organoids in a Perfusion Bioreactor as Model to Evaluate Drug-Induced Liver Toxicity

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Current *in vitro* systems do not allow accurate predictions of drug-induced liver injury (DILI), which occurs even post-marketing. The recent development of organoid and biofabrication holds great potential for the development of novel *in vitro* analogs that can reliably model liver functions and predict DILI, aiding drug discovery. Adult hepatic progenitor cells (HPCs) can form self-organizing 3D organoids *in vitro*, which can then differentiate into hepatocytes. However, organoids are pure epithelial structures, and are heterogeneous in size, differentiation potential and functionality. Additionally, to fabricate analogs of relevant sizes, nutrients should be provided through active perfusion, to potentially recapitulate the function of blood vessels. In this study a culture system was developed aiming to capture native liver metabolism by mimicking the multicellular composition of the niche of hepatic progenitor cells (HPCs). To approximate liver complexity, organoids were processed either with or without co-culture with non-parenchymal cells, bioprinted into a perfusion system, and tested as devices to predict DILI.The combination of liver organoids with MSCs and endothelial cells resulted in decreased expression levels of the liver stem cells marker LGR5, and increased expression levels of differentiation markers of hepatocytes (albumin) and cholangiocytes (GGT1) compared to liver organoids only. Cell aggregates remained viable for up to eight days after printing. Additionally, a three-fold increase in ATP levels in culture supernatants was detected for printed constructs subjected to treatment with triton, indicating that the analogs can be suitable for testing acute liver toxicity. Next, a bioreactor was fabricated to allow co-printing of poloxamer and gelatin methacryloyl-bioinks with liver organoids into the perfusion chamber of the device, while preserving cell viability and functionality of the liver analogs. Our results indicate that perfusable 3D biofabricated constructs in a bioreactor system recapitulate salient features of native liver tissue. Importantly, the choice of tissue specific stem cells and their interaction with non-epithelial cell population is fundamental in steering organoid fate. Combining organoid technology with biofabrication offers unmatched possibilities to create complex, perfusable liver-on-a-chip constructs under highly controllable and reproducible conditions, which can be used for studies predicting DILI.

a92517

49-O-3 Pig Spleen as the site of human hepatocyte regeneration

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We succeeded a novel protocol to establish the pig whose spleen has human origin hepatocytes. The spleen as the engraftment location for human hepatocyte transplantation was examined and chimeric human hepatized spleen was successfully generated by use of human iPS cell derived hepatic progenitors in pigs. The splenic artery and vein of adult minutia pig were cannulated and separated from the systemic circulation, so that temporary extracorporeal circulation could be performed. As a pretreatment to attenuate spleen immune-cell activity, perfusion with mitomycin C was performed, followed by a thorough rinsing. Secondly, human hepatocytes or iPS cell derived hepatic progenitors were transplanted using this route, after which splenic arteriovenous blood flow was resumed under the triple immunosuppressive drug administration. Human serum albumin level was increased in the first week after hepatocyte transplantation, after which, although it slightly decreased, it could be detected for one month after transplantation. The macroscopic findings of the spleen after one month of transplantation revealed tissues that can be considered as the transplanted hepatocytes. Furthermore, in the human albumin immunostaining and HE (hematoxylin and eosin) staining, the cells considered to be hepatocytes were positive for human albumin. In most tissues and organs, blood flows from an artery to a vein through capillaries. However, the liver and spleen do not have capillary vessels, but a looser tissue portion called sinusoid or sinusoidal structure. The spleen can be considered as an excellent location for hepatocyte transplantation and engraftment. It is worthwhile saying that we succeed to reconstruct chimeric hepatized spleen derived from human originated cells in the adult pig.

a92222

49-O-4 Bioengineered human fetal livers: a new tool for the production of hematopoietic progenitor cells

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Despite the advances made in the *ex-vivo* expansion of cord blood-derived hematopoietic stem/progenitor cells (CB-HSPC), challenges remain regarding our ability to generate an appropriate number of cells capable of treating an adolescent or adult patient from a single unit of cord blood[1,2]. During fetal development, the liver is the central site of HSPC expansion and erythropoiesis. Therefore, we examined and studied whether suitable expansion of HSPC could be obtained *in vitro* under physiologic conditions provided by surrogate fetal liver microenvironments.

In order to test this, we produced bioengineered liver constructs made of matrigel[3] where human hepatoblasts, hepatic stroma cells, and endothelial cells were seeded. Once the organoid was formed, we added CB-HSCP with different media formulations for 7 days. The largest expansion of HSPC was observed with a mixture of hematopoietic stem cell and endothelial media. To characterize this further, we produced human embryonic stem cell-derived hepatoblasts (hES-HB)[4] and co-cultured them for 7 days with CB-HSPC. In static conditions, maintenance of the number of burst-forming unit-erythroid (BFU-E) was observed. However, in dynamic conditions with continuous flow of culture medium, a significant large expansion of BFU-E was detected, indicating a recreation of the hepatic microenvironment and its role in erythropoiesis during fetal development.

Hence, bioengineered human fetal livers represent a novel tool for the production of hematopoietic progenitor cells *in vitro*, which could have a great impact in the treatment of hematological diseases and in the study of their intrinsic biology.

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a93217

49-O-5 The paracrine effect of human visceral adipose tissue on primary human hepatic stellate cells in human 3D liver scaffolds

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Background: Emerging data indicate that the progression from simple steatosis (NAFL) to non-alcoholic steatohepatitis (NASH) results from converging pathophysiological events originating in the liver, the adipose tissue, and the gastrointestinal tract. Adipose tissue is recognized as an endocrine organ that secretes adipokines controlling systemic metabolism and energy homeostasis. We previously showed that adipose tissue macrophages are activated in NASH and secrete pro-inflammatory cytokines which play a detrimental role during NAFL/NASH development.
Aim: In this study the paracrine effect of human visceral adipose tissue (VAT) on primary human hepatic stellate cells (hHSC) cultured on 3D human liver scaffolds was explored.
Method: Freshly obtained VAT biopsies derived from obese patients and control patients undergoing bariatric surgery and cholecystectomy, respectively, were used for short-term culture (LEAN, NAFL and NASH patients). Similar-sized VAT explants were cultured in serum-free medium and conditioned medium was collected after 48hrs. Human liver 3D scaffolds were obtained by the decellularization of healthy human liver unsuitable for transplantation. Primary hHSC were seeded for 7 days on 3D scaffolds and exposed to adipose tissue conditioned media (AT-CM) for 2x24hrs. Histology and gene expression was performed.
Results: hHSC homogeneously engrafted in the 3D scaffolds. No significant differences in the expression of pro-fibrogenic and pro-inflammatory genes was found in hHSC exposed to AT-CM derived from LEAN, NAFL and NASH patients and they were similarly upregulated when compared to non-treated cells. Importantly, within the group of samples from NASH patients, the upregulation of ACTA2 and COL1A1 was significantly more evident in samples with evident liver fibrosis. On the other hand, AT-CM derived from diabetic patients induced a significantly higher increase in pro-fibrogenic gene expression in hHSC 3D culture when compared with samples derived from non-diabetic patients, irrespective of the stage of liver fibrosis. HSC-related adipokines expression and characterization of the adipose tissue secretome is ongoing to further explore the mechanism of action on hHSC.
Conclusion: AT-CM-derived from NAFL and NASH patients, marked by different stages of hepatic steatosis, showed a paracrine effect on hHSC. This is the first study exploring the relationship between VAT and hHSCs on human liver 3D scaffold.

a92312

49-O-6 Primary human Hepatic Stellate Cell phenotype is differently regulated by pro-fibrogenic and pro-inflammatory stimuli in cirrhotic and healthy human liver 3D ECM scaffolds

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Background: Hepatic stellate cells (HSCs) are key mediators during liver fibrosis. A common platform to investigate HSCs activation is the usage of classical 2D cultures. However, the ECM microenvironment plays a crucial role in reason of the liver-specific 3D microarchitecture, which favours essential cell-ECM interactions.
Aim: To evaluate a novel in vitro 3D model based on human liver scaffolds in order to combine the advantage of the liver's specific 3D architecture as well as the preserved bioactive cues to achieve a more accurate in vitro phenotype of primary human HSCs.
Method: Human liver 3D scaffolds were obtained by the decellularization of healthy and cirrhotic livers. Scaffolds were repopulated with the hHSC cell line LX2 or with primary human HSCs for 10 days. Cells were exposed to profibrogenic agents: PDGF-BB (1-10ng/mL), TGF β1 (2-5ng/mL) for 24hrs or LPS (1ng/mL) for 1-3hrs. In parallel, LX2 cells or primary hHSCs were grown in 2D cultures and treated as above. Histology was performed to compare hHSCs cell phenotype/engraftment in the 3D scaffolds and qRT-PCR was performed.
Results: Significant differences were observed in gene expression of hHSCs cultured on 2D vs 3D healthy scaffolds, especially when exposed to LPS, PDGF-BB and TGF β1. Human HSCs in 3D cultures treated with LPS showed a strong increase in Acta2, CYGB, COL1A1, IL-6 whereas LOX gene expression remained unchanged. TGF β1 induced a significant increase in Acta2, Col1A1 and LOX gene expression, but not CYGB and IL-6. In contrast, PDGF-BB significantly decreased Acta2 in comparison to non-treated hHSCs in 3D cultures. Furthermore, HSC activation markers Acta2 and COL1A1 were significant upregulated in in hHSCs cultured in cirrhotic scaffolds compared to healthy scaffolds. In contrast, LX2 cells on cirrhotic scaffolds showed a downregulation of Acta2, Col1A1, and Col3A1 gene expression compared to LX2 cells on healthy scaffolds, indicating that, when cultured in a natural 3D ECM microenvironment, LX2 cells, a cell line, do not show the same gene expression pattern of primary hHSCs.
Conclusion: This study shows that primary hHSCs have different gene expression patterns when cultured in 3D scaffolds derived from healthy and cirrhotic human livers and in comparison to 2D cell culture. By using human tissue-specific 3D scaffolds, as models of human physiology and pathophysiology, specific and novel drug targets and biomarkers will be identified.

a92144

49-O-7 Glycosaminoglycans (GAGs) in pathological extracellular matrix networks

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The extracellular matrix (ECM) network is extensively altered in pathological tissues. Structural and biochemical remodelling of ECM components result in altered biomechanical tissue characteristics. GAGs are essential carbohydrate-rich non-fibrillar components of the ECM. The impact of GAGs on tissue biomechanics is not well understood. In this study, collagen fiber networks that are rich in GAGs were investigated using immunofluorescence staining, atomic force microscopy (AFM) and Raman microscopy. Histochemical and immunofluorescence staining was performed to identify GAGs in cryo-preserved sections of normal colon tissues and colon cancer tissues. AFM nanoindentation and Raman microscopy was employed on ECM-rich sites to assess biomechanical and biomolecular data from control and cancer tissues. Immunofluorescence staining revealed significantly increased levels of GAGs within the collagenous network of cancer tissues compared to control tissues. Notably, the pathological ECM was stiffer compared to the respective controls. Raman spectra of the ECM confirmed increased signals from carbohydrates within the collagenous network. Our results indicate a significant impact of GAGs on the biomechanics of the ECM network. The label-free detection of GAGs using Raman microscopy offers new possibilities for the diagnosis and monitoring and of diseased tissues in vitro.

a93445

49-O-8 Crohn's disease patient-derived small intestinal organoids reveal disease-status related modification of stem cell properties

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Intestinal stem cells (ISCs) play indispensable roles in the maintenance of the intestinal epithelium, and their dysfunctions can deeply relate to the pathophysiology of gastrointestinal diseases including Crohn's disease (CD). Studies have shown that patient-derived intestinal organoids can maintain its disease-related cell properties in vitro and serve as a useful tool to identify the underlying dysfunctions of key cell populations. In this study, we performed a single-cell level analysis of the organoids that were established from CD patients, to reveal the possible modification of their ISC properties. In our current analysis, CD patient-derived small intestinal organoids were established from enteroscopic biopsy specimens taken from active lesions (aCD-SIO), or from mucosa under remission (rCD-SIO) by following the method described by Sato T. et al (Gastroenterology, 2011). Expressions of ISC-marker genes in those organoids were examined by immunohistochemistry, and also quantified by microfluid-based single-cell multiplex gene expression analysis. For the evaluation of ISC-specific function, organoid cells were subjected to a single-cell organoid reformation assay under the assistance of our newly developed 3D-scanner system. Analysis of patient-derived tissues revealed that ISC-marker genes, OLFM4 and SLC12A2, were expressed by an increased number of small intestinal epithelial cells in the active lesion of CD. aCD-SIOs, rCD-SIOs or those of non-IBD controls (NI-SIOs) showed a comparable level of OLFM4 and SLC12A2 expression in all organoids. By our current method, single-cell gene expression data of 12 ISC-markers were acquired from a total of 1215 organoid cells. t-distributed stochastic neighbor embedding (tSNE) analysis identified clusters of candidate ISCs, and also revealed a distinct expression pattern of SMOC2 and LGR5 in candidate ISCs cells of aCD-SIOs. In addition, 3D-scanner assisted single-cell organoid reformation assays showed significantly higher reformation efficiency of aCD-SIO-derived cells, compared to that of NI-SIO-derived cells. In conclusion, small intestinal organoids established from active lesions of CD maintain potential ISCs with modified marker expression profiles, and also with high organoid reformation ability. Our results indicate that unidentified factors in the inflammatory environment may modify small intestinal stem cell properties and thereby contribute to the pathophysiology of CD.

g00002

49-O-9 Microchip-based spheroid formation enhances differentiation function and engraftment of insulin-producing cells derived from human liver cells for diabetes treatment

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The shortage of donor and the low engraftment efficacy are the main limitations of islet transplantation. A number of studies have been reported to make insulin-producing cells (IPCs) from stem cells. In this study, the following techniques were used to improve the differentiation efficiency of IPCs: 1) Use of liver cells sharing a common developmental origin with pancreatic cells and free from safety risk of stem cells; 2) Direct trans-differentiation using adenovirus vectors carrying the human PDX-1, NeuoD and MafA genes, all of which are important transcription factors in the pancreatic developmental stage; and 3) Enhancement of differentiation efficacy using 3-dimensional (3D) spheroid formation based on microchip technology.

3D IPCs spheroids were prepared using concave microwell. It was confirmed that the size of the spheroid was increased ($R^2 = 0.998$) in proportion to the number of cells. The diameter of the spheroid seeded with 10^6 cells was 175.8 $\pm$ 18.0 μm, which it the desired size of isolated islets. Gene expression of transcription factors related with beta cell differentiation (PDX1, NeuroD, MafA, NKX6.1, NGN3, and FOXA2), and pancreatic endocrine hormone (insulin, glucagon, somatostatin) were evaluated. Compared with 2D culture, insulin gene expression was increased more than three-fold in 3D spheroid and glucose was decreased ten-fold. Insulin production confirmed by immunohistochemistry and ELISA, and insulin was increased in IPC spheroids. IPCs and IPC spheroid with $2x10^6$ cells were transplanted into the kidney capsule in the diabetic nude mouse. Blood glucose level of IPC spheroid group was decreased to 200 mg/dl after transplantation, but it gradually increased during 4 weeks. However, in the mouse with single IPC transplants, blood glucose showed the tendency of an increase for 4 weeks without any decrease of blood glucose. At the 3rd and the 14th days after transplantation of IPCs, the kidney was harvested to confirm the transplanted cells by immunohistochemistry. PDX1 expressing cells were stained to confirm that the transplanted cells survived after 3 and 14 days of transplantation, and it was confirmed that some of these cells produced insulin. With such results, that mass production of 3-dimensional spheroids with a designed shape and size of IPCs using micro-concave well was confirmed. Moreover, 3D spheroid formation enhanced differentiation efficacy of IPCs in vitro and the function of glucose control in vivo.

a90590

59-O-1 How Do Stem Cells Find Their Niche?

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Stem cells reside in specialised cellular niches that regulate their function. Wnt ligands are niche signals that are essential for the maintenance of many types of stem cells. Localised Wnt signals can induce self renewal by orienting asymmetric cell division to produce one stem cell and another linage restricted daughter cell. Large-scale quantitative analysis of single embryonic stem cells reveals a mechanism employed by stem cells to recognise and actively recruit directional Wnt signals that drives asymmetric cell division.

We found that the stem cells detect localised Wnt proteins by actin-enriched and beta-catenin dependent nanoprotrusions. To characterise further the interaction of the cells and localised Wnt, we apply the theory of simple liquids bridging the gap between living and dead matter. We quantify the biophysics and the dynamics of this interaction in terms of effective potentials and pair correlation functions. Our findings show a positive correlation between the cellular levels of beta-catenin and the affinity to the localised Wnt. Additionally, beta-catenin levels influence the location and duration of the interaction between localised Wnt and the plasma membrane. Finally we demonstrate that in the presence of localized Wnt signals, beta-catenin is dispensable for polarizing the receptor lrp6 but essential for polarizing the down stream effector APC, spindle orientation and consequently asymmetric stem cell division. This system provides a novel mechanism of the Wnt-niche detection and response and can be extended to other stem cell types and niche signals.

a90490

59-O-2 Acidity in the heart: how pH affects iPSC-derived cardiomyocytes and strategies to harness it for therapeutic intervention

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Background and aims. After myocardial infarction the cardiac tissue acidifies, with the pH dropping to 6.5-6.8 due to a switch from aerobic metabolism to anaerobic glycolysis. This acidic environment can be harnessed for designing biomaterials aimed at delivering pro-survival factors and/or therapeutic cells, such as human Induced Pluripotent Stem Cell (hiPSC)-derived cardiomyocytes (CMs). The goal of this study was two-fold; first, we sought to investigate the effect of acidic pH on differentiation of hiPSCs into CMs and on terminally differentiated hiPSCs-CMs in order to assess effects of the ischemic environment on the cells, and to determine if the addition of a pro-survival factor can protect the cells in an acidic environment. Next, we aimed to develop a pH-responsive and injectable hydrogel consisting of a peptide-coupled copolymer designed to form a viscous solution at room temperature and physiological pH, and converting to a physically crosslinked gel at 37° C and physiological pH due to formation of physical crosslinks via hydrophobic interactions. Additionally, pH-induced cargo release should occur at 37° C and pH 6.8 due to protonation of the polyhistidine peptide.
Results. Results showed that an acidic culture pH of 6.8 (compared to the control pH of 7.4) significantly decreased cardiac differentiation of hiPSCs in terms of viability, metabolic activity, cardiomyocyte yield, and cardiac-specific gene expression, which was similarly observed in terminally-differentiated hiPSC-CMs. Moreover, we demonstrated that administering the pro-survival factor Insulin-like Growth Factor 1 (IGF-1) to the culture medium rescued the low pH phenotype back to the values observed for the control culture condition. In parallel, a thermo-responsive triblock copolymer formed of polycaprolactone - poly(ethylene glycol) – polycaprolactone was synthesized and characterized by rheometry, confirming gelation at 37° C. The terminal caprolactones were end-functionalized to enable covalent coupling to polyhistidine peptides, resulting in a 5-block copolymer. The pH-responsiveness of the peptide at pH 6.5-6.8 was verified by UV-vis.
Future directions. The next steps include assessing the encapsulation and delivery efficiency of IGF-1 and hiPSC-CMs within the gel. Ultimately, this hydrogel will be used as a platform for delivery of growth factors and/or cardiomyocytes in the infarcted area, which will be assessed in a mouse model of myocardial infarction.

a91199

59-O-3 Quantitative Assessment of Pluripotent Stem Cells by an Improved RM-DIC Imaging System

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Pluripotent stem cells (PSCs) are known to have various degrees of pluripotency due to the differences in derivation methods and maintenance conditions. This variability of pluripotency necessitates isolation of PSCs with high pluripotency for their application to regenerative medicine, but the quality control processes are costly and time-consuming. Therefore, development of inexpensive and less laborious selection methods is essential for translation of PSCs into clinical applications.
Here, we developed an imaging system, termed Live-cell Phase Distribution (LiPD) imaging system, which visualizes live cells without staining or labeling based on retardation modulated-differential interference contrast (RM-DIC) imaging system. The LiPD image and its derived PD index were found to reflect the mitochondrial content, enabling quantitative estimation of the degrees of somatic cell reprogramming and PSC differentiation. Moreover, the PD index allowed unbiased grouping of PSC colonies into those with high or low pluripotency without the aid of invasive methods. Finally, three-dimensional images of PSC colonies generated by LiPD imaging provided an aspect ratio as a further criteria to assess pluripotency of PSCs.
Thus, the LiPD imaging system may be utilized for quantitative screening of live PSCs with high pluripotency before more rigorous quality control processes.

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The authors have no conflict of interest.

a91491

59-O-4 Analysis of structural and functional parameters in mesenchymal and induced pluripotent stem cells during differentiation using new methods of high-resolution microscopy and fluorescence imaging

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The effective control of the structurally-functional changes during stem cell differentiation is a big problem because of the complex relationship between different signaling pathways, extracellular microenvironment and metabolic cell requirements. FLIM (Fluorescence lifetime imaging microscopy) and high-resolution microscopy in the combination with exogenous and endogenous markers allow non-invasive and long-term study of these changes underlying the cellular metabolism, pH, viscosity, and cytoskeleton.
In this study cellular metabolism was studied on the basis of the fluorescence lifetimes of NAD(P)H using FLIM method. The intracellular pH level was analyzed using transient transfection of cells with the pH-sensor SypHer-2 and fluorescence microscopy; the plasma membrane viscosity - using the staining of cells with the molecular rotor BODIPY 2 and the FLIM method; the cytoskeleton - using transient transfection of cells with TagRFP and STORM (Stochastic optical reconstruction microscopy).
Based on the data on the fluorescence lifetime contribution of protein-bound NAD(P)H, we registered a metabolic consistent switch from glycolysis to OxPhos to glycolysis in mesenchymal stem cells (MSCs) duing osteogenic differentiation and switch to more glycolytic status in MSCs during chondrogenic differentiation. At both differentiations, a shift of the pH values to the acid side also was observed. The analysis of the fluorescence lifetime of BODIPY2 showed an increase in membrane viscosity in MSCs during chondrogenic differentiation and its decrease in MSCs during osteogenic differentiation. At the cytoskeleton ultrastructure assesment in chondrogenically and osteogenically differentiated cells was found the change in the actin fibers orientation and an increase in their terminal parts when compared with undifferentiated MSCs.
Preliminary data about the structural and functional parameters in induced pluripotent stem cells testified glycolytic metabolism, more acid pH (7,2±0,7) and weakly developed actin cytoskeleton.
So these results expend the general understanding of functional changes in the stem cell differentiation process and open up the new ways for developing treatment strategies in regenerative medicine and stem cell therapy. This work has been financially supported by Russian Science Foundation (grants No. 17-75-20178).

a91093

59-O-5 Visible laser-induced detachment of iPS-derived cardiac cells from Gold Nanoparticle-embedded Collagen Gel

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Cell sorting techniques have recently attracted much attention in the field of cell biology with the development of regenerative medicine. Here, we report that iPS-derived cardiac cells are cultured on a visible light-responsive cell scaffold, and the beating iPS-derived cardiac cells are selectively detached by photoirradiation without any cytotoxicity.
The 50 nm gold nanoparticles (AuNP) were prepared by the seeded growth method, using hydroquinone, as described previously[1]. AuNP were embedded in collagen gels (AuCols) , and AuCols were used for coating on a 96-well plate and incubated at 37°C for 30 min. The iPS-derived cardiac cells[2] were cultured on AuCols for 4 days. A target cell, beating cardiomyocytes, was detected on an inverted microscope equipped with a laser irradiation system. The target cell was irradiated by a laser (561 nm, 3 mW, 10 sec) and aspirated using a micromanipulator system with a 60 μm glass capillary. The detached cell was recultured on another 96-well plate for 4 days and beated again. We succeeded in selective less damaged detachment of beating cardiomyocytes cultured on AuCols, in principle 100% selectivity by this method To collect enough amount of cardiomyocytes for cardiac cell sheet tissues, we are developing a high speed sorting technique using but the fluidic system instead of micro-manipulator system.
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a91506

59-O-6 Towards Building a Patient-Specific Endothelium for Patients with Peripheral Artery Disease

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Small diameter prosthetic vascular grafts to bypass occluded arteries for patients with peripheral artery disease (PAD) are associated with low patency rates, compared with autologous vein grafts. Tissue-engineered vascular grafts with patient-specific, autologous endothelial lining may provide a solution to the limitations associated with synthetic vascular grafts. In this study, we established patient-specific endothelium from 4 patients diagnosed with PAD (age 68-80, 2 males, 2 females), using induced pluripotent stem cell (iPSC) technology. We show that iPSCs can be successfully generated from all patients by reprogramming their peripheral blood mononuclear cells with integration-free Sendai viruses encoding Yamanaka factors, under feeder-free and xeno-free conditions. Patient-specific iPSCs have the hallmarks of pluripotency, exhibit normal karyotype and can be differentiated into vascular endothelial cells (ECs), also under feeder-free and xeno-free conditions. After purification with fluorescence-activated cell sorting, patient iPSC-ECs demonstrate typical endothelial cobblestone morphology, express EC markers (PECAM-1, VE-Cadherin, vWF, and Flk-1), and exhibit endothelial functions, including tubulogenesis, anti-platelet adhesion, and nitric oxide production. However, compared with ECs from healthy subjects, patient iPSC-ECs exhibit partially impaired vascular endothelial functions, such as disorganized tube formation and increased platelet adhesion, especially when the patients are presented with diabetes. Seeding of patient-specific iPSC-ECs onto clinically used expanded poly(tetrafluoroethylene) vascular grafts coated with poly(1,8-octamethylene citrate) generates a patient-specific neo-endothelium in the lumen. Collectively, these results are a step toward future clinical translation of an autologous EC-seeded vascular graft, as well as future therapeutic development using patient iPSCs for disease modeling.

a91964

59-O-7 Spatio-temporally patterned neuroectoderm tissues recapitulate early neural morphogenesis and pathogenesis

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Human pluripotent stem cells (hPSCs) have the remarkable differentiation and self-organization ability to mimic early embryonic tissue development. However, hPSC tissue patterning is often spontaneous and uncontrollable in conventional spheroid or monolayer cultures, which limits their translation into standardized experimental models for developmental diseases. To direct tissue patterning *in vitro,* it is imperative to apply the spatio-temporal principles underlying embryonic development *in vivo* that facilitate a controlled tissue patterning. Recent advances using stem cell micropatterning techniques have provided a spatial control over hPSC differentiation fates, however, there aren't any reports on the formation of a spatially organized hPSC structure that can be related to specific developmental structures. Here, we demonstrate the directed formation of a spatially patterned 3D neuroectoderm (NE) tissues from hPSCs. The NE is an important embryonic developmental structure that undergoes differentiation and morphogenesis to form a neural tube, which constitute the rudimentary central nervous system. We combined cell micropatterning and a temporally-sequenced induction protocol to achieve spatio-temporal control over hPSC differentiation and morphogenesis. The micropatterned PSCs were induced to undergo a mesoendoderm pre-patterning step before NE induction to mimic the temporal embryonic developmental events. Strikingly, this method resulted in the formation of a structurally organized and reproducible 3D multi-cellular structure, which exhibited a stratified organization of a polarized NE tissue. The 3D NE tissue structure displayed spatial layering of the all three germ layers with exterior $SOX17^{+}$ endoderm, middle layer of Brachyury $^{+}$ mesoendoderm and innermost $SOX2^{+}$ neuroectoderm layer of cells, recapitulating early embryonic patterning in 3D. Importantly, the 3D NE structure was observed to recapitulate morphogenic characteristics of early neural tube folding, such as apical constriction and E-to-N-cadherin switching. The consistency at which we can generate spatially ordered organization of the different germ layers in this model provided an unprecedented opportunity to shed insights into how mesoendoderm orchestrates NE morphogenesis via TGFb signaling. Finally, we demonstrate practical translation of this micropatterned NE model to phenotypically model the effects of drug or genetically-induced (e.g. Fragile X syndrome) neuropathogenesis *in vitro*.

a92744

59-O-8 Embryonic stem cell-derived hapatocyte-based therapy to patients with hyperammonemia

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Embryonic stem cells (ESCs) are another good raw material for cell products. I aim to manufacture an investigative new drug from ESCs to treat patients with hyperammonemia. Hyperammonemia is a metabolic disturbance characterized by an excess of ammonia in the blood, and is a dangerous condition that leads to mental retardation. Primary hyperammonemia is caused by inborn errors of metabolism such as ornithine transcarbamylase (OTC) and carbamoyl phosphate synthetase I in the urea cycle. OTC deficiency (OTCD) is an X-chromosome-linked disorder and is severe in hemizygous boys. Hemizygous boys often develop this disease during the neonatal period, and the patient often dies during this period. Living donor liver transplantation has been indicated for neonatal-onset type OTCD, and outcomes are favorable. However, neurological impairment associated with hyperammnonemic episode of OTCD often occurs before liver transplantation that is usually performed at an age of 5 months. For bridging to liver transplantation, I aim to perform embryonic stem cell-based therapy to a patient with OTCD to prevent hyperammonemia. To this end, we prepared hepatocytes derived from human ESCs as an investigational new drug. Sequencing analysis revealed that the ESCs as a raw material have intact the urea cycle-associated enzymes. The products expressed enzymes and exhibited metabolic activity of ammonia in vitro. To perform proof of concept (POC) studies in a disease model, we generated immunodeficient mice with OTCD, which can receive human embryonic stem cell-derived products. In this study, I introduce results of our preclinical POC data and non-clinical pharmacology and toxicology.

a93131

59-O-9 Equine stem cell therapy for musculoskeletal injuries

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Stem cells have gained attention not only in human but also in veterinary biomedical research due to their potential as therapeutic agents for musculoskeletal injuries and/or disorders. MSCs, ESCs, and iPSCs have been actively studied as a source for stem cell therapy in horses. We, previously, established equine induced pluripotent stem cells (iPSCs) from equine Adipose-derived Stem Cells (ASC) using a Lentiviral vector encoding four transcription factors, Oct4, Sox2, Klf4, and c-Myc. With the established E-iPSC, we tried to differentiate it into MSCs by serial passaging using MSC differentiation media. Differentiation of MSC was confirmed by RT-PCR and FACS analysis with CD44 and CD29 which are MSC surface markers. Subsequently, stem cell therapies for the injured horses were applied through the injection of e-MSC, e-iPSCs, and e-iPSC-MSCs respectively expecting to improve tissue regeneration and the condition of the damaged parts was observed by radiography. In addition, TGF-beta2 for immunecompromise was treated while culturing to reduce the expression level of MHC class Ⅰ and class Ⅱ which are related to immune rejection. Through qPCR, it was confirmed that the expression level of MHC class Ⅰ and class Ⅱ was downregulated by TGF-beta 2 treatment. As a result, overall effect of the equine stem cell transplantations including e-MSCs, e-iPSCs, and e-iPSC-MSCs has improved the musculoskeletal injuries.

a92986

99-O-1 Direct cell reprogramming as a new emerging strategy in cardiac regeneration

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Myocardial infarction (MI) is the current leading cause of mortality in the industrialised world. It is due to the irreversible death of billions of cardiomyocytes, secondary to a condition of ischemia. This leads to the formation of a stiff fibrotic tissue, mainly populated by cardiac fibroblasts (CFs). Currently, the only available therapy addressing the irreversible loss of functional cardiomyocytes is heart transplantation. Different tissue engineering approaches and cell therapies are under investigation, aimed at recovering myocardial contractility. Main issues in these strategies are the poor grafting and survival ability of implanted cells as well as the limited endogenous regenerative potential of adult heart.

A new strategy is now emerging based on direct reprogramming of CFs into induced cardiomyocytes (iCMs) using transcriptional factors and/or microRNAs (miRNAs) (miR-combo) [2-4]. Proof of concepts results of *in vitro* and *in vivo* conversion of mouse CFs into iCMs have been published and *in vitro* direct reprogramming of human CFs has also been reported [1-3]. However, such strategy is still an immature approach: reprogramming efficiency is low and partially reprogrammed non-beating cardiomyocytes have been generally obtained. Recently, *in vitro* direct reprogramming efficiency of mouse CFs cultured in 3D fibrin hydrogels using miR-combo has resulted significantly increased compared to 2D culture systems [4].

Based on these preliminary results, in this work we studied the miR-combo mediated reprogramming efficiency of human dermal and cardiac fibroblasts cultured on hydrogel matrices, including fibrin, fibrin/laminin, fibrin/fibronectin and fibrin/cardiac biomatrix [5], by analysing cell morphology, cell viability, change in gene expression (PCR analysis) and presence of markers of trans-differentiation by immunohistochemistry.

The 3D biomimetic hydrogels were able to increase reprogramming efficiency respect to 2D culture environment, both at a genetic and protein level, with an enhancement in the expression of cardiac genes and cardiac proteins such as cardiac troponin I and alpha sarcomeric actinin.

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a91384

99-O-2 Human cardiac derived cells (CardAP cells) for the treatment of chronic myocardial diseases – from individualized autologous to universal allogeneic cell-based therapies

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Cell therapy represents a promising treatment option for myocardial diseases. We identified a specific cell type in human endomyocardial biopsies (EMB): cardiac-derived adherent proliferating cells (CardAPs) with distinct cardioprotective features. However, as individualized autologous therapies are rather difficult and costly, universally applicable off-the-shelf CardAPs batches would be a preferred alternative. Since the amount of CardAPs is limited due to the small size of EMB, we introduced the atrial appendages (AA) as a promising cell source for allogeneic cell therapies. We demonstrated by immune cell co-cultures that EMB- and AA-CardAPs have similar low immunogenic and immunomodulatory properties.

The aim of this study was to evaluate and confirm the cardioprotective properties of AA-CardAPs in vitro and in vivo.

In vitro, AA-CardAPs showed pro-angiogenic potential indicated by high secretion levels of VEGF and IL-8. VEGF values amounted to 47.14 (min) and 385.54 (max) pg/10^5 cells. IL-8 secretion was measured as 37.64 (min) and 169.90 (max) ng/10^5 cells. Furthermore, AA-CardAPs exhibit improved properties of vascular structures. In matrigel-based tube formation assays, human umbilical vein endothelial cells (HUVECs) treated with conditioned medium (CM) of AA-CardAPs had a mean number of branching points of 52.8 ± 13.4, which was significantly increased in comparison to the mean of the negative control of 22.1 ± 6.3. Also we observed a significantly elevated mean total branching length of CM-treated HUVECs of 9225 ± 1941 in comparison to the negative control of 4572 ± 1608.

In an in vivo model of Coxsackievirus B3 (CVB3)-induced myocarditis in C57BL/6J mice an intravenous application of AA-CardAP cells led to an improved left ventricular function shown by a 1.4-fold ($p<0.0005$) increase of the contractility parameter dP/dt_{max}, and a 1.5-fold ($p<0.0005$) improvement of dP/dt_{min}. Also, AA-CardAPs decreased cardiac collagen I expression. Moreover, the number of splenic TGF-ß-expressing CD68 cells in CVB3 mice was reduced by 2.6-fold ($p<0.0001$) as well as the percentage of T regulatory cells, defined as CD4CD25FoxP3 cells, was reduced to the level of control mice; a finding which was also seen for CD4-IL10- and CD8-IL10-expressing cells.

Our findings indicate that AA-CardAPs have comparable properties to EMB-CardAPs and represent a potential scalable and cost-efficient cell source towards a future off-the-shelf myocardial cell therapy.

a92641

99-O-3 A novel cell alignment method of human induced pluripotent stem cell-derived cardiomyocyte by cell sheet extension

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Introduction : The native cardiomyocytes are highly aligned and those contraction force is well organized. On the other hand, human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) cultured in a well are randomly aligned, and those contraction force distributes in various direction. So, hiPS-CMs application in transplantation, drug screening and disease model are limited. In this study, we developed a novel cell alignment method in which hiPS-CM sheet extend in one direction by pulling, without scaffolds including nanofiber. Method: A Square hiPS-CM sheet is harvested from a temperature-responsive culture dish. The square cell sheet was put on an adhesive silicon which is part of a cell sheet extension device, then, the silicon was pulled and extended, with which the cell sheet also extended along. Next, the extended cell sheet was transplanted on the subcutaneous layer, to verify the extended hiPS-CM sheet was aligned. Results: Immediately after extension, the sheet was extended and thin more than non-extended cell sheet (aspect ratio: 1.72±0.03 vs. 1.08±0.04; P<0.05, thickness of cell sheet; 30.6± 2.8μm vs. 47.7±0.9μm; P<0.05), and in immuno-histological analysis, cardiomyocyte in the extended cell sheet had unidirectionality, though cardiomyocytes in the non-extended cell sheet had random directionality. In vivo experience, one month after transplantation to nude rat, a direction of electrical propagation in the extended cell sheet was unidirectionality which was same with extended direction in the calcium ion transient imaging. In the immuno-histological analysis, one week after transplantation, myocardial fibers in the extended cell sheet were aligned unidirectionality, though cardiomyocytes in the non-extended cell sheet were randomly aligned. These results indicated cardiomyocyte directionality before transplantation in the extended cell sheet was preserved and the extended hiPS-CM sheet was matured as an alignment cardiac tissue. Conclusion: The cell alignment method using the cell sheet extension device is a promising method to fabricate the mature hiPS-CM tissue controlled cardiomyocyte alignment.

a92154

99-O-4 Influence of fiber orientation and fibronectin coating on cardiomyocyte organization in electrospun Polyactive scaffolds

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Introduction

Cardiovascular diseases affect a vast majority of the world's population and commonly results in the irreversible loss of myocardial tissue. Developing a regenerative model for myocardium may provide alternative treatment methods. Scaffolds with aligned fibers may enable guided orientation of cardiomyocytes *in vitro,* which can be useful to develop mature cardiac tissue. This study aims to understand the effect of fiber alignment on cell organization *in vitro*. For this, we tested the potential of Polyactive, a class of biocompatible polymers that has never been used for cardiac regeneration.

Materials and Methods

Electrospun fibrous scaffolds with different degrees of alignment were fabricated using 25% Polyactive (300PEOT55PBT45) dissolved in a solvent mixture comprised of chloroform:hexafluoro-2-propanol (7:3). Scaffolds were coated with fibronectin diluted to 0.5% v/v in 0.02% w/v gelatin solution. Immortalized mouse HL-1 cardiomyocytes were seeded onto the scaffolds for 16 days. Cells cultured on plastic culture plates were used as controls. Cell proliferation was determined by metabolic activity and DNA assay. Live/dead imaging was used to assess cell viability over several time-points. Immunofluorescence staining was performed for several markers (SERCA2, actin and sarcomeric α-actinin).

Results

Scaffold alignment was achieved by electrospinning scaffolds on a rotating mandrel of varying rpm (150rpm, 3000rpm and 5500rpm). Scaffolds were analyzed by SEM imaging and classified as random (coherency 0.35), semi-aligned (coherency 0.31) and aligned scaffolds (coherency 0.79). Fiber diameters were 1.36 ± 0.197 for random, 1.639 ± 0.149 for semi-aligned and 1.763 ± 0.368 for aligned scaffolds. Metabolic activity showed increasing activity over successive time-points at days 1, 2, 4, 8, 12 and 16 for all samples. DNA assay also showed highest DNA content at Day 16. Live/dead staining confirmed few dead cells in culture over time. Immunofluorescence staining with actinin and phalloidin markers revealed cell cytoskeleton (actin filaments) alignment parallel to the direction of electrospun fibers.

Conclusion

HL-1 cardiomyocytes seeded on scaffolds with aligned fibers and fibronectin coating showed better cell structural organization compared to cells seeded on random fibers. Hence, scaffolds with aligned structure are a promising biomaterial for cardiac tissue regeneration.

a91708

99-O-5 Personalized micro-3D human heart tissues for orphan drug development

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Cardiovascular Disease (CVD) is the number one cause of death globally, costing $30.7 billion every year in the United States alone. The massive cost, including extensive clinical trials for CVD drug development, has impaired the development of new and innovative treatments. There is an outstanding need to create new CVD drugs for inherited conditions (i.e., orphan drugs). Orphan approaches require fewer resources, and once approved, a new drug can be applied to a broader population. For the orphan approach, a system that faithfully recapitulates CVD disease in a dish can provide an unprecedented opportunity to discover new treatments.
Here, we have developed semi-automated processes by which investigators can: 1) optimize growth and differentiation condition of patient-specific or gene-edited human induced pluripotent stem cell (hPSC) lines, 2) grow micro hearts in 96/384-well plates using cardiomyocytes derived from each line, and 3) analyze their excitation-contraction-energy coupling using a high-throughput phenotyping device. The process has been applied to establish disease modeling of dilated and hypertrophic cardiomyopathies caused by troponin-T mutations or dystrophin mutations as seen in Duchenne muscular dystrophy (DMD). Preliminary data identifies a significantly reduced cardiac contractility of the micro hearts derived from a DMD subject with cardiomyopathy (E.F. < 51%) compared to those derived from DMD subject without cardiomyopathy. Furthermore, we will discuss approaches to analyze micro heart physiological properties including their action potentials, calcium transients, and mitochondrial activities. We then applied this knowledge to validate our disease models and to screen responses to libraries of compounds or gene therapies.

a92150

99-O-6 VEGF- and PDGF-BB-Decorated Fibrin Matrices ensure Persistent and Therapeutic Vascular Growth

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Therapeutic angiogenesis by Vascular endothelial growth factor (VEGF) delivery suffers from 2 major limitations: at least 4 weeks of delivery are required to avoid vessel regression, but sustained and uncontrolled expression can cause angioma growth. We previously found that the maturation factor Platelet-Derived Growth Factor-BB (PDGF-BB) can prevent aberrant angiogenesis by VEGF gene delivery (1). To overcome safety concerns of gene transfer, here we investigated the delivery of VEGF and PDGF-BB proteins from a state-of-the-art matrix-bound system, based on the transglutaminase (TG) reaction to bind the modified factors into fibrin hydrogels (2).
TG-engineered $VEGF_{164}$ and PDGF-BB were cross-linked into fibrin hydrogels and different doses of each, both together or no factors (control) were injected in limb muscles of SCID mice. We found that: 1) gels were completely degraded within 10 days in all conditions; 2) by 2 weeks, TG-PDGF-BB completely normalized aberrant angiogenesis by high TG-VEGF doses, yielding only mature and functionally perfused capillary networks, with PDGF:VEGF ratios as low as 1:20; 3) 10 days of TG-PDGF-BB co-delivery with both low and high VEGF doses caused stabilization and long-term persistence of new vessels, whereas >90% regressed with VEGF alone; 4) in a mouse hindlimb ischemia model, co-delivery at 1:20 ratio (50 μg/ml VEGF), but not either factor alone, caused 2-fold increases in both microvessel density and collateral arteries after 4 weeks, fully restoring blood perfusion.
Therefore, controlled co-delivery of TG-VEGF and TG-PDGF-BB proteins provides a convenient (off-the-shelf), safe (no genetic modification) and clinically applicable approach for therapeutic vascular growth with a short-term 10 days' treatment.

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Acknowledgments

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The fibrin gel immobilization scheme presented is the subject of patents upon which J.A.H. is named as inventor and has been licensed by a company in which J.A.H. is a shareholder.

a93342

99-O-7 A tissue chip model mimicking the right ventricular hypertrophy in pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) is a rare disease that causes narrowing of pulmonary arteries. Consequently, the heart must work hard to push the blood through the arteries into the lung and thus PAH patients die from right heart failure resulting from right ventricular hypertrophy (RVH). Currently there is no model that can accurately recapitulate RVH resulting from PAH. Here, we developed and validated a microfluidic device to mimic human PAH-induced RVH. We first prepared a polydimethylsiloxane (PDMS) chip comprising five channels to mimic the perivascular, adventitial, medial, intimal, and luminal layers of an artery. The two outermost layers, luminal and perivascular, work as access points to instill growth media, drugs and non-vascular cells, and to establish growth-factor gradients and fluid flow. The trapezoidal pillars, placed in every 200 µm, separate the channels but allow cell-cell interactions. We later modified the chips to grow cardiomyocytes (CMs) in an anisotropic fashion similar to the cells' growth pattern in the heart. We designed a micropattern comprising arrays of 30 × 30 μm^2 rectangles, used photolithography to engrave the pattern on a PDMS layer, coated the patterns with human fibronectin, and finally attached this fibronectin-coated micropattern engraved PDMS layer on one of the channels of the chip. We then seeded healthy human CMs on micropatterned channels, normal endothelial cells (N-ECs) and PAH afflicted endothelial cells (PAH-ECs), collected from healthy donors and PAH patients, on separate channels. When we seeded N-CMs alone or in the presence of N-ECs, CMs grew in an anisotropic pattern with a preferential alignment within the fibronectin-coated micropatterns and produced an array of CM bundles. However, seeding of PAH-ECs along with CMs made CMs hypertrophic, as evidenced by the narrowing of the space between two adjacent fibronectin micropatterns. CMs became hypertrophic under influence of hypertrophy inducing agents such as angiotensin II and IL-6 that are known to be release by PAH-ECs. This study is first to seed PAH-EC and CMs on a chip and demonstrate that PAH-EC communicate to make normal CMs hypertrophic. This study also established that micropattern engraved multilayer chips can be used to emulate PAH-induced RVH in humans and which can be deployed as a model for studying the influence of PAH therapy on RVH and for elucidating communication between CMs and PAH afflicted cells of pulmonary arteries.

a93471

99-O-8 Transplantation study of human alveolar stem cells derived from iPS cells

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Alveolar epithelial type 2 (AT2) cells, which produce pulmonary surfactant, are tissue stem cells in alveoli. Although human AT2 cells play critical roles in alveologenesis, process of damage repair and pathogenesis of refractory lung diseases, it has been challenging to elucidate their mechanism in human due to difficulty in expanding primary AT2 cells from human tissues. We have recently established the following methods of generation and long-term expansion of human iPS cell-derived alveolar stem cells in organoids. First, ventral anterior foregut endoderm (VAFE) cells were stepwise induced from hiPSCs in 2D culture. Following preconditioning culture in the medium involving CHIR99021, FGF10, KGF, and DAPT for promoting subsequent AT2 cell differentiation, CPM+NKX2.1+ lung progenitor cells were isolated and co-cultured with fibroblasts in 3D Matrigel to form alveolar organoids (AOs). SFTPC, a specific AT2 cell marker, positive cells were induced efficiently in AOs. Other AT2 cell markers such as SFTPA, SFTPB, SFTPD, DCLAMP, and ABCA3 were consistently expressed in AOs. In electron microscopy, lamellar bodies, which are the specific organelle of AT2 cells, were also detected in AOs. Alternatively, we developed a method of fibroblast-free AO induction from CPM+NKX2.1+ lung progenitor cells by inhibiting GSK3β and TGFβ. Furthermore, long-term culture of iPS cell-derived alveolar stem cells more than six months was successful by repeating AO formation. SFTPC+ cells were expandable with keeping their gene expression profile consistent with AT2 cells and the potential to differentiate into alveolar epithelial type 1-like cells

Finally, we tried to engraft human iPSC-derived alveolar stem cells (hiASCs) in immunodeficient mice to ask about their organoid-forming ability *in vivo*. After cell aggregates were formed by co-culturing hiASCs, human lung fibroblasts and human umbilical vein endothelial cells (HUVECs) in non-adherent well plates, they were engrafted in murine renal subcapsular space. 3D structure was observed macroscopically in the murine renal subcapsular space two months after engraftment. Immunostaining of harvested kidney showed that renal subcapsular cells involved human NKX2.1+ cells but surfactant protein makers were negative.

These data suggested that hiASCs could be used for transplantation but some additional strategies are required to prevent their differentiation after transplantation.

a93155

99-O-9 Precision Cut Lung Slices (PCLSs) as a model for testing lung gene therapies

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The lung remains an attractive target for the gene therapy of monogenetic diseases such as Cystic Fibrosis. Despite over 27 clinical trials, there are still very few gene therapy vectors that have shown any improvement in lung function; highlighting the need for *in vitro* human-derived assays which retain or mimic native tissue. Thus far such systems are under developed and pre-clinical testing relies on animal models which are not ideal. Herein, we have developed a method for generating precision cut lung slices (PCLSs) which uses agarose to stabilise tissue before sectioning to create a tractable clinically-relevant lung assay. In order to examine efficiency and duration of gene therapy strategies directly in PCLS models we treated mouse and human-derived lung tissues with a novel cell penetrating peptide (CPP)-based non-viral vector that utilises glycosaminoglycan (GAG)-binding enhanced transduction (GET) for highly efficient gene delivery[1]. We demonstrated that GET peptides exhibit enhanced gene transfer of a reporter luciferase plasmid in comparison to polyethylenimine (PEI) and lipid-based commercial transfection reagents. In addition, we showed that cryopreservation of PCLS did not inhibit gene transfer or negatively affect the viability of tissues. This approach promotes reducing animal use by creating multiple PCLS per animal and by facilitating pre-clinical testing of lung gene therapies in more relevant human tissue models.

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a92361

10-SYIS OPA-1 Early stage wound healing of osteochondral defects: an *in vivo* comparison of adipose tissue-derived CD271 positive versus plastic adherent mesenchymal stem cells

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Introduction: A focus of current research in regenerative medicine has been examining the potential of defined subpopulations of MSCs from the heterogeneous pool of cells towards a defined lineage. CD271 has been extensively studied as a suitable marker for selectively isolating MSCs from bone marrow[1]. Whether CD271+MSCs from adipose tissue (AT) have enhanced capacity to heal cartilage is currently unknown. We investigated the wound healing potential of plastic adherent MSCs (PA MSCs) versus CD271+MSCs *in vivo*.
Methods: PA MSCs and CD271+MSCs were isolated from AT that had been harvested as surgical waste products. MSCs were isolated from the mononucleated cells through plastic adherence or by magnetic associated cell sorting for CD271 immunopositivity. Pellets of PAMSCs and CD271+MSCs were assessed for chondrogenesis *in vitro* prior to *in vivo* testing. A total of 15 female athymic nude rats were divided into the following groups: (i) PA MSCs (n=6); (ii) CD271+MSCs (n=6); (iii) control group of carrier alone (no cells, n=3). An osteochondral defect of 2 mm diameter was created in the femoral patellar of each animal. Alpha Chondro Shield® was used as the cell carrier. At 3 weeks post-transplantation, animals were sacrificed to examine the extent of wound repair macroscopically and histologically.
Results: ECM positive for collagen type II was only seen in the pellets of CD271+MSCs. Macroscopic scoring revealed a significantly better repair tissue in animals that received CD271+MSCs compared to animals that received PAMSCs. Histologically, no significant differences were observed in the three groups; however, the repair tissue in CD271+MSCs group showed less vascularisation compared to PA MSCs. There were also low levels of pain molecules in rats transplanted with CD271+MSCs compared to PA MSCs indicating a paracrine effect of the secretome of MSCs. Further work in our group is indicative of a reduced angiogenic potential of CD271+MSCs (manuscript under preparation).
Conclusion: CD271+ MSCs transplanted into athymic rats enhanced the repair of osteochondral lesions with reduced blood vessel ingrowth. Both PA MSCs and CD271+MSCs survived post transplantation; however, there was little evidence of new articular cartilage formation. The low angiogenic potential of CD271+ MSCs makes them the ideal cell candidates for articular cartilage; although, this would require further research to substantiate.

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a91710

10-SYIS OPA-2 Induced pacemaker spheroids as a model of the heart sinoatrial node

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Introduction: Remarkably, it only takes ~10,000 pacemaker cells to pace-and-drive the entire heart (billions of cardiomyocytes). Although we have a general understanding of how individual cardiac pacemaker cells beat automatically there is a lack of understanding on the "source-sink mismatch" seen in the heart. This concept has been difficult to study due to it being painfully low-throughput to study these native pacemaker cells in vitro. We have created 3-dimensional induced pacemaker spheroids to understand the underlying design principles of cardiac pacemaker tissue's source-sink mismatch.
Methods: The iPMs were created by viral transient expression of one single transcription factor, TBX18, to neonatal rat ventricular myocytes as we have previously demonstrated [1]. The iPM or GFP-spheroids (control) were created by subjecting the NRVMs to the AggreWell™ plate at 1,000 cells/well, and cultured for three days in suspension.
Results: iPM-spheroids had a 17-fold increase in sinoatrial node (SAN) specific gap junction, Cx45, transcripts and a 2-fold decrease in myocardial gap junction, Cx43, compared to GFP-spheroids. When cultured for >2 weeks, iPM-spheroids demonstrated small α-sarcomeric actinin positive cells organized as a mesh in the core, similar to the pacemaker cells in the native SAN. iPM-spheroids demonstrated long term spontaneous pacing, over >4 weeks, while its monolayer control beats within the first four days. Co-cultures of one iPM-spheroid (1,000 cells) showed propagation consistencies over 40% while the control spheroid could not pace and drive 150,000 cells at this percentage. Lastly, when spheroids were injected into rat apex, we see that iPMs can consistently pace-and-drive the myocardium in an ex vivo optical mapping visualization.
Conclusion: iPM-spheroids can pace and drive surrounding quiescent myocardium, overcoming the source-sink mismatch. The iPM-spheroids are viable in long-term and exhibit native SAN-like pacemaker cell organization. These data provide an in vitro platform on which the design principles of native SAN could be tested.

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a91112

10-SYIS OPA-3 Biofabrication of 3D Structures using Cell Fiber prepared with Micro-mold

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In tissue engineering and biofabrication, one of the emerging issues is how rapid 3D structures can be produced. This paper describes rapid biofabrication of 3D structures using cell fibers prepared with micro-molding method. Long-sized cell fibers were facilely prepared with specially designated micro-mold within 12 hrs after seeding cells. These cell fibers were easily handled with tweezers. Hereby, we succeeded in fabricating tubular structures by reeling cell fibers around cylindrical device, and also succeeded in fabricating bundle-like structures by bundling them within 1 day. We deduce that our method would be helpful to fabricate various type of 3D structures in bioengineering fields.

Recently, many researches have focused on biofablication or bioassembly to fabricate 3D structures in vitro. And cell spheroids, cell sheets and cell fibers are used as building units or bio-parts for 3D-tissue fabrication. We have focused on cell fibers for the following reasons; it is easy to process fibers into various 3D shapes, For example, by reeling, bundling, waving, or some other way. And several human tissues are composed of bundles, cords and fibers of cells, such as muscular tissues.

We prepared spiral-shaped micro-mold using polydimethylsiloxane (PDMS). This micro-mold has a sequential spiral groove with 500-μm depth and 200-μm width, and the length of the spiral groove reached 5-m with micro-mold in size of 6-cm culture dish. After treating PDMS micro-mold with cell non-adhesive polymer, C2C12 myoblast cells were seeded onto the micro-mold at the initial cell density of 5.0×10^6 cells/1-m of groove. Seeded cells aggregated and formed cell fiber with approximately 5-m length within 12 hrs. Almost all cells were confirmed alive after aggregating into fiber-shape. These cell fibers were easily handled with tweezers; therefore cell fiber would have sufficient strength for 3D bioassembly.

Next, we tried to fabricate 3D structures by assembling cell fibers. We succeeded in fabricating tubular-like structures by reeling cell fibers around the cylindrical device we designed and made. Furthermore, by bundling up cell fibers with tweezers and immersing them into biocompatible hydrogel, we succeeded in fabrication of bundle-like structures. We could perform these bioassembly processes within 1 day. These results indicated that various 3D structures can be fabricated very rapidly by assembling with long-sized cell fibers produced with micro-fabricated molds.

a91599

10-SYIS OPA-4 Adipose tissue-derived stromal cell conditioned medium modulates endothelial-to-mesenchymal transition induced by IL-1β /TGF- β 2 co-stimulation, but does not restore endothelial cell function

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INTRODUCTION: Endothelial cells (EC) are important contributors to cardiac fibrosis by virtue of their easy of transdifferentiation to myofibroblasts. The TGF-β-driven endothelial-to-mesenchymal transition (EndMT) of cardiac EC may represent up to 25% of cardiac myofibroblasts in ischemic hearts. Our group previously showed that inflammatory stimuli (IL-1 β) synergize with fibrotic stimuli (TGF- β) in EndMT and that the conditioned medium of adipose tissue-derived stromal cells (ASC-CMe) blocks the TGF- β -driven differentiation of fibroblasts into myofibroblasts. Thus, we hypothesized that ASC-CMe also downregulates EndMT and may suit to control cardiac fibrosis in future therapy. **METHODS:** Human umbilical vein endothelial cells (HUVEC) were cultured as six experimental groups: 1)ECM, HUVEC cultured with endothelial cell medium (ECM), without added factors; 2)ECM/IL-1 β , HUVEC cultured with ECM added with IL-1 β ; 3)ECM/IL-1 β /TGF- β 2, HUVEC cultured with ECM added with IL-1 β and TGF- β 2; 4)ASC-CMe, HUVEC cultured only with ASC-CMe, without added factors; 5)ASC-CMe/IL-1 β , HUVEC cultured with ASC-CMe added with IL-1 β ; and 6)ASC-CMe/IL-1 β /TGF- β 2, HUVEC cultured with ASC-CMe added with IL-1 β and TGF- β 2. Gene expression of inflammatory, endothelial and mesenchymal markers were analyzed by RT-qPCR. The protein expression of endothelial and mesenchymal markers was evaluated by immunofluorescence microscopy and Western Blot. EC function was measured by sprouting assay. **RESULTS:** IL-1 β and TGF- β 2 treatment induced EndMT, as evidenced by the change in HUVEC morphology – which became disrupted, enlarged and elongated - and the increase in expression of mesenchymal markers in the groups receiving stimulation, both at the gene and protein levels. Treatment with ASC-CMe affected EndMT, observed as reduced expression of mesenchymal genes *TAGLN* (p=0.0008), *CNN1* (p=0.0573) and protein SM22α (p=0.0511) that coincided with increased protein expression of VE-Cadherin (p=0.0563). Although ASC-CMe could block the progression of the EndMT process, the EndMT-induced impaired angiogenesis of HUVEC could not be restored, as the disrupted cell-to-cell adhesion could not be rebuilt. **CONCLUSION:** The present study supports the anti-fibrotic effects of ASC-CMe through the modulation of the EndMT process. We demonstrated that ASC-CMe reduces EndMT induced by co-stimulation with IL-1 β and TGF- β 2 as evidenced by the reduction of mesenchymal and increase in endothelial markers.

a91943

10-SYIS OPA-5 Endogenous gene activation of BMP4 for osteogenic differentiation with CRISPR/Cas9 activation system

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Since reported as a powerful genome editing tool, CRISPR/Cas9 system has been applied throughout biological research field. However, in regenerative medicine, there has not yet been reported applications using the CRISPR/Cas9 system. It seemed to stem from safety issues such as unpredictable mutations by Double Strand Breaks(DSBs) of current CRISPR/Cas9 system. Here, we show a bone regeneration system with Adipose-derived Stem Cells(ADSCs) through the Light Inducible CRISPR Activation System(LICAS) which consist of catalytically inactive Cas9(dCas9), transcription factors(VP64), and light-inducible heterodimerizing proteins CRY2 and CIBN. CRISPR activation system uses CRISPR/Cas9 system's target-specific binding feature without occurring DSBs, which can overcome the safety issues by epigenetic approach. To eliminate the risk of long-periodic activation, the LICAS is transiently transfected to stem cells. Our system was used as a boosting module for osteogenic differentiation of ADSCs for improvement of bone regeneration treatment. We targeted BMP4 gene as an inducing factor for bone formation. From *in vitro* to *in vivo* study, results demonstrated that endogenous gene activation of an osteogenic differentiation marker can induce multi-signaling of bone formation and improvement of bone regeneration effect can be achieved by the LICAS. Consequently, Our system is one of approaches which can solve not only safety problems, but also efficiency problems. It establishes new avenues for application of CRISPR/Cas9 systems in regenerative medicine.

a91811

10-SYIS OPA-6 Biocompatible snake venom-induced designer fracture hematoma for the healing of large bone defects

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INTRODUCTION The fracture hematoma is a natural fibrin scaffold and is crucial for the initiation of bone healing, serving as a reservoir for growth factors and a space for (stem) cell infiltration. However, it is not clear if hematomas in normal fractures, which heal, differ structurally from the ones in large bone defects, which do not heal. Therefore, the aim of this study was to determine the structural properties of hematomas in normal and large bone defects, and to assess if the structure of a normal fracture hematoma can be mimicked *ex vivo* using a snake venom enzyme (SVE) to enhance the repair of large bone defects.
METHODS Defects of 1 and 5 mm were created in rat femurs. *In vivo* fracture hematomas and whole blood were collected on day 3. The CE was added at various concentrations to citrated whole blood to form *ex vivo* hematomas. The structure of the samples was imaged using scanning electron microscopy (SEM). Presto Blue assay was used to assess the effect of the CE on the cell viability of rat bone marrow MSCs, which were also cultured within *ex vivo* hematomas, both for 7 days.
RESULTS SEM images of *in vivo* hematomas revealed that fibrin fibers in 5 mm defects (320±64 nm) were 50% thicker compared to 1 mm defects (209±20 nm) 3 days after surgery. Increasing concentrations of CE led to thinner fibrin fibers in *ex vivo* hematomas, which ranged from 156±8 nm at the lowest concentration to 94±3 nm at the highest. The cell proliferation rate decreased with an increasing concentration of CE, showing a 6.9±0.9-fold growth at the lowest concentration, a 3.3±0.3-fold at the highest, and a 7.6±1.1-fold increase without the CE. However, exponential cell growth was observed for all groups. Cells cultured within *ex vivo* hematomas caused a contraction of the hematoma and were found to be homogeneously distributed.
DISCUSSION This study is the first to quantify structural differences of hematomas between normal fractures and large bone defects, which demonstrated the CE's ability to modulate the structure of fibrin fibers in *ex vivo* induced hematomas. Cell-mediated contraction of the hematomas resembled physiological cell behavior of wound healing, while the cell viability assays confirmed biocompatibility of the CE at lower concentrations. Taken together, this study showed that mimicking the structure of normal fracture hematomas could be the first step towards developing new treatment strategies that improve the healing of large segmental bone defects.

a91073

10-SYIS OPA-7 CRISPR-mediated dual programming of bone marrow-derived mesenchymal stem cell for calvarial bone defect regeneration

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Calvarial bone regeneration remains a hurdle in clinic due to poor spontaneous healing. Bone marrow-derived mesenchymal stem cell (BMSC) has been widely utilized for bone/cartilage regeneration. Our previous studies showed that stimulating chondrogenic instead of osteogenic differentiation may switch the repair pathway from the native intramembranous to endochondral ossification pathway and boost calvarial bone healing. CRISPR activation (CRISPRa) and CRISPR inhibition (CRISPRi) are newly developed technology that exploits dCas9 protein and single guide RNA (sgRNA) for programmable gene manipulation. In this study, we developed a single baculovirus (Bac-CRISPRai) that harbored the CRISPR activation and inhibition modules to simultenously activate Sox9 (essential for chondrogenesis) and PPARg (essential for adipogenesis). We showed that Bac-CRISPRai transduction of rat BMSC was able to upregulate Sox9 and downregulate PPARg expression. The gene manipulation effect of Bac-CRISPRai was further enhanced in terms of duration and magnitude when combined with our Cre/loxP baculoviral hybrid vector. Furthermore, simultenous upregulation and downregulation of Sox9 and PPARg significantly stimulates the downsteam chondrogenic differentiation and hinders the adipogenic differentiation. Finally, implantation of the engineered BMSCs into SD rats significantly promoted the calvarial bone healing. These data altogether demonstrate that CRISPR-mediated dual programming of BMSCs can improve the chondrogenic differentiation and calvarial bone healing.

a91068

10-SYIS OPA-8 Developing biomaterial-based therapeutic applications for induced pluripotent stem cell derived-endothelial cells using non-invasive bioluminescence imaging

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Induced pluripotent stem cell derived-endothelial cells (iPSC-ECs) are a promising cell type for therapeutic promotion of angiogenesis, however, inadequate understanding of their *in vivo* behaviour has hindered their clinical translation. To address this, we developed a non-invasive imaging method, based on bioluminescence (BLI), to track the real-time engraftment of injected cells towards injuries and implanted biomaterials *in vivo*[1]. Using this method, we separately studied iPSC-EC engraftment in the context of wound repair and efficacy when combined with implanted electrospun scaffolds. In an established mouse wound model, injected iPSC-ECs were tracked using BLI imaging and showed a steady decline within the wound over 14 days, indicating poor engraftment. Despite the short survival time, iPSC-EC treatment resulted in a 195% increase in blood perfusion assessed via laser doppler, which was consistent with a 650% increase in neo-capillary density as well as a 324% and 857% increase in the expression of angiogenic genes, PECAM-1 and Tie-1, respectively. Histological analysis also showed 32% and 103% increase in collagen deposition and fibroblast recruitment, respectively, underlying the full closure of iPSC-EC treated wounds occurring 4 days faster than controls. Poor engraftment rates led us to develop biomaterial scaffolds to enhance iPSC-EC engraftment and function. BLI imaging was again used to assess *in vitro* growth of iPSC-ECs cultured on a electrospun scaffolds comprised of varying PCL/gelatin blends and fiber thicknesses. The highest iPSC-EC compatibility was found to be with PG73 (PCL/gelatin at 70:30 ratio) scaffolds. Using a subcutaneous implant mouse model, PG73 scaffolds were shown to increase iPSC-EC engraftment 70-fold compared to iPSC-ECs injected alone. This correlated to enhanced angiogenesis, collectively indicated by a 134% increase in blood perfusion, a 316% increase in neo-vessel density, and a 140% and 312% increase in angiogenic cytokine, PlGF and VEGF release, respectively. Together, these studies show the regenerative potential of iPSC-ECs while also providing a valuable tool to closer examine their *in vivo* behaviour to aid in the development of future biomaterial solutions that may potentially augment their function, enabling a broader range of therapeutic applications.

1. Tan R, et. al. Non-invasive tracking of bone marrow mononuclear cells to injury and implanted biomaterials. Acta Biomaterialia 53, 378-388, 2017.

a91474

10-SYIS OPA-9 A Placenta-Fetus Model to Evaluate Maternal-Fetal Transmission and Fetal Neural Toxicity of Zika Virus

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The recent global epidemic of viral infections and the resulting birth defects from maternal-fetal viral transmission, such as the 6-10+% of Zika virus (ZIKV)-associated birth defects in the US, showcases the critical unmet need for experimental models that recapitulate the physiology of the human maternal-fetal interface and downstream fetal development. To address this need, we used a tissue engineering approach to develop an *in vitro*, 3D model of the maternal-fetal interface and downstream fetal cells. We incorporate placental trophoblast and endothelial cells into an extracellular matrix environment and validate formation of this interface through evaluating expression of E-Cadherin, VE-Cadherin, zonula occludens-1 (ZO-1), vascular endothelial growth factor (VEGF), and human chorionic gonadotropin (hCG). We also validated our model by assessing molecular transport of glucose across the barrier, finding a diffusion coefficient of 5.80×10^{-6} cm^2/s for a cell-laden model compared to 1.33×10^{-5} cm^2/s for an acellular gel. Following validation, we utilized this model to study ZIKV exposure to the placenta and neural progenitor cells. Our results indicated that ZIKV infects both trophoblast (8.57%) and endothelial cells (0.36%), leading to higher amounts of virus ($>4 \times 10^6$ PFU at 12 hours) downstream of the barrier. Chloroquine inhibits ZIKV infection and reduces downstream viral load ($<1 \times 10^6$ PFU at 12 hours). Further, viability of neural cells downstream of the barrier was maintained with the full cell-laden barrier model present (80% for both ZIKV-exposed and control), compared to significantly reduced viability when directly adding ZIKV to neural cells (<30% for ZIKV-exposed and >70% for control), suggesting that placental cells sequester ZIKV and may modulate ZIKV exposure-induced fetal damage. These findings are consistent with clinical observations and support that tissue models, such as this, can enable rigorous assessment of potential therapeutics for maternal-fetal medicine.

a93237

10-SYIS OPA-10 Fabrication of a human implantable vascular bed using decellularized porcine small intestine re-endothelialized with human cells

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[Introduction]
Fabricating three-dimensional tissues *in vitro* is known to be difficult due to a lack of sufficient nutrition to the cells. Our laboratory developed vascular beds, which enabled introduction of perfusable vasculature into tissues. It solved the problem of nutrient shortage and allowed construction of thick myocardial tissue using cell sheet engineering. This study aims at creating a implantable vascular bed derived from porcine tissue to realize future transplantation therapy. We used a gentle decellularization method to maintain the scaffold's vascular network and reseeded with human endothelial cells to produce a construct for clinical transplantation.

[Methods]
Porcine small intestine with intact arteriovenous loop was selected for the vascular bed. After harvesting, the tissue was decellularized by sequentially perfusing three reagents through the artery and intestinal lumen. The amount of DNA was measured to evaluate whether the tissue met established decellularization criteria. Subsequently, GFP-positive HUVECs were seeded into the decellularized intestinal vessels and cultured to re-endothelialize the lumina using a bioreactor to promote cell adhesion. The effect of perfusion-culture on re-endothelialization was assessed by morphological observations.

[Results]
The amount of DNA of the native and decellularized intestine was 141 ± 17.0 ng/mg, 42.7 ± 6.6 ng/mg respectively. We confirmed that the decellularized intestine satisfied the standard level of decellularized tissue. In addition, the intestinal vasculature remained intact after decellularization as confirmed by perfusion of epoxy resin. Moreover, the results of re-endothelialization after culturing with the bioreactor indicated that the cell adhered to the blood vessel walls and formed complete luminal structures in the decellularized intestine.

[Discussion & Conclusion]
We have found a promising way to create an implantable vascular bed using decellularized intestine. These results are promising, although some vessels were not fully re-endothelialized, likely due to a non-uniform resistence of the blood vessels. To solve this, a bioreactor which can perfuse throughout the tissue is under development.

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a93335

10-SYIS OPA-11 Mimicking the islet extracellular matrix niche in microwell islet delivery devices using collagen-IV and laminin 111 leads to improved insulin secretion in human islets

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Intrahepatic islet-transplantation is a promising therapy for treatment of type 1 diabetes. During islet isolation, collagenase is used to extract islets from the pancreas, leading to loss of important cell-matrix interactions. Loss of the native pancreatic microenvironment is associated with apoptosis of islet cells, early graft failure, and poor islets function. In short, the degradation of the pancreatic microenvironment limits the clinical success of islet transplantation. The islet extracellular matrix is comprised of a specific combination of collagen, laminin, and fibronectin molecules. Reintroducing these molecules has been shown to boost the function, viability, morphology, and proliferation of β-cells. In this research, the effect of combinatorial ECM on islet function and survival was investigated. Specifically, thin film microwell array scaffolds made from two distinct biomaterials were coated with fibronectin, collagen type IV, laminin-111, laminin-332 or a combination thereof. We reveal that coatings containing a single type of ECM molecule, e.g. fibronectin or collagen, can improve short term islet function. However, these single proteins do not prevent loss of morphology and subsequent loss of islet function afterwards. In contrast, combining collagen-IV with laminin-111 at a ratio of 8:2 not only improved short term islet function, but also preserved islet structure and islet function on a longer term. This effect was reproducibly shown on poly(ester urethane) and poly(ethylene glycol terephthalate-b-butylene terephthalate) microwell islet delivery devices as well as tissue culture polystyrene. We concluded that bio-functionalization of inert biomaterials regardless of their molecular composition with a specific combination of islet ECM molecules can support and improve islet function over longer time-periods. Our data suggested that creating a biomimetic islet niche by bio-functionalization of biomaterials can significantly improve the endocrine function of β-cell s. The creation of islet mimicking niches in islet delivery devices leads to an improvement of islet function by restoring part of the islet's extracellular matrix in these devices.

a90078

10-SYIS OPA-12 Substance P and SDF-1 α Peptide Eluting Grafts Promote In Situ Blood Vessel Regeneration

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The objective of this study was to develop small-diameter vascular grafts capable of eluting SDF-1 α peptide (SDF-1 α P) and substance P (SP) for *in situ* vascular regeneration. Polycaprolactone/collagen type 1 (PCL/Col) grafts containing SP or SDF-1 αP were fabricated by electrospinning. SP and SDF-1 α P-loaded grafts recruited significantly higher numbers of mesenchymal stem cells than that of the control group. The *in vivo* potential of PCL/Col grafts with or without SP and SDF-1 α P was assessed by implanting them in a rat abdominal aorta for up to 4 weeks. All grafts remained patent as observed using laser Doppler and stereomicroscope. Host cells infiltrated into the graft wall and the neo-intima was formed in peptides-eluting grafts. The lumen of the grafts was covered by the cobblestone-like cells, which organized along the direction of the blood flow in SP grafts, as discerned using scanning electron microscopy. Moreover, SDF-1 αP and SP grafts led to the formation of a confluent endothelium as evaluated using immunofluorescence staining with von Willebrand factor antibody. SP and SDF-1 α P grafts also promoted smooth muscle cell regeneration, endogenous stem cell recruitment, and blood vessel formation, which was the most prominent in the SP-eluting grafts. Evaluation of inflammatory response showed that three groups did not significantly differ in terms of the numbers of pro-inflammatory (M1) macrophages, whereas SP grafts showed significantly higher numbers of pro-remodeling (M2) macrophages than that of the control and SDF-1 α grafts. SP and SDF-1 α P eluting grafts can be potential candidates for *in situ* vascular regeneration and are worthy for future investigations.

a91754

20-SYIS RFA-1 Development of an Artificial Eyelid Device for Corneal Culture Models

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Dry eye is one of the most prevalent eye diseases. It is characterized by unstable tear film. Low tear production, excessive tear evaporation, and infections on the ocular surface all contribute to the instability of the tear film and thus its breakdown. The continuous eyelid motion and the pressure it exerts on the ocular surface further contributes to pain and irritation of eyes in dry eye patients.

Most in vitro corneal models used in evaluating dry eye disease are static. They do not take into account the dynamic nature of the eyelid motion. Our research thus focuses on developing a sliding artificial eyelid device to evaluate the effect of eyelid motion and tear volume on corneal surface. To start with, we are working on a linear model whereby a linear motor actuator with a polydimethylsiloxane (PDMS) head slides past a flat corneal surface. For this we made a linear actuator which can be programmed to change stroke length, speed, and frequency to emulate variations in blinking pattern in normal and dry eye subjects. In addition, we have integrated a small pressure sensor to measure the normal force exerted by the PDMS bulb on the corneal surface while actuating the blinking motion. Further, we made a wirelessly controllable mini microscope with adjustable magnification to observe changes in cell morphology as a result of sliding eye lid. So far we have tested the device on a fibroblast seeded collagen gel sheet. The preliminary result indicates that cell morphology is unaffected until certain limiting force. We want to further calibrate the shear force and test the change in morphology and cell detachment on a corneal culture model. Moreover, we are also working on integrating a tear chamber to evaluate the effect of tear volume and shear stress on the corneal surface.

a90977

20-SYIS RFA-2 Modeling of amyotrophic lateral sclerosis with a tissue-engineered spinal cord

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Background: Amyotrophic lateral sclerosis (ALS) is an incurable neurodegenerative disease causing motor neurons (MN) death, and triggering patient death due to respiratory failure within 3 to 5 years of diagnosis. In some patients, a mutation in the Superoxide Dismutase 1 (SOD1) gene, that induces protein misfolding and aggregation in MN, has been identified as a cause of ALS. Recent evidence has shown that the combination of MN, astrocytes, microglia and myoblasts, may constitute a metabolic unit and that non-neuronal cells could contribute to the ALS development. **Objectives:** Our aim is to develop and characterize a tissue-engineered mouse model of ALS based on the use of MN, astrocytes, myoblasts and microglia extracted and purified from transgenic mice overexpressing the wild type (SOD1WT) or mutant (SOD1G93A) form of the human SOD1 protein. The purpose of this approach is to develop an in vitro ALS model using well characterized mutant cells to successfully recapitulate the disease phenotype through the follow-up of MN degeneration. **Methods:** MN have been extracted from spinal cord mouse embryos aged of 14 days of development, and astrocytes and microglia, from transgenic SOD1G93A or wild type SOD1WT mice. These cells have been purified by gradient density separation and co-cultured on 3D collagen/chitosan sponges. Tissues were kept in culture for a period of 36 days. **Results**: The cell extraction method has been optimized to obtain a high yield (> 1 million per embryo) of purified (> 90%) MN. The 3D model showed the close cell-cell interaction between astrocytes and MN. We also noticed that when SOD1G93A MN were cultured in the 3D model, in presence of mutant astrocytes, mutant microglia or both, there was a drastic reduction in TUJ1-positive neurites and these latest were less branched, compared to controls made of SOD1WT astrocytes and microglia. **Conclusion:** Diseased MN are able to organize into nerve fibers in presence of normal glial cells, but not with diseased glial cells. In vitro ALS modeling should provide a better understanding of the disease mechanism, and could serve as a screening platform for future drugs. Moreover, this 3D model would be adaptable to other types of mutations involved in ALS.

a93337

20-SYIS RFA-3 Insect Muscle Tissue Engineering for Bioactuator and Food Applications

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Alongside medical uses, skeletal muscle tissue engineering tools can be applied to bioactuators and cultured meat technologies. Insect cells are a promising material source for these applications due to simple culture requirements (e.g., does not require carbon dioxide exchange or a humidified atmosphere, room temperature growth), rich nutrient density (e.g., high in protein, fiber and unsaturated fats) and superior contractile force relative to mammalian cells [Baryshyan et al., 2012]. Although insect cells are commonly cultured for recombinant protein production, the generation of three-dimensional tissue constructs from insect cells has not yet been explored. As a step towards in vitro insect tissue culture, a genetically immortalized Drosophila adult muscle progenitor cell line is employed to explore development regulation, biomaterial interaction and viability in three-dimensional scaffolds. First, the effect of insect hormones analogs (methoprene and 20-hydroecdysone) on proliferation and differentiation is determined by multi-day proliferation assays and immunostaining for muscle cell markers. So far, results from in vitro experiments support observations noted in the literature that methoprene can promote proliferation while inhibiting differentiation induced by 20-hydroecdysone [Chebas et al., 1989]. Insect-derived biomaterials were used to investigate interactions of the cells with scaffold geometries. Thin films were fabricated with silk (2, 4, 6%) or chitosan (0.5, 1, 2%) solutions and subsequently seeded with Drosophila cells. So far, we have observed that cells attach to and proliferate on silk films but do not spontaneously differentiate while cells attach to and spontaneously differentiate on chitosan films without high levels of proliferation. Next steps include stimulating differentiation of cells on micropatterned silk and chitosan films to induce myofiber alignment, producing myofiber contraction by L-glutamate and transitioning cell-film constructs to three-dimensional scaffolds to observe long-term viability and muscle development.

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a90658

20-SYIS RFA-4 CRISPR Activation for BMSC Engineering and Enhanced Calvarial Bone Healing

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Today critical-sized calvarial bone-defect repair remains a challenging task. CRISPR activation (CRISPRa) is an emerging technology that exploits deactivated Cas9 protein and single guide RNA for programmable activation of endogenous gene expression. Here we developed a hybrid baculovirus vector to express the CRISPRa SAM system for simultaneously activation of multiple genes to modulate both canonical and non-canoical Wnt signaling pathway. We demonstrated that the expression of CRISPRa system in the rat bone marrow-derived mesenchymal stem cells (rBMSCs) enabled the activation of several genes (e.g. Wnt10b, Foxc2, BMP2, etc.) for several hundred fold. In particular, CRISPRa-mediated activation of Wnt10b stimulated the expression of osteogenic differentiation markers such as Runt-related transcription factor 2 (RUNX2), alkaline phosphatase (ALP), and Osteopontin (OPN) and successfully drove rBMSC fate towards the osteoblast. Moreover, the activation of Wnt10b inhibited adipogenesis in rBMSC by suppressing adipogenic transcription factors PPARγ and C/EBPα. Implantation of the baculovirus-CRISPRa system-engineered rBMSCs successfully accelerated and improved calvarial critical-sized defect (6 mm) healing at 8 weeks after implantation. These results altogether that the CRISPRa system can activate endogenous gene expression in rBMSC and potentiate the ability of rBMSC to differentiate towards osteogenic pathway and repair calvarial bone defects.

Keywords : CRISPRa, calvarial bone-defect, BMSC, wnt pathway, osteogenesis

a90799

20-SYIS RFA-5 Inkjet Printing of Graphene Oxide for Cell Patterning: The Application in Bio-Subretinal Chip

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Abstract:

Three-dimensional printing technology has attracted much attention in various fields in the recent years. Studies of inkjet printers in 3D printing, especially electronic components, biosensors and cell patterns have been extensively reported for biomedical engineering related applications. Here, we employed an inkjet printing technology to recreate important interactions in the implantable retinal chip. This GO-patterned substrate was served as a platform that allowed human retinal pigment epithelium cells (RPE) to attach and grow in the microelectrode array. We demonstrated that after 7 hours cell culture on printed graphene oxide (GO) of 80 μm diameter, which revealed a lower non-specific cell adhesion and a higher cell number than on 30 μm diameter. In addition, the cell proliferation, viability, and tight junction barrier function (ZO-1) of RPE cells were well maintained on micropatterned substrate during culturing. Consequently, the concept of inkjet printing was applied to establish a promoted cell patterning of bio-subretinal chip. Due to the excellent reproducibility of the Inkjet-printing provided a suitable technology for precisely control the size of bio-subretinal chip arrays. Printed-GO micropatterns could enhance cell adhesion density of the bio-subretinal chip. These results indicate that our inject-printed GO micropatterns has a great potential for a bio-subretinal chip.

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a91071

20-SYIS RFA-6 Microfabrication of In Vitro Alveolar-Capillary Barrier Model by Inkjet-based Bioprinting

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An in vitro alveolar-capillary barrier is one of the essential model systems for pulmonary drug and particle tests in disease studies, drug discovery and toxicology. An alveolar-capillary barrier in the gas exchanging region of the lung consists of epithelial and endothelial layers with a thickness of 2 μm. This thin structure is critical to sustain pulmonary function such as gas diffusion. There has been efforts to fabricate the biomimetic human alveolar-capillary barrier model, using microfluidic devices and bioprinting technology. However, none of the works has achieved to mimic this thin membrane, a key feature for the model. Here, we present a human alveolar-capillary model with a sub-10 mm-thick membrane, containing multi-type alveolar cells. We fabricated the alveolar-capillary barrier model with four types of human alveolar cell lines, including type 1 alveolar cell (NCI-H1703), type 2 alveolar cell (A549), lung fibroblast (MRC5), and lung microvascular endothelial cell (HULEC-5a). High-resolution drop-on-demand inkjet printing enabled the fabrication of the thin alveolar-capillary barrier model under sub-10 mm thickness for the optimal structure by drop-on-demand deposition of multi-type alveolar cells as a thin layer. We evaluated the functions of the fabricated models by histology, barrier integrity test, and barrier permeability test to demonstrate the level of biomimicry. Inkjet-based bioprinting enabled the fabrication of reproducible in vitro alveolar-capillary models, which have biomimetic microstructures with customized and functionally designed micro-patterns. The inkjet-bioprinted alveolar-capillary models have a potential to replace animal testing as expecting to be applied in disease models for pathology, drug discovery, and toxicology.

a92041

20-SYIS RFA-7 The Strategy of Molecular Therapy to Promote Tissue Regeneration by the Synergy of BMP-2 and SHH

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Tissue regeneration often involve growth factors effect, but due to stem cell without sufficient receptor for binding growth factor, tissue regeneration difficult to complete in single growth factor stimulation to achieve tissue regeneration. Bone morphogenetic protein 2 (BMP-2) plays an important role in bone remodeling that involve bone differentiation and maturation in different bone stage. But BMP-2 also is confronting lack of differentiated efficiency in stem cells osteogenesis. In this strategy, we apply two kinds of molecular (BMP-2 and sonic hedgehog (SHH)) to involve different pathways, by individual pathway induce osteogenesis by BMP-2 and SHH respectively and integrate these signals on downstream to promote bone-tissue regeneration enhance mineralization and bone formation. In differentiated early stage, mesenchymal stem cells differentiated osteo-progenitor cells, and we had confirmed SHH enhance osteo-progenitor cells proliferation, and its could provide more amount progenitor cells involve osteogenesis to promote tissue regeneration. We prove BMP-2 and SHH were individual affect on SAMD and Gili2 respectively and integrate on Runx2. It demonstrates that in BMP-2 and SHH active different pathways and its function could be synergy. The synergy of BMP-2 and SHH also observed on ALP stain that enhance osteogenesis. So that it could overcome the limit signal pathway differentiation. Mesenchymal stem cells cultured in bioreactor with BMP-2 and SHH immobilized-sinbone that it could enhance mineralization. it indicated that BMP-2 combine SHH promote not only osteogenesis also bone maturation. *In vivo* study, we implanted BMP-2 and SHH immobilized-sinbone on rabbit skull also could promote mineralization that achieve enhance bone tissue regeneration. In this study, we demonstrated integration of different pathways by BMP-2 and SHH could enhance cell messaging to achieve osteogenesis even affect on mineralization. The synergy also increases new bone formation in animal experiment to show that the synergy by molecular therapy could promote tissue regeneration.

a93055

20-SYIS RFA-8 Development of a Crosslinkable Hydrogel Derived from Human PlacentalTissue

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Placenta-derived extracellular matrix (ECM) is a promising biomaterial because it provides the microstructure and biochemical cues that are important for restoring and repairing tissue in regenerative medicine. The cytokine and growth factor-rich environment of the chorionic villi, as well as the abundance of natural cell-instructive proteins make this material an excellent candidate for tissue engineering applications. However, hydrogels derived from placental ECM are usually formed by thermo-reversible gelation, severely limiting control of physicochemical and biological properties. To overcome this drawback, we developed a semi-synthetic hydrogel derived from the chorionic villi of the human placenta which allows for photo-crosslinking under cytocompatible conditions. Decellularization of the chorionic villi was performed using either Triton X-100 or Triton X-100/Sodium dodecyl sulphate and the efficacy of cell removal was confirmed using DNA quantification assays. Following decellularization, the ECM was enzymatically digested and chemically functionalised with methacryloyl groups to result in methacrylated placental ECM (PlacMA). Successful functionalization of PlacMA was confirmed with ^{1}H-NMR and a 2,4,6-Trinitrobenzene Sulfonic Acid assay. In the presence of a photoinitiator and visible light, PlacMA hydrogel precursor solutions readily formed covalently crosslinked hydrogels with tuneable mechanical properties. The potential for cell encapsulation and bioprinting are currently under investigation. Our results demonstrate that placental tissue can be functionalised to allow for the formation of tuneable and mechanically stable hydrogels for tissue engineering applications.

a90357

30-SYIS CS-1 Bi-layered Biodegradable Scaffolds for Bone and Cartilage Tissue Engineering

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Electrospinning has emerged as a versatile, cost effective and reliable technique for fabrication of micro and nanofibers that provide biomimetic microenvironment for growth and specific lineage differentiation of mesenchymal stem cells (MSCs). Nevertheless, the induction of specific lineage differentiation of MSCs often requires the use of a formulated culture medium, and its effect will diminish when removed from the culture medium. To date, there is minimal evidence of a bi-layered electrospun scaffold that can simultaneously release multiple bioactive molecules at different time points. Here we established a bioactive bi-layered scaffold through scalable electrospraying/spinning techniques that are capable of co-delivering both hydrophilic and hydrophobic bioactive molecules in a controlled manner. We hypothesize that customizable loading of different essential bioactive molecules within the scaffold can direct *in-situ* specific lineage MSC differentiation (e.g. osteogenic and chondrogenic) in a minimally supplemented culture environment. Scaffold was characterized with Scanning Electron Microscopy, and release profiles of all bioactive molecules were quantified with the High Performance Liquid Chromatography (HPLC) and UV-Vis spectroscopy. These scaffolds were used to evaluate the differentiation capabilities of mesenchymal stem cells (MSCs) into osteogenic and chondrogenic lineage. The bioactive molecules loaded scaffolds exhibited enhanced gene expression for osteogenic and chondrogenic markers and have immense potential in the field of tissue regeneration and drug delivery.

a90578

30-SYIS CS-2 Biomechanical analysis of muscle wound healing in a murine model

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INTRODUCTION

Skeletal muscle tissue engineering is a new strategy of tissue repair which aims to reconstruct skeletal muscle defect. We have already shown that human progenitor muscular cells seeded on a collagen scaffold are able to be integrated in a wounded mouse muscle without immune rejection. We want to show the functional recovery of the repaired tissue repair of the same wounded mouse muscle treated with progenitor skeletal muscle cells associated to the same collagen scaffold. The aim of this study is to analyze the biomechanic properties of this muscle wound healing in our mouse model.

METHODS

Human muscle progenitor cells (14 w.of gestation) were expanded for the experimentation These cells were associated with a collagen scaffold. The right gastrocnemius muscle of C57BL/6 mice were injured with a punch of 4 mm diameter. The injured thigh muscles were partially replaced (hole of 4 mm) either with a collagen scaffold disc with human marked fetal muscle cells ($1x10^5$), with the scaffold disc alone or with nothing. The gastrocnemius muscle on the opposite thighs served as control-samples. An analysis of the force of the muscle contraction as well as the tetanization and the endurance of the muscle was performed at day one and at 8 weeks.

RESULTS

Our mouse model is well adapted to test the muscle contractility even after a severe injury. Our preliminary results show that the absolute peak twitch obtained from the injured muscle is lower than the one obtained from the non injured contra-lateral muscle at different time point after the intervention. The use of fetal cells in an injured muscle seems to improve the absolute peak twitch.

CONCLUSIONS

Progenitor human cells are able to engraft on mouse muscle. These cells associated with a collage scaffold are able to improve the functional recovery after an muscle injury.

a90654

30-SYIS CS-3 Development of gelatin-DBM core shell bone tissue engineering microtissue for rat critical sized bone defects and its mechanism study

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Large segmental bone defects, of which the causes include trauma, infection and other diseases, is the common hurdle of clinical treatment. Compared with traditional treatment methods (autologous bone and biomaterial filling), tissue engineered bone graft (TEBG) based on mesenchymal stem cells (MSCs) has a good osteogenesis, small trauma and low immunogenicity. However, Current TEBG fabrication methods mainly based on top down(TD) strategy are hindered with problems including low throughput and limited diffusion properties, thus decrease the treatment efficacy. The microtissue technology based on the hydrogel system might be able to overcome this problem, for the biomimetic environment of extracellular matrix reducing the possible damage during the implantation, the uniformly distribution of seed cells and the advances of angiogenesis. However, the suboptimal mechanical property and osteoinductivity of hydrogel system make it unsuitable for bone reconstruction. Here we designed a core-shell structured tissue engineered microtissue. Demineralized bone matrix(DBM) microcarrier, with well osteoconductivity and mechanical property, was chosen as the core materials, with which rhBMP2 was coated to enhance its osteoinductivity. Gelatin was used as the shell to support the growth of seed cells. It was demonstrated that MSCs were efficiently seeded onto gelatin-DBM core shell microscaffolds and quickly proliferated while retaining a high viability. After culturing for 7 days, the cells were then directly induced to undergo 14 days of osteogenic differentiation in the same bioreactor. The osteogenic differentiation of MSCs was demonstrated with increased calcification by detecting ALP activity, calcium content and gene expression of osteogenic proteins. At three months post-treatment in rat cranial defect mode, the core shell microtissues based TEBG inserted within the defect generated significantly more bone tissue compared to gelatin microtissues based TEBG as demonstrated by the micro-computed tomography and histological analyses. This study provided a promising cell delivery platform for repairing bone defects.

a91141

30-SYIS CS-4 Modulation of Bone Morphogenetic Protein-2 Release via gelatin microparticels and coacervate loaded Thiolated Gelatin-PEGDA Interpenetrating Hydrogels for Carvarial Bone Regeneration

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Bone morphogenetic protein-2 (BMP-2) is commercially available therapeuticgrowth factor for bone tissue regeneration. However, adirect administration of BMP-2 into a critical sized calvarial defect with no support could exhibited many limitation, especially less effective bone repair. In this study, we developed a composite interpenetrating (IPN) hydrogel containing polycation-based coacervate (Coa) and gelatin microparticles (GMPs) as BMP-2 delivery vehicles, for a guided skullboneregeneration. IPN hydrogels were fabricated with thiolatedgelatin/poly(ethylene glycol) diacrylate (PEGDA), with varying mechanical and degradation properties based on the composition. Released BMP-2 from each vehicles effectively upregulated*in vitro* osteogenic differentiation of human mesenchymal stem cells by showing significantly enhanced alkaline phosphate expression as compared with bolus BMP-2 treatment without delivery vehicles.For *in vivo* rat calvarial defect regeneration, five different groups (i.e., (1) control, (2) only IPN gel, (3) IPN gel with bolus BMP-2, (4) IPN gel with BMP-2 loaded Coa (5) IPN gel with BMP-2 loaded GMP) were bilaterallyimplanted into 5 mm sized calvarial defects. After 4 weeks of transplantation, micro-CT analysis indicated significantly higher scores of bony bridging and union in Coa and GMP groupsas compared to other groups, and a ratio of bone volume/total volume in GMP group was significantly higher than other groups. Histomorphometric analysis using hematoxylin/eosin and masson'strichrome staining also demonstratedthat facilitated bone formation was observed in Coa and GMP groups. Taken together, it could be concluded that (1) the utilization of BMP-2 delivery vehicles (i.e., Coa and GMP) in IPN hydrogels achieved a controlled and sustained release of BMP-2 with maintainingits bioactivity, (2) In vivo carvarial bone regeneration could be controlled by the BMP-2 release pattern, and consequently, (3) our composite IPN hydrogel systemcould be a feasible tissue engineering platform ropromote craniofacial bone regeneration.

a92221

30-SYIS CS-5 Interfacing hard and soft biomaterials for simultaneous regeneration of bone and cartilage

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The mismatch between the mechanical properties of the biomaterials that are used for regeneration of bone and cartilage in osteochondral defects creates stress concentration at their interface. That stress concentration together with the inherent difficulty of connecting metals to hydrogels make it very challenging to create the required combination of biomaterials. Here, we propose novel approaches to overcome those challenges. We made an osteochondral plug model with two regions: a metallic osteal region with a hierarchical architecture made by selective laser melting (SLM) and a fibre reinforced hydrogel chondral region made by melt electrospinning writing (MEW). Two chemical surface treatments have been proposed for attachment of the reinforced hydrogels to metallic scaffolds, namely ion-assisted plasma polymerization (IAPP) and carboxylic coating (CC). In IAPP, covalent immobilization is achieved through radical chemistry. Carboxylic groups are reactive functional polymer terminations that offer favourable structural and interfacial performance.

Titanium hierarchical architectures were manufactured by SLM machine equipped with a YLM-400-AC Ytterbium fiber laser with a wavelength of 1070 nm. IAPP was applied on the titanium substrate via a custom-made setup. The RF input power and DC bias voltage were 50 W and –500 V, respectively (deposition time=2 min). For CC coupling, the polymer consisted of propoxylated bisphenol dissolved in styrene generated by curing with liquid methyl ethyl ketone peroxide. Once the ingredients were mixed, the uncured polymer system was applied on the Ti surfaces to make a 1 mm thick layer using a spin coater. Fibre scaffolds were then written with a custom-built MEW device directly onto the coated metallic implants. Fibre constructs were then embedded with GelMA hydrogels.

The IAPP surface was composed of carbon, nitrogen, and oxygen with atomic concentrations of approximately 71, 20 and 9%, respectively (XPS). No titanium signal was recorded, indicating that the thickness of the coating is at least 10 nm and also the coating provides complete coverage of the underlying titanium surface. The existence of high concentrations of radicals in the IAPP structure was confirmed by EPR. The cured polymer created by the CC method showed the presence of C-C and C-O vibrational bands, while a substantial amount of the carbonyl groups, i.e. C=O, remained intact. CC resulted in higher interface strength as compared to IAPP.

a92476

30-SYIS CS-6 Homologous use of a decellularised biomaterial in a urinary bladder auto-augmentation large animal model

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INTRODUCTION: New approaches are needed to improve the clinical outcome and quality of life in complex urological paediatric patients with primary and secondary end-stage bladder disease. Numerous surgical techniques have been proposed including auto-augmentation, where the detrusor muscle is split to allow the urothelium to bulge, increasing bladder capacity and compliance. Although considered a useful intervention when performed in the pre-end-stage failing bladder, the risk of perforation has inhibited widespread uptake of the auto-augmentation technique. In this study, we report the use of a porcine acellular bladder matrix (PABM) to support the exterior aspect of the auto-augmented bladder in a large animal surgical model.

MATERIALS AND METHODS: PABM was produced from full thickness porcine bladders. Six Landrace female pigs (average weight: 23.65 kg) underwent urinary bladder auto-augmentation, and the bulging mucosa was covered with a patch of PABM held in place by absorbable sutures. Macroscopic and microscopic appearances were evaluated alongside immunohistochemistry to assess tissue integration in animals after a four-month follow-up.

RESULTS: No intra-operative complications occurred and all pigs recovered and voided normally post-operatively. Five animals were followed-up for 4 months, but one animal was sacrificed after 18 days due to an indirect complication. Histology showed that the patch material underwent extensive cellular integration, with no evidence of inflammation.

CONCLUSIONS: The cellular integration properties of PABM combined with its natural strength and compliance make it an ideal biomaterial for use in reconstructive urological surgery. These first surgical results support a role for PABM in homologous urinary bladder auto-augmentation.

a92967

30-SYIS CS-7 Protease-sensitive Elastin-Like Recombinamers-based hydrogels able to stimulate angiogenesis

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Tissue engineering takes a multidisciplinary approach seeking to repair, improve, or replace biological tissue function in a controlled and predictable manner. Proteolysis has been indicated as one of the first and most sustained cell activity responsible for structural remodelling and functional plasticity of tissues. Elastin-Like Recombinamers (ELRs) based hydrogels shown a high potential in terms of biocompatibility, possibility to simulate mechanical properties of the ECM, codify into their backbone bioactive sequences as well as to control their proteolytic activity. In this work, four ELRs containing proteolytic target sites that degrade in response to tPA and uPA enzymes with tunable degradation rates have been designed, produced and characterized. We aimed to study how degradation rate could influence the integration with the host tissue as well as to compare the effects of degradation rate *in vivo* demonstrating a clear difference in cellular invasion likewise in the formation of new blood vessels and formation of new tissue. ELRs were designed, synthetized and produced using recombinant protein technology. Comparative degradation rates were evaluated using human recombinant tPA and uPA enzymes. Their cytocompatibility was assessed using an AlmarBlue assay employing HUVEC cells. Firstly, vascularization was evaluated *in vitro* using a 3D cell culture model of sprouting angiogenesis employing a co-culture made of HUVECs and HFF1 cells. *In vivo* studies were performed injecting the hydrogels intramuscularly in a hind limb zone using Swiss CD-1 CR male mice and analysed subsequently by histology and immunohistochemistry. *In vitro,* these constructs are sensitive to proteases-mediated cleavage relying on their degradation to smaller fragments throughout the course of the reaction. *In vivo* they exhibit a clear difference between their degradation rate characterized mainly by a different inflammatory cell invasion as well as a distinct neo-vascularization. In addition, it was demonstrated that in the absence of growth factors, cleavage sites have a profound impact in enhancing hydrogel invasion *in vivo* likewise in their vascularization. They exhibited a prominent vascular host tissue response with formation of a densely vascularized granulation tissue despite a distinct cell infiltration. These results make them promising tissue substitutes for biomedical applications as their degradation can be tailored to tissues that may require more rapid regeneration.

a93312

30-SYIS CS-8 Do the Carrageenan-Gelatin scaffolds provide sufficient biological cues for a cell-free articular cartilage regeneration?

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Introduction:

A scaffold which mimics structural component of the articular cartilage and regenerates the tissue using endogenous stem cells is indispensable. In this study, we tested in *in vitro* and *in vivo* a carrageenan-gelatin (CG) scaffold which mimics glycosaminoglycan and collagen framework respectively for the articular cartilage regeneration

Materials and Methods:

Rabbit bone marrow mesenchymal stem cells (MSCs) were seeded on CG scaffold and differentiated into chondrogenic lineage. Quality of *in vitro* generated cartilage was quantitatively assessed by Total Collagen and GAG production and cartilage-specific gene expression (COL2A1 and SOX9). For in vivo testing, a 4 mm osteochondral defect was created in the patellar groove of the rabbits' femora (n=5) bilaterally and treated either with scaffold alone or tissue engineered construct (TEC). The outcome was compared with chondrocyte transplantation and untreated defects on the second set of animals (n=5). Four months after treatment the extent of articular cartilage regeneration was evaluated histologically by O'Driscoll score and immunostaining of the neocartilage.

Results:

The tested scaffold supported cell adhesion on confocal microscopy and showed >95% viability. There was a significant increase ($P<0.05$) in total collagen and GAG content in TEC over the course of chondrogenic differentiation. Gene expression analysis confirmed the increase ($p<0.001$) in cartilage-specific genes (COL2A1 and SOX9) at day 28 of differentiation. Histological analysis showed defects treated with both TEC and scaffold alone groups regenerated with hyaline cartilage ($P<0.05$) with profound safranin O staining as compared to controls. Collagen type 2 immunostaining was positive in the neocartilage of TEC and scaffold alone groups whereas the controls stained poorly.

Conclusion:

This study demonstrated that CG scaffold alone or with MSCs support articular cartilage regeneration. This opens up a possibility for using CG as a standalone graft for articular cartilage repair.

a93313

30-SYIS CS-9 Tissue-engineered abdominal wall: the future of ventral hernia repair?

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Background: Hernia recurrence is a serious complication of mesh ventral hernioplasty operations. The use of tissue-engineered muscle to repair ventral hernias may be an alternative. Here, we tested the efficacy of culture-expanded autologous muscle-derived stem cells (MDSC) over a biologically coated electrospun polycaprolactone (PCL) scaffold in a rat ventral hernia model.

Methods: Rat MDSC isolated from gastrocnemius were characterized by flow cytometry for CD34, Stem cells antigen -1 (Sca-1), syndecan-4 and cell cycle analysis. Cells seeded on PCL (0.1x10^6 per cm2) were sutured with supporting polypropylene (PP) mesh (group 1) and transplanted into a 2x1cm defect (n=12 each). Animals that received PP mesh alone (group 2) and PP mesh+ PCL (group 3) served as controls. The outcome at six and ten weeks (n=6 each) was assessed by adhesions (Hollinsky score) and histology. Immunostaining for desmin, PAX7 were performed to assess nascent skeletal muscle regeneration while TUNEL assay was performed to quantitate cell apoptosis in long term.

Results and Discussion: Cells were over 90% positive for Sca-1 and CD34, and 69% for syndecan-4; average of 90% cells were in G0/G1 phase. In vivo, mean adhesion score at six and ten weeks was less ($P<0.05$) in MDSC (3.1 and 3.8) as compared to PCL (6.5 and 6.0), PP mesh (5.4 and 7) alone. Histological analysis revealed muscle regeneration in 9/12, 1/12 and 0/12 animals in group 1, group 2 and group 3, respectively ($P<0.001$). An average of nineteen regenerated muscle bundles/low power field (LPF) were present in MDSC group while only 2 bundles/LPF were in controls. Desmin immunostaining on the neo-abdominal (MDSC group) wall showed 19.4% positive of total area and PAX7 positive cells were also detected; controls were negative. Apoptosis assay in MDSC group (10 weeks) revealed increased cell death as compared to 6 weeks ($P<0.01$).

Conclusion: This study establishes the feasibility of using tissue engineered anterior abdominal wall for the treatment of ventral hernias in an animal model; however long-term transplant cell viability is a matter of concern requiring further investigation.

a90093

40-SYIS CS-1 MSCs implantation does not rescue the degeneration of intervertebral disc allograft after transplantation

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Fresh-frozen intervertebral disc (IVD) allograft transplantation has been successfully performed in human cervical spine with acceptable clinical outcomes; nevertheless, rapid degeneration of IVD allograft was observed in some cases. Based on the regenerative potential of mesenchymal stem cells (MSCs), this degeneration might be benefited from MSCs injection. To test this hypothesis, using a goat model, 1×10^5 human bone marrow MSCs were injected into the IVD allograft immediately (n=5) and at 1.5 months after transplantation (n=5); IVD allografting only was used as control(n=5). Radiographs were taken immediately and at 1, 3, 4.5 and 6 months after transplantation; disc height of the allograft and mobility of the lumbar spine were measured. After 6 months, the goats were sacrificed and the lumbar spines from L2 to L6 were harvested *en bloc* for *ex vivo* MRI scanning (T1w, T2w and UTE). Histological staining (H&E, Masson and TB) was then conducted to observe its structural change. With and without MSCs injection, complete healing of the IVD allograft as well as the restoration of the segmental and global mobility of the lumbar spine was seen. Nevertheless, MSCs injection immediately or post bony healing could not reduce the decrease of disc height; it was not able to rescue the degenerative changes of allografts based on the T1w MRI scanning or to prevent the loss of endplate integrity after UTE scanning, although hydration of the allograft in these three groups were still observed. Histological results further determined this negative effect. Therefore, IVD allografting was able to heal well with the recipient slot and to restore the motion of the lumbar spine, but its rapid degeneration could not be rescued by MSCs injection immediately or post bony healing.

a90954

40-SYIS CS-2 Immunomodulatory effects of adipose-derived stromal cells on adipose tissue regeneration in decellularized adipose tissue scaffolds

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Decellularized adipose tissue (DAT) scaffolds provide a highly adipo-inductive microenvironment and are promising cell-instructive matrices for volume augmentation in reconstructive surgery. Seeding DAT with allogeneic adipose-derived stromal cells (ASCs) has previously been shown to promote soft tissue regeneration in an immunocompetent rat model. Moreover, preliminary immunostaining suggested that the ASCs may modulate the response of infiltrating host macrophages involved in remodeling the scaffold into fat. However, the role of macrophages in adipose tissue regeneration remains poorly understood. The objective of this work was to investigate the interactions between ASCs and macrophages *in vivo*, with the goal of developing a deeper understanding of the immunomodulatory effects of ASCs on soft tissue regeneration, including the formation of new blood vessels and adipose tissue within the DAT.DAT scaffolds were prepared from discarded human fat. To facilitate cell tracking, ASCs were isolated from Actb-dsRed transgenic male mice, seeded onto the DAT, and implanted into the inguinal region of female MacGreen mice, in which cells of the myeloid lineage express EGFP. At 4, 8, 12 and 16 weeks, angiogenesis, adipogenesis and the macrophage response (IBA-1, CD163, CD80, iNOS, Arg-1, TNFα, IL-10) were assessed relative to unseeded controls through immunohistochemistry.ASC-seeding significantly enhanced host cell infiltration and angiogenesis within the DAT. For example, at 4 weeks, total cell infiltration and blood vessel counts were increased by 19% and 24% relative to the controls, respectively. Seeding the DAT with ASCs also augmented adipogenesis within the implanted region, with analysis of dsRed+ expression indicating that the newly-formed adipocytes were host-derived. IHC staining demonstrated that host macrophages had infiltrated the DAT implants. These DAT macrophages exhibited increased expression of pro-regenerative markers in the seeded group as evidenced by a 2-fold higher frequency of Arg-1+ cells at the 4-week time point in the absence of observable differences in iNOS cell frequency.Through their capacity to promote cell recruitment and modulate the immune response, ASCs can be a powerful tool to promote host-derived tissue regeneration within tissue-engineered bioscaffolds. By understanding the process of regeneration mediated by ASCs, we will be better able to harness their capacity to enhance tissue repair.

a91004

40-SYIS CS-3 Primary human corneal endothelial cell transplantation in an *ex vivo* system

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Purpose: The corneal endothelium is responsible for maintaining corneal clarity. However, this cell layer poses great challenges for clinicians due to its lack of regenerative potential and reducing cell population with age. This study investigates the potential of human corneal endothelial cells (HCEC), loaded with iron-based nanoparticles, to be magnetically-directed to injured regions of the cornea.

Methods: Primary cultures of human HCEC from CellProgen were maintained in human endothelial serum free media containing 10 ng/ml FGF-2 and plated at 75,000 cells in a 48 well plate for 24 hours. Cells were then exposed to 50nm dextran-coated biotin conjugated super paramagnetic iron oxide nanoparticles (SPIONP) at 37° C for up to 48 hours in serum free media. PCR and Prussian Blue staining were utilized to evaluate uptake of nanoparticles and cellular response by SPIONP dose. Mathematic modeling based upon stokes law, gravity, and magnetic field strength as well as fluid flow dynamics and outflow of the aqueous chamber were used to determine optimum SPIONP cell loading in relation to magnetic field strength for induced cellular movement within the aqueous chamber. Mathematical modeling efficacy was evaluated by injecting SPIONP loaded HCECs onto a denuded human corneal endothelium in the presence of an applied magnetic field.

Results: HCEC were successfully cultured and maintained their in-vivo marker expression of CD200. PCR revealed there was dose dependent impact on cell pump expression with SPIONP loading and an optimized loading dose was determined. When SPIONP loaded-HCEC were placed in solution with the denuded cornea, up to 1 million cells/mL, the cells showed targeted movement through the solution towards the externally applied magnetic field of 1.23 Tesla.

Conclusions: Proof of concept studies performed here indicate that cells with internally-loaded SPIONP can be directed and manipulated through an aqueous solution to a predetermined area when a magnetic field is applied. Mathematic modeling of the cell loading capacity and magnetic strength needed for this movement to occur can be an effective tool for tailoring specific ocular therapeutic needs for patients. Results of this study may lead to the development of a non-surgical technique to replenish this vital cell layer.

a91235

40-SYIS CS-4 Sophisticated cell microfactories co-encapsulating osteoblastic and adipose stem cells for bone regeneration

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The successful regeneration of large bone defects remains a significant challenge in orthopedic research. Along with tissue engineering and regenerative medicine (TERM) field evolution, new technologies aiming tissue repair have been emerged. Stem cells are the focus of many TERM applications and adipose derived stem cells (ASCs) quickly became attractive for bone tissue engineering. However, controlling stem cell multipontency and engineering bone *in vivo* remains a challenge, as it often leads to heterotypic and inferior osseous tissues. So, inspired by the multiphenotypic cellular environment of bone, we hypothesized that self-regulated liquified and multilayered capsules [1], loaded with ASCs and human osteoblasts (hOBs) cells are a promising attempt. The multilayered membrane ensures permeability to essential molecules for cell survival. Furthermore, the surface functionalized poly(ε -caprolactone) microparticles loaded into the liquefied core act as cell adhesion sites, allowing cells to construct their own 3D cell culture assembly system. For that, using the electrospraying technique, capsules encapsulating only ASCs or a co-culture with hOBs were cultured with or without osteogenic differentiation factors. Here, we aim to promote a well-orchestrated cell-to-cell interaction, evaluating the osteogenic potential of hOBs on ASCs. Moreover, the proposed capsules were tested using a rotary cell culture system to better mimic the dynamic environment of native tissues. Static culture conditions were tested as control. Results show the successful development of microtissues inside the controlled environment of capsules, even in the absence of osteogenic differentiation factors. We also observed that the bioreactor developed larger aggregates of cells and microparticles, providing biophysical stimulation and robust improvements in bone formation over static culture. Accordingly, we intend to use the proposed system as hybrid devices implantable by minimally invasive procedures for TERM applications.

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a91295

40-SYIS CS-5 Modelling Cell Survival and VEGF Gradients in Engineered Tissue Using a Joint Theoretical-Experimental Approach

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Therapeutic cell survival and the formation of VEGF gradients are crucial for the revascularization of engineered tissue, and the regrowth of neurites in the context of peripheral nerve repair. Mathematical modelling offers a time and cost efficient way of investigating this and similar complex problems in tissue engineering, especially when integrated with experimental work. In this study, a multidisciplinary approach was used to create a mathematical model of interactions between cells and oxygen and VEGF concentrations in a peripheral nerve repair construct (NRC). Schwann cell-like differentiated adipose derived stem cells were seeded at different densities in plastic compressed collagen gels and maintained at different oxygen levels. Viability and VEGF concentration after 24h were assessed using CellTiter-Glo (Promega) and ELISA. The mathematical model consists of three partial differential equations with terms representing processes including cell proliferation and VEGF secretion, and was parameterized against the data using COMSOL Multiphysics. Model simulations over a geometry representative of an NRC were run to predict the influence of different designs upon the spatio-temporal distributions of cells and VEGF. Results suggest seeded cell densities of fewer than 1.5E8 cells/ml may be optimal for achieving both cell survival and adequate VEGF concentrations, and that varied distributions of seeded cells could help induce steeper VEGF gradients. This framework could be used in other tissue engineering settings and expanded to include blood vessel growth in response to gradients. Model predictions like these could help reduce the number of experiments required and direct the form of future engineered tissue designs.

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a92006

40-SYIS CS-6 Engineered Fibronectin-based Hydrogels for Integrin-VEGF Promotion of Vascularisation

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Hydrogels are of growing interest for Tissue Engineering applications not only due to their intrinsic properties - similar to some characteristics of soft tissues - but also because of their versatility and tunability potential. Hydrogel can be functionalised with a variety of biological cues (e.g. cell-adhesive or protease-degradable peptides) to control cell behaviour. In this manner, substantial efforts have been made to sophisticate these systems as ECM mimetics in combination with proteins or protein fragments. These new systems have the capability to recapitulate complex interactions of cells with ECM proteins by presenting binding sites for several molecules, which can't be achieved by functionalising with small peptides. Here we show the incorporation of fibronectin (FN) to a poly(ethylene) glycol (PEG) hydrogel backbone. FN is a glycoprotein of the ECM that contains binding sites for several molecules such as collagens, heparins or growth factors. It has been recently shown that the exploitation of FN-growth factor synergistic interactions efficiently triggers stem cell differentiation and vascularisation [1, 2].
We have engineered new hydrogels based on the full FN protein. FN was covalently linked to a synthetic PEG polymer using Michael-type addition reaction, achieving gelation at physiological pH and temperature. FN concentration was controlled independently of the stiffness of the gel. This system showed cytocompatibility when cells were encapsulated within the hydrogels and it could be further engineered to control degradation rates. VEGF-loaded FN-PEG hydrogels showed a lower release profile compared to their homologous without protein, being able to retain up to 60% of the VEGF loaded. Moreover, VEGF binding isotherms showed the ability of these FN-based hydrogel systems to withhold more VEGF than their counterparts with no FN — acting as reservoirs for this molecule. We studied the angiogenic effect of these FN-VEGF interactions using 3D *in vitro* models, loading endothelial cells (HUVECs)-coated microcarriers and finding enhanced sprouting compared to non-FN gels. This system has also shown its angiogenic performance in chorioallantoic membrane assay. In addition, vascularisation has been investigated encapsulating HUVECs within VEGF-loaded FN gels showing cell rearrangement and early tube formation.

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a92723

40-SYIS CS-7 Tissue Engineering of a 3D Prevascularized Dermo-Epidermal Skin Model with Wound Healing Potential

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Tissue-engineered dermo-epidermal skin grafts hold a promising role regarding coverage of wounds in patients or as *in vitro* wound healing model. The latter implies avoidance of cost-extensive animal experiments and thus enables flexible and fast investigations of symptoms, new drugs or skin regeneration. Till date, there are only few fully-vascularized dermo-epidermal skin grafts, but still functionality and regeneration are inadequate as *in vitro* testing models. Therefore, we hypothesize that macrophage induction in a fibrin based skin model leads to a fully regenerated epidermis after laser treatment in a dermo-epidermal skin graft. For hydrogel moulding, endothelial cells from human umbilical vein and dermal fibroblasts aligning to capillary-like structures were embedded into a fibrin hydrogel. After 24 h, human keratinocytes forming the epidermal layer were seeded on top of the fibrin gel surface. After another 24 h, human macrophages were seeded as middle layer on the keratinocytes and 24 h later the second keratinocyte layer was added. Skin models were cultivated 10 days in air-liquid interface conditions before treated with a CO_2 laser. Hydrogels were terminated at day 0, 2 and 3 after laser treatment. As control group untreated samples with and without macrophages were chosen. Samples were stained (immune-) histologically visualizing the vascular network via CD31 staining as well as the stratification and regeneration of the keratinocyte layer with pan-cytokeratin staining. Macrophage localization was visualized by CD68 and CD163 staining. The use of GeneChip array and qRT-PCR revealed down-regulation of wound healing related genes at day 2 after lesion and up-regulation at day 3 in macrophage containing hydrogels vs. hydrogels without macrophages. Qualitative results from staining support the statement that macrophage induction accelerated the wound healing process compared samples without macrophage seeding. This study provides a fully vascularized fibrin gel scaffold with regenerative potential and early indications of wound healing after laser lesion.

a93097

40-SYIS CS-8 Functional integration of human ESC-derived retinal sheets after transplantation into immune-deficient retinal degeneration mice

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Abstract

Cell based therapy has recently emerged as a promising strategy to treat retinal degeneration (RD). Transplanted retinal cells have been shown morphologically integrated into the host in several visually impaired animal models. However, quantitative evaluation of recovered visual functions remain arduous. Also, the demand for proof-of-concept studies warranting clinical use of human ESC/iPSC-retinal cells is ever increasing. We have previously characterized the signs of visual function restoration in the advanced RD mouse model after allo-transplantation of mouse iPSC-retinal sheets. An immune-deficient RD mouse model (NOG-*rd1-2J*) we recently established has allowed us to similarly characterize xeno-transplantation of human ESC-derived retinal sheets. Here we present the following quantitative evaluation of visual functions by electrophysiology recording of light responses originated in the reconstructed retinal circuitry of xeno-transplantation.

The transplantation suitability of NOG-*rd1-2J* was tested with xeno- (human) and allo- (mouse) retinal grafts. Human ESC-retinal sheet maturation was confirmed with long-term survival and structured photoreceptor layer, and without sign of rejection or tumorigenesis in the host. Multi-electrode array (MEA) recording was conducted concurrently to assess the light response of reconstructed retinal circuitry by detection of both field potentials and host retinal ganglion cell (RGC) spikes. We largely found retinal ON-pathway dependent light responses from the host RGCs, which was consistent with whole-mount immunostaining suggestive of host-graft synaptic connection at the responding sites. The viably retained and/or reconstructed retinal circuits were further dissected out based upon the RGC light response patterns. Results from present xeno-transplantation were also subjected to comparison with allo-transplantation using mouse iPSC-retinal sheets we previously reported.

This study demonstrates a proof of concept for clinical use of human ESC-derived retinal sheets. In addition, the evaluation of restored retinal light response may serve as a standardized assessment for future pre-clinical practice.

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a90061

60-SYIS CS-1 Strong Adhesive for Broken Artery and Heart

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Hemostasis is a significant problem during surgical procedures and after major traumas. None of the existing hemostatic agents can rapidly stop massive hemorrhage like arterial damage and penetrated cardiac injuries, due to their weak adhesive strength on wet tissue surfaces. We designed a photoreactive tissue-adhesive matrix gel mimicking the natural extracellular matrix (ECM) composition of the connective tissue that could adhere strongly on wet and dynamic tissue surfaces and is not compromised by pre-exposure to blood. It could endure up to 236.44 ± 48.33 mmHg blood pressure, which was much higher than the normal arterial blood pressure (120 mmHg). Most importantly, this matrix gel provided instant hemostatic seal, within one second under UV light exposure (365nm, 60 mW/cm^2), when applied to carotid arteries with a 2~3 mm defect and hearts with a whole-layer cardiac penetration hole (3-mm diameter). The pigs survived normally after carotid artery and heart bleeding hemostasis, which makes the matrix gel a promising adhesive for clinical hemostasis and repair of vascular defects.

a90764

60-SYIS CS-2 Regenerative potential of exosomes isolated from clinical grade oral mucosal epithelial cell sheet production

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The oral mucosa exhibits a unique wound healing potential, which is sometimes referred to as "fetal-like wound healing". Wounds that would result in scars in other anatomical locations can often be healed without any scar formation in the oral mucosa. Members of our institute have used oral mucosal epithelial cell (OMEC) sheets in clinical regenerative medicine applications to replace corneae and prevent stricture formation in the esophagus after superficial cancer resections (endoscopic submucosal dissection). Although the underlying mechanism of these cell sheet therapies remains unknown, increasing evidence suggests that paracrine factors, including exosomes, are major contributors to the regenerative potential of many cell therapies. To this end, we isolated exosomes from media that was used for cell sheet culture ("conditioned") and left-over media that was never in contact with the epithelial cells (control) from clinical grade OMEC sheet production. The exosomes were characterized by western blot (+CD9, +Flotillin, -GRP94), nano tracking analysis (~120nm), protein quantification and electron microscopy. We found that the exosomes suppressed fibroblast proliferation, without being cytotoxic, and led to a remarkable increase in growth factor production (qPCR: HGF, VEGFA, FGF2, CTGF, ELISA: HGF). The exosomes also induced a small, but significant, reduction in *S. aureus* growth. We further investigated wound healing in a rat model. Fluorescent exosomes were topically added to full-thickness wounds. They adhered spontaneously and their signal could be detected until the final day (day 6). Preliminary histological analyses revealed a greatly reduced wound area (60% reduction compared to vehicle control) in the conditioned group only. In conclusion, we have here made the first steps towards understanding the OMEC sheets' mode-of-action from an exosome perspective.

Conflict of interest

The Authors declare no conflict of interest.

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a91017

60-SYIS CS-3 Interleukin-4 Functionalised Bioactive Vascular Grafts Modulate Inflammation and Inhibit Neointimal Hyperplasia

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Introduction: Synthetic vascular grafts uniformly fail in low diameter (<6mm) applications due to inflammation driven neointimal hyperplasia (NH). Macrophage phenotype, dictated by interaction with the graft surface, is critical to regulation of this local immune response. Classical pro-inflammatory M1 macrophages are polarised in vivo by interleukin-4 (IL-4) to the anti-inflammatory M2 phenotype. We have developed a novel vascular graft with an IL-4 functionalised surface that enhances M2 macrophage polarisation, strikingly reducing NH.

Methods: Small diameter conduits and flat sheets of polycaprolactone (PCL) were produced by electrospinning. Bombardment of the PCL graft surface with nitrogen ions through plasma ion immersion implantation (PIII), resulted in the creation of reactive radical groups on the surface of the electrospun PCL [1]. These reactive groups facilitated covalent immobilisation of IL-4 from solution onto PCL surface in a single-step reaction. Functionalised PCL with the appropriate controls were assessed in vivo via a mouse subcutaneous implant and mouse carotid artery interposition grafting [2].

Results: In a mouse carotid grafting model, M2 macrophages were enhanced in IL4-functionalised grafts (89±22% vs 33±2% in PCL, $p<0.05$), corresponding to a 49±6% decrease in pro-inflammatory IL-1β ($p<0.05$) and 114±9% increase in anti-inflammatory IL-10 ($p<0.05$). These immunomodulatory effects resulted in a 57 ± 10% ($p<0.05$) reduction in NH. Similar in vivo immunomodulatory effects were confirmed in a second well-established model of acute wound inflammation.
Conclusion: Imparting the biological effects of IL-4 onto graft surfaces locally regulates macrophage phenotype and production of inflammatory cytokines, observed in two separate mouse models. In a model of vascular grafting, we observed these effects were consistent with striking reductions in NH demonstrating significant implications for the improved long-term performance of synthetic vascular grafts.

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a91370

60-SYIS CS-4 Inhibition of NF-κB Signaling by Controlled Release of Selective IKKα Inhibitor from Silk Fibroin Scaffold Effectively Promotes Keratocyte Phenotype and Corneal Regeneration

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The expression of IL-1β in the cornea is increased in various corneal diseases including chemical burns and herpetic stromal keratitis, which leads to corneal inflammation and angiogenesis. In the current study, upregulation of IL-1β is detected in rat cornea alkali-burn model and ex vivo human cornea culture model following sodium hydroxide treatment. IL-1β induces the loss of keratocyte phenotype and extracellular matrix formation in cultured keratocytes through the activation of NF-κB signaling pathway. Inhibition of NF-κB signaling by selective IKKα inhibitor BMS 345541 blocks the effect of IL-1β in a dose-dependent manner. Therefore, we develop a strategy of controlled release of BMS 345541 from silk fibroin scaffold to alkali burns corneas in rats, which effectively promotes keratocyte phenotype and corneal regeneration. These findings demonstrate that selective IKKα inhibitor BMS 345541 dampens IL-1β-induced abnormal keratocyte phenotype via blocking NF-κB signaling and therefore is a promising strategy for the treatment of IL-1β - related corneal diseases.

a91717

60-SYIS CS-5 Stem cell derived extracellular vesicles for lung tissue regeneration

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Current treatment regimens for lung injuries are mainly supportive and rely on self-regeneration processes for recovery. The aim of this study was to **demonstrate the regenerative capability of stem cell-derived extracellular vesicles (EVs) for the lung tissue repair** and their potential use in inflammatory and fibrotic conditions of the lung. Our previous work demonstrated that **mesenchymal stromal cells facilitate functional tissue regeneration** after smoke inhalation injury, which was concluded to be **orchestrated by paracrine signals (including EVs)** produced by the stem cells. EV-based therapy for tissue repair (e.g. acute and chronic lung injuries) is increasingly investigated because of their anti-inflammatory and immunomodulatory properties.

We isolated EVs from placenta mesenchymal stromal cells (DMSC23) and validated their physico-chemical and molecular properties using ISEV-recommended protocols (NanoSight, qNano, SEM, TEM). **Moreover, we characterised individual vesicles and their populations using atomic force microscope infrared spectroscopy (AFM-IR) for the first time, to identify nanoscale differences in molecular and structural composition of EVs, at a resolution <20 nm.** The use of nanoscale characterisation is critically important as EVs are heterogeneous, which affects the nature and quantity of the biological payload that signals recipient cells, and ultimately modifies their function. The nanoscale analyses of DMSC23-EV structure and composition enabled us to select the optimal specialised EVs to facilitate tissue regeneration.

We demonstrated that EVs derived from placenta stem cells contain key miRNAs (OpenArray profiling) that control tissue repair. EVs were shown (real time imaging and wound closure assay) to modulate cell migration. We developed a cell injury model and demonstrated reduced cellular stress (nitric oxide expression) and inflammatory cytokines (IL-6) when cell were treated with the EVs. In summary, these results highlighted the role of EVs in tissue repair and their **potential to enhance the effectiveness of therapy for accelerated repair and regeneration of the lung.** The study achieved an important milestone for future applications of EVs in tissue repair and in their use for other lung diseases, crucial to devising more **personalised and effective therapies.**

a92129

60-SYIS CS-6 Biomimetic Recombinant Coatings with Antimicrobial Properties for Preventing Implant Infections

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Peri-implant inflammations are on the increase, challenging research across numerous fields towards coatings that aim to counter this pathology. One of the main threats for implants is bacteria colonization and biofilm formation. In this regard, developing covalent coatings for titanium implants that prevent the bacteria attachment and the development of the infection, ensuring the biointegration is crucial. In this study, the antimicrobial and anti-biofilm activity of recombinant coatings was evaluated covalently attached onto Titatium discs. The novel recombinant materials are based on a polycationic Elastin-Like Recombinamer (ELR) backbone and the antimicrobial peptide (AMP) GL13K, combining the extracellular-matrix (ECM) mimicking property of the ELR and the antimicrobial properties of the GL13K. In this work, we investigated the antimicrobial capacity of the chimeric recombinamer and the effectiveness of the coatings.

Titanium grade II discs were covalently functionalized via silanization with the recombinant biomaterials and incubated in the presence of relevant pathogens in the implant colonization and biofilm formation (*S. gordonii* ML-5, *S. mutans* ATCC® 700610™, *S. epidermidis* ATCC® 35984™ and *S. aureus* ATCC® 25923™) and also against complex multi-species oral stocks that allow simulate oral microbiota. Afterwards, ATP-activity, CFUs, SEM visualization and live-dead staining of the biofilms were assessed. The cytocompatibility was tested by an AlamarBlue® assay after the incubation of primary human gingival fibroblasts over the surfaces. Bacterial analysis revealed that new coatings provides strong anti-fouling and anti-biofilm activity, even against multi-species stocks, enhancing cell adhesion and proliferation of oral cells. These results suggested that these chimeric recombinamers enable the formation of covalent coatings for medical materials with high resistance and antimicrobial activity.

We developed an innovative recombinant strategy for the production of a new chimeric biomaterial that combines the properties of ELRs and AMPs. The covalent functionalization of Ti discs and the antimicrobial and anti-biofilm activity of the coatings were demonstrated. Thus, these novel coatings could lower the severity of bacterial infections, preventing biofilm formation and improving the implantation process due to ECM-mimicking properties of the ELR in dental implants or in other medical devices, such as prosthesis or vascular devices.

a92554

60-SYIS CS-7 Local Delivery of Flavopiridol Repairs Spinal Cord Injury by Regulation of Astrocytes and Inflammation

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The repair of spinal cord injury (SCI) is closely related to inflammatory cytokines, among which quite a few have been demonstrated detrimental or beneficial to repair. The sequential changes of local inflammatory cytokine protein levels after rat SCI are still not clear. So we first studied the sequential changes by multiplex immunoassay and found 4 cytokines that might be beneficial to repair decreased after SCI, and 9 cytokines that might be detrimental to repair increased.

Flavopiridol has been reported to significantly improve motor recovery and decrease reactivity of astrocytes which are the important source of inflammatory cytokines. So we asked whether flavopiridol could regulate the synthesis of inflammatory cytokines in astrocytes. We found that flavopiridol inhibited proliferation, scratch-wound healing, and inflammatory factor synthesis in astrocytes, while permitting the survival of neurons. Hence, flavopiridol has differential effects on astrocytes and neurons, indicating its potential promotion on SCI repair.

Flavopiridol is a broad-spectrum inhibitor. Its high systemic dose may cause strong side-effects. The mini-osmotic pump used for intrathecal flavopiridol delivery is costly and may cause problems with histocompatibility. Biodegradable and injectable Poly (lactic-co-glycolic acid) (PLGA)-based methylprednisolone nanoparticles (NP) have been used in SCI repair, and the NP-enabled local delivery is significantly more effective than systemic delivery. So we aimed to develop a strategy for the local delivery of flavopiridol and to evaluate this strategy. We showed that flavopiridol NP had a sustained release of up to 3 days. Flavopiridol NP significantly decreased the pro-inflammatory factor synthesis by astrocytes, while the IL-10 expression was elevated. Flavopiridol NP decreased the cell-cycle related protein expressions of astrocytes, neurons and macrophages in vivo. They also increased the integrity of spinal cord gross tissue structure, inhibited the glial scarring and cavitation, and facilitated neuronal survival and regeneration. Multiplex immunoassay showed that flavopiridol NP affected local inflammatory cytokine profile. They increased GM-CSF while decreased IP-10. Administration of flavopiridol NP eventually improved the functional recovery of injured rats. These findings demonstrated that local delivery of flavopiridol in PLGA NP improves recovery from SCI by regulation of astrocytes and inflammation.

a92594

60-SYIS CS-8 Safety and efficacy of sustained release of basic fibroblast growth factor using gelatin hydrogel: Phase I–IIa clinical trials in patients with limb or myocardial ischemia

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BACKGROUND: Our previous studies demonstrated that sustained release of basic fibroblast growth factor (bFGF) using gelatin hydrogel enhanced angiogenesis in animal models of hindlimb or heart ischemia. We conducted two phase I–IIa clinical trials to examine the safety and efficacy of bFGF-incorporated gelatin hydrogel in patients with limb or myocardial ischemia.
METHODS: Ten patients with critical limb ischemia (CLI) underwent a 200 μg intramuscular injection of bFGF-incorporated gelatin hydrogel microspheres (males, 7; mean age, 66.9 ± 12.2 years). Primary endpoints were safety and transcutaneous oxygen pressure ($tcPO_2$) at 4 and 24 weeks after treatment. Two patients, a 47-year-old man and a 76-year-old woman, with ischemic heart disease (IHD) had coronary artery bypass grafting (CABG) with concomitant implantation of gelatin hydrogel sheet (GHS) containing 200 μg of bFGF on the surface of the avascular myocardium. Primary endpoint was safety at 24 weeks after implantation. Secondary endpoints included symptoms, cardiac function evaluated by magnetic resonance imaging (MRI), and myocardial perfusion evaluated by myocardial perfusion scintigraphy at 4 and 24 weeks after treatment.
RESULTS: Neither death nor serious adverse event was observed in both studies. In the study for patients with CLI, $tcPO_2$ significantly improved at 24 weeks after treatment (28.4 ± 8.4 vs. 46.2 ± 13.0 mmHg for pretreatment vs. after 24 weeks, respectively, $p < 0.01$). In the study for patients with IHD, New York Heart Association functional class was improved in both patients at 24 weeks after treatment (pretreatment to 24 weeks after treatment; class II to class I, class III to class I). MRI revealed that left ventricular ejection fraction increased in both patients (41.5% to 47.7%, 63.4% to 77.2%), indicating that CABG improved cardiac function. Per-segment reversibility score measured by scintigraphy, defined as the difference of a score per GHS-implanted myocardial segment between at rest and at stress, was unchanged or reduced (0 to 0, 1.5 to 0), suggesting that the implantation of bFGF-incorporated GHS may have possibly improved myocardial perfusion.
CONCLUSIONS: The sustained release of bFGF using gelatin hydrogel may offer a safe and effective modality of therapeutic angiogenesis for patients with CLI or IHD. Further study with a large number of patients is warranted toward broad clinical application.

a91551

60-SYIS CS-9 Solutions Towards Implementing a Multisystem Cell Therapy for Improvement of Urinary Continence: MUSIC project

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Stress Urinary Incontinence (SUI) is a disease affecting over 200 million people worldwide and is twice as common in women as in men. Childbirth and menopause are major reasons of the increased prevalence in women, whereas prostatectomy is one of the main causes in men. The significantly reduced quality of life; 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.
Early clinical trials using stem, or progenitor cells in both male and female patients have promising functional results with minimal adverse effects. However, as simple as it seems to be, the precise identification, isolation and transplantation of these cells seems to be more complex than originally expected.
Within our international, H2020-funded, project consortium MUSIC, we identified some points, which seem to be fundamental for the long-term success of this TE therapy: (1) the production of autologous cells needs to skip the costly GMP-site to become more affordable and efficient;(2) the final product needs to be completely xeno-free;(3) the cell-transplantation needs particular precision and (4) it needs to be supported by pelvic floor stimulation with neuromuscular-electromagnets for effective functional regeneration of the urinary sphincter.
Together, with combined expertise and efforts, we are working towards providing such a novel autologous progenitor-cell-based multisystem-therapy, which already shows great potential as a feasible solution for the current bottlenecks of applying a cellular therapy to SUI patients, that we currently face in a PhI clinical trial.

a90129

70-SYIS CS-1 Nanospaced adhesive peptide domain governs mesenchymal stem cell fate via YAP/TAZ pathway modulation

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The ability of mesenchymal stem cells to sense changes in extracellular matrix (ECM) composition in their local microenvironment is crucial to their survival. Transmembrane receptors that have specificity for peptide sequences within individual ECM molecules permit cells to rapidly assess the mechanical properties of the ECM, and respond by recruiting intracellular proteins to form focal adhesion (FA) complexes. Our previous data showed that increased lateral nanospacing (30 to 60 nm) of RGD peptides led to lower cell spread areas and less mature FA formation in human mesenchymal stem cells (hMSCs). However, the exact underlying mechanism driving these substantial differences remains unclear. In this work, we created tailored surfaces of self-assembled, azide-functionalized polystyrene-block-poly (ethylene oxide) copolymers (PS-PEO-N_3) with controlled lateral spacing of RGD peptides (~30 and 60nm) on PEO-N_3 nanodomains. We observed that smaller spacings led to nuclear localisation of YAP/TAZ, however, YAP/TAZ remained cytoplasmic for larger spacings. Smaller (30nm) nanospacing resulted in lower vinculin tension sensor (VinTS) FRET activity, indicating the development of higher levels of tension at the site of focal adhesions compared to larger (60nm) nanodomain spacings. By combining fluorescence-lifetime imaging microscopy (FLIM) and VinTS FRET, we observed that the recruited vinculin also remained in these FAs for longer at smaller nanodomain spacings, with fluorescence life times of up to τ =2.78ns, compared to τ =2.26ns on larger nanodomain spacings. More FAK-GFP expression was found to colocalize at FA sites with longer FA lifetimes on smaller nanodomain spacings when compared to larger nanospacings, suggesting the formation of more mature FAs. In terms of modulation of intracellular signaling pathways associated with mechanotransduction, higher RhoA and Src FRET activity and lower Rac FRET activity were noted when the lateral spacing of peptides was decreased from 60nm to 30nm. Moreover, nuclear YAP/TAZ localisation correlated with higher nuclear RUNX2 expression after osteogenic differentiation on both spacings. It was found that YAP/TAZ expressed mainly cytoplasmic when hMSCs underwent adipogenic differentiation.
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a90812

70-SYIS CS-2 NOT AVAILABLE

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NOT AVAILABLE

a90963

70-SYIS CS-3 Discovering New Biomaterials that Influence Mesenchymal Stem Cell Function and Fate: Working at the Crossroads of Chance and Knowledge

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Cell-material interactions can be used as a regulator of stem cell fate through properties such as chemistry and surface topography. High throughput screening of large numbers of diverse chemistries can speed up the discovery of biomaterials for various applications, including regenerative medicine and medical devices. Polymer microarrays are a key enabling technology for the discovery of new biomaterials, and have been previously used to identify novel polymers that resist bacterial attachment[1] and substrates on which to mature cardiomyocytes[2].

Polymer microarrays consisting of 298 different polymers were fabricated by UV curing of methacrylate and methacrylamide monomers deposited using a pin printer (Biodot). These large, chemically diverse libraries were screened to identify materials capable of inducing osteogenesis in primary human mesenchymal stem cells (hMSCs). Polymers were ranked by attachment after 48 hours in culture to identify hits. Cell attachment and spreading were quantified by α-tubulin & Hoechst 33342 staining using automated image analysis. The influence of the polymer substrates on cell fate was also investigated by assessing expression of early and late markers of osteogenesis in hMSCs derived from several donors, such as alkaline phosphatase and osteocalcin, using high-throughput imaging and analysis techniques.

Cells displayed a range of morphologies on polymer surfaces, including rounded, polygonal and fibroblast-like morphologies. A wide range of cell attachment and spreading responses have been observed. Quantitative methods for assessment of osteogenesis on microarrays have also been developed to screen for polymers that induce increased expression of osteogenic markers. Surface analysis and cell attachment and differentiation data will be used together to identify patterns and interactions that may be associated with lineage commitment of hMSCs.

Looking ahead, combinations of 2D and 3D approaches will be used to identify polymers that influence stem cell fate and advance our understanding of the effects of chemistry on MSCs. The hundreds of different polymer-cells interactions observed on microarrays can be used to elucidate underlying structure-activity relationships and give us an insight into how they function. This will be vital to help develop materials for regenerative medicine applications and scaffolds for stem cell delivery.

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a90983

70-SYIS CS-4 Using additively manufactured auxetic structures to simplify the application of biaxial tensile strains to cells cultured in 2D and 3D

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The application of cyclic strains to cells *in vitro* is an important technique for understanding how the *in vivo* environment of dynamically loaded tissues such as blood vessels, heart and lungs affect the cells[1]. It can also provide a way to condition engineered tissue or create improved tissue mimics for drug testing. The main modalities to apply strains to cells include stretching objects clamped between linear actuators and pneumatic stretching. Stretched cells are cultured in 2D, by attaching them on membrane or hydrogel surfaces, or in 3D by embedding them in hydrogels.

Applying strains to cells in 3D is often challenging due to difficulties in clamping hydrogels to linear actuators or binding them to stretchable membranes. Also, with current techniques, it is challenging to apply biaxial strains using linear actuators due to the need for multiple actuators, which is especially challenging for small samples.

To overcome these challenges, we have combined additively manufactured auxetic structures[2] with hydrogels. The resulting constructs were loaded to provide controlled straining on cells cultured in 2D (on the hydrogel surface) or in 3D (embedded in the hydrogel). The hydrogels are formed around the fibers of the auxetic structures and stay in place or stretch with the fibers by confinement gripping. No binding of gels to fibers is needed, thereby making the technique non-specific to the materials used. The auxetic nature of the structures allows for the generation of biaxial tensile strains using a single axis linear actuator.

Auxetic meshes have been additively manufactured, hydrogels (collagen and polyacrylamide) have been formed around them, and strains have been characterized using COMSOL modeling. Empirical validation will be done by tracking embedded beads. Cells have been cultured on and in gels, and strains with various anisotropies and magnitudes have been applied. The effects of the strains on the cells are currently being studied. While initial stretch testing was performed in a commercial biodynamic chamber on a mechanical tester, low-cost stretching chambers that work inside cell-culture incubators have now been developed and are currently being tested.

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a91273

70-SYIS CS-5 Dact1 And Dact2: Novel Regulators Of Mesenchymal Stromal Cells

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Mesenchymal Stromal Cells (MSCs) are thought to participate in tissue repair and regeneration. MSCs can be isolated from adult tissues like bone marrow, fat pad, and synovium, and are consequently promising candidates for regenerative therapies in osteoarthritis (OA). A number of cell signalling pathways regulate MSCs with Wnt and TGF β believed to play crucial roles. The Dishevelled Binding Antagonist of Beta-Catenin (Dact) gene family encodes several proteins that modulate Wnt and TGFβ signalling, however no study has investigated the role of Dact proteins in MSCs and during chondrogenesis.

We initially performed immunohistochemistry for DACT1 and DACT2 and observed DACT proteins in stromal compartments of the developing joint in mouse embryos (E13.5 to E18.5). In 2 weeks-old mice, DACT1 and DACT2 proteins were detected in bone marrow, synovium and fat pad. These results suggested that DACTs are involved in joint development and are present in the tissue compartments where MSCs can be found. We then assessed the role of DACT1 and DACT2 in human MSCs in vitro. For the first time, we show that iRNA knock-down of DACT1 in hMSCs promotes cell death via apoptosis (downregulation of Birc5). Whilst the knockdown of DACT2 does not impact on cell survival. We, therefore, assessed the chondrogenic potential of DACT2 knockdown MSCs in 3D culture. After 3 weeks in the absence of TGFβ, we observed by histological analysis that DACT2 knockdown modulates chondrogenic differentiation of MSCs. In conclusion, DACT1 plays a role as a negative regulator of MSCs apoptosis, whereas DACT2 is involved in MSCs chondrogenic differentiation. With this work, we have identified for the first time DACT1 and DACT2 as important mediators of MSCs survival and chondrogenic differentiation.

a91338

70-SYIS CS-6 The Immune Modulating Properties of Extracellular Vesicles (EVs) from Human Cardiac-derived Adherent Proliferating (CardAP) cells

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Inflammation plays a Janus-faced role in the regenerative process of damaged tissues, like in the myocardial infarcted area. While an initial pro-inflammatory milieu is essential for the initiation of regeneration, a prolonged inflammation will oppose repair and replacement. Accordingly, a suitable tool would be therapeutics, which are capable to diminish a long-lasting inflammation.

In this study, we investigated the potential of extracellular vesicles (EVs) from cardio-protective human cardiac-derived adherent proliferating cells (CardAP) to modulate immune responses. Therefore, EVs have been isolated and analysed concerning their phenotype as well as for their modulatory effects on human immune cells *in vitro*. In general, EVs were isolated by differential ultracentrifugation of the conditioned medium from CardAP cells (n = 4 - 6 donors), which were kept for 20 hours under serum-free conditions or with an additional cytokine stimulation of IFN γ , TNF α and IL-1ß. Both EV-types showed a mean diameter of about 95nm as determined by nanoparticle tracking analysis and electron microscopy. Proteins, like tetraspanins or major histocompatibility complex (MHC) class I molecules, could be detected on their surface by flow cytometry. Other markers were expressed exclusively on the cytokine-induced EVs, as intercellular adhesion molecule 1 (ICAM-1). For the functional studies, peripheral blood mononuclear cells (PBMCs) were co-cultured with EVs from CardAP cells for 3 or 5 days with either phytohemagglutin (PHA), aCD3, aCD3/CD28 or without stimulation. The unstimulated co-culture of both EV-types with PBMCs did not induce any T cell proliferation. Analysis of antigen presenting cells (APCs) in co-cultures of complete unstimulated PBMCs or purified CD14+ cells with EVs revealed a significant downregulation of MHC class II molecules. Furthermore, the PHA-, aCD3- and aCD3/aCD28-induced T cell proliferation was diminished by the treatment with both types of CardAP EVs as compared to the PBS treated control. This was accompanied by lower levels of the pro-inflammatory cytokine IFN γ in supernatants of PHA-stimulated PBMCs with EVs as compared to the controls.

Our results indicate that CardAP EVs are capable to modulate ongoing immune responses towards a lower inflammatory state *in vitro*, which might be of relevance for therapeutic purposes. Future *in vitro* studies are necessary to evaluate the mode of action, especially the implication of the EVs interaction with APCs.

a92653

70-SYIS CS-7 Oscillating magnetic fields post-transfection can promote endosomal escape and enhanced transgene expression for GET-mediated transfection

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Nucleic acid therapies aim to augment, inhibit or correct endogenous gene activities which can programme cell behaviour or repair molecular defects in specific disorders. Efficient delivery, for plasmid (pDNA) transgenes, meaning cell uptake and nuclear trafficking, still remains a challenge. For in vivo gene therapy strategies the lack of targeting and concentration achieved in the focus tissue, as well as difficulty in maintaining significant long-term expression has confounded non-viral approaches. To overcome these challenges magnetic targeting of the non-viral system has been developed, which involves the delivery of nucleic acids associated with magnetic (nano) particle (MNPs) carriers under the influence of a magnetic field.

Most optimisation of magnetofection has been focused on the application of magnetic fields for targeted delivery, efficiently enhancing the kinetics and concentrating the therapeutic to a specific area both *in vivo* and *in vitro* [1]. However, very little work has aimed to understand the transfection process after uptake by the cell. We have developed a peptide based transfection system which can be electrostatically or covalently grafted onto the surface of dextran coated MNPs. This system is termed GET (GAG-binding enhanced transduction) and its transfection efficiency relies on the synergistic combination of a cell penetrating peptide and a membrane docking peptide to heparan sulphate glycosaminoglycans (GAGs) together with a LK15 sequence for pDNA condensation[2].

In this work, we have optimized the GET-MNPs-DNA system for fast (<1min) and efficient (>50%) transfection by applying a magnetic field. We assessed the effect of oscillating magnetic fields to further enhance DNA transfection efficiency[3]. We demonstrated that once uptaken the GET-MNPs-DNA complex is located in endosomes and that oscillation of a permanent magnet array post transfection can prolong the long-term expression of the delivered gene (by several days), suggesting that the mechanical force exerted by the particles under the oscillating magnetic field promotes endosomal escape. These phenomenon has so far not been described and will provide better understanding of intracellular trafficking and a tool to enhance magnetic gene delivery towards clinical application.

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a93167

70-SYIS CS-8 The synergistic effect of mechanical and biochemical stimulation on 3D multi-layered scaffolds for tendon tissue engineering

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Tendon injuries and degeneration have high incidence and subsequent negative impact on people activities and quality of life [1]. Tissue engineering aims to overcome limitations of autografts, allografts, xenografts and prosthetic devices through designing engineered biological substitutes which are capable of reproducing the native tissue structure and promoting repair, healing and regeneration. Fiber-based approaches have been considered for tendon regeneration due to the possibility of manufacturing fine filaments which can mimic the natural collagen fiber orientation [2]. In this study, we have designed and fabricated three-dimensional (3D) multi-layered scaffolds formed by electrospun nanofibrous structures coated with thin layers of human mesenchymal stem cells (hMSC)-laden hydrogel. The fibrous matrix provided adequate mechanical properties, while the hydrogel properly emulated the microenvironment and properties of the native extracellular matrix, guaranteeing good cell spreading, proliferation and an adequate 3D cellular distribution. A bioreactor model was also designed and developed to apply biomechanical stimulation to the cells encapsulated within the engineered scaffolds. An optimized amount of bone morphogenetic protein 12 (BMP-12) was added to the constructs to promote hMSC proliferation and tenogenic differentiation. Our results demonstrated that the addition of BMP-12 induced tenogenic differentiation more effectively during dynamic stimulation compared to static conditions. Moreover, dynamic culture promoted cellular integrin-mediated focal adhesions and cytoskeleton deformation response which resulted in a preferential, longitudinal cell orientation and collagen I deposition. The synergistic effect of mechanical and biochemical stimulation resulted into MSC proliferation, alignment and differentiation as well as collagen I deposition, suggesting that the proposed 3D multi-layered system can be used for engineering functional tendons.

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a93487

70-SYIS CS-9 Effect in proliferation and differentiation of osteoblast *in vitro* under electrical field

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Several studies have proved that bone tissue may have the ability to respond to biophysical stimuli such as electric and electromagnetic fields. However, one of the greatest difficulties in understanding how these affect the functions of the osteoblast is matter of study yet; because there is a high variability in the design of the systems and therefore in the electrical fields (EFs) applied to the in vitro models. Additionally there is no evidence about the procedure to calculate electrical field (EFs). Previously, we have proved a new framework to calculate EFs and designeda novel device based on a parallel plate capacitor, to generate and distribute the EFs homogeneously over cell culture of condroblast. In the present work, the goal was to evaluate the effect of applied EFs of 4 mV/cm and 8mV/cm with three exposure times (30min, 1 and 5 h) in osteoblast-like cells isolated from new-born mouse, using the same system. We evaluate the effect of EFs on cell proliferation with MTS assay, as well as on the osteogenesis process when evaluated the formation of mineralization nodules, alkaline phosphatase activity and osteopontin expression by immunostaining. We have determined that EFs applied for 30 min and 1h improve cell proliferation; while, if applied for 5h the proliferation is inhibited. Additionally, it was shown that EFs seem to promote cell differentiation. In conclusion, this system confirm the effect of EFs on bone cells, and this device design can be used to modulate the osteoblast physiology in vitro and therefore we can develop better biomimetics systems to use in tissue engineering. Keywords: osteoblast, electrical field, tissue engineering, alkaline phosphatase (ALP) and osteopontin

a90581

80-SYIS CS-1 Engineering and Analysis of Normal and Malignant Neurogenesis Microenvironment for Physiologically Reproducible Therapeutics Screening Facilitated by Perfusion

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Pre-clinical testing of new therapeutics relies on the use of *in-vitro* and *in-vivo* models. One major weakness of this system is the inadequate recapitulation of physiological conditions *in-vivo*, including the extracellular matrix and the consistent supply of nutrients and oxygen via the microcirculation perfusion, which then adds to the spectrum of selective pressure that leads to the heterogeneity and ultimately fate of both normal and malignant neural tissues[1-3]. Tissues and organs artificially grown using engineered devices are seen as a promising type of advanced *in-vitro* models to initially reduce and eventually replace current conventional *in-vitro* and *in-vivo* models[1-2]. Nevertheless, most of the prototypes have so far lacked perfusion, without which these advanced *in-vitro* models cannot recapitulate key aspects of tissue microenvironment such as the interstitial fluid, the microcirculation of blood vessels and immune system, which are particularly crucial for neurogenesis[1]. In Oxford we are establishing a perfusion culture and analysis platform involving collaboration among three departments. Previously, a micro-bioreactor maintaining tissues with 200-400μm thickness via long-term observation (14-35 days) has been developed[1,2]. Co-culture of tumour cells with angiogenesis-related cells has also been applied[2,3], and the relations between interstitial flow dynamics with tumour growth kinetics have been elucidated[2]. We demonstrated that differentiation status and therapeutic responses were significantly different under perfusion, and heterogeneous co-culture suggested selective cell-killing under therapeutic perfusion versus episodic delivery. We will also present some preliminary data from a microfluidic platform, developed based on the above bioreactor, with an improved spatial-temporal control of the cultured micro-tissues.

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a90682

80-SYIS CS-2 Creating micron-size 3D living biointerfaces for stem cell differentiation induced by non-pathogenic bacteria

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In this study, we demonstrate the fabrication of micron-sized 3D environments for stem cells in which their fate can be temporally controlled via inducible growth factors expressed by co-existing bacteria (previously established by our group). Using a droplet-based microfluidic platform, we produce alginate hydrogel constructs of high mono-dispersity and high encapsulation efficiency while maintaining high cell-viability of both prokaryotic and eukaryotic cells. The developed platform allows for the fabrication of customised alginate constructs with different chemical composition (i.e.; functionalised or entrapped growth factors) and physical features (size, width and stiffness). In this study, we show the differentiation of encapsulated MSC cell line into osteoblast in presence of inducible Lactococcus lactis for BMP-2 and FN7-10 over the course of 2 weeks. Furthermore, we applied the droplet-based microfluidic platform as a bio-printing system, where three different type of cells are introduced via individual microfluidic channels. This feature enables experiments in which cells population density can be fine-tuned.

a90747

80-SYIS CS-3 Synthetic embryos generated solely from stem cells

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The blastocyst is the early mammalian embryo, which forms all embryonic and extra-embryonic tissues, e.g., the placenta. It consists of a spherical thin-walled layer, the trophectoderm, that surrounds a fluid-filled cavity sheltering the embryonic cells. From mouse blastocysts, both trophoblast and embryonic stem cell lines can be derived, which are *in vitro* analogs of the trophectoderm and embryonic compartments, respectively. Here, we report that trophoblast and embryonic stem cells cooperate *in vitro* to form structures that morphologically and molecularly resemble blastocysts (*'blastoids'*). Like blastocysts, blastoids form *via* inductive signals originating from the inner embryonic cells and driving the outer trophectoderm development. The nature and function of these signals are largely unexplored. Genetically and physically uncoupling the embryonic and trophectoderm compartments, along with single cell transcriptomics, reveals that the embryonic cells maintain trophoblast proliferation and self-renewal, while fine-tuning trophoblast epithelial morphogenesis. Finally, we show that these embryonic signals are paramount for blastoids to robustly implant and nest *in utero*. Altogether, this data shows that the communication occurring between embryonic and trophoblast stem cells can be recapitulated *in a dish* to form synthetic embryos.

a90962

80-SYIS CS-4 Novel primary fibroblast-derived 3D constructs to examine the role of the extracellular matrix in oral cancer progression

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Introduction: During cancer progression regulation is determined by interactions between tumour cells, stromal cells (fibroblasts and leukocytes) and other components of the microenvironment such as the ECM. The ECM is predominantly deposited by cancer-associated fibroblasts (CAF) and plays a key role in cancer progression. Although considerable evidence exists demonstrating a role for CAF in oral squamous cell carcinoma (OSCC), little is known about their influence on ECM: tumour interactions.
Aims: Generate novel tissue-engineered 3D constructs using normal oral fibroblast (NOF)- and CAF-derived ECM, as native scaffolds to accurately model tumour:ECM interactions in OSCC progression.
Methods: Utilising a transwell technique, culture conditions were optimised to stimulate NOF- and CAF-derived ECM deposition. Full-thickness epithelium models were produced by culturing normal (FNB6) or OSCC (H357) cell lines seeded onto ECM scaffolds. Key markers were characterised by immunohistochemistry (Ki67, AE1/3, COLIV, E-cadherin and α SMA) and immunoblotting for ECM proteins. Collagen fibres in NOF- and CAF-derived matrices were analysed using second harmonic generation microscopy (SHGM). Atomic force microscopy (AFM) and live-cell fluorescence imaging microscopy (LFIM) determined ECM biomechanics and the complex behavioral interplay of cancer cells in these ECM scaffolds respectively.
Results: NOF stimulated to produce ECM generated an organised matrix with an average thickness of ~200 µm compared to CAFs which produced a thicker (350 µm), highly irregular ECM. Immunoblotting of the matrices revealed a significantly different protein deposition of CAF matrices compared to NOF matrices. SHGM revealed collagen width, length, and linerisation as statistically different in CAF-derived matrices than NOF-derived matrices. Addition of FNB6 and H357 cells generated a stratified epithelial layer histologically resembling normal and cancerous tissue *ex vivo*. Further immunohistochemical analysis revealed differential expression of ECM proteins and evidence of CAF-mediated tumour progression. AFM determined the micromechanical properties of the ECM and alterations of tissue stiffness. LFIM provided information on the migration and invasion of cancer cells within these models.
Discussion: Using tissue-engineering techniques it is possible to model fibroblast-mediated ECM deposition providing a novel, physiologically relevant *in vitro* tool for the study of OSCC progression.

a91272

80-SYIS CS-5 In Vitro Tumor Models and Exosomes: 3D Culture Changes Everything

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Interest in extracellular vesicles (EVs; e.g. exosomes) has soared due to their promising utility in numerous clinical applications including as biomarkers for cancer detection via liquid biopsy. Analysing the molecular cargo of EVs may allow for discovery of biomarkers with sufficient specificity and sensitivity for a given cancer type to be diagnosed from a simple blood draw. The source of EVs used for such a discovery effort is critical. Here, we demonstrate the striking effect that a 3D culture model can have on both the secretion behaviour and the molecular cargo found in EVs. We used a biopolymer-based scaffold (3D) to simulate the tutor microenvironment for culture of prostate cancer cells in vitro. For a range in comparisons we focused on 4 cell types: PC3 (aggressive prostate cancer cells), LNCaP (less aggressive prostate cancer cells), PNT1 (benign prostate epithelial cells), and adipose-derived stem cells (ASCs; non-prostate cells included as controls). Cvs secreted by cells in 3D were compared to those secreted by the same cells cultured in 2D (tissue culture plastic). All cell types showed heightened EV production when cultured in 3D, ranging from 2-5x increased EV-protein per cell spending on cell type. The size distributions in terms of EV-diameters did not vary across cell types or culture conditions as measured by nanoparticle tracking analysis, DLS, and TEM. Next generation (NGS) and proteomics analyses revealed dramatic alterations in EV content as a results of 3D culture. Principle component analysis performed on NGS data of the 500 genes of highest variance exhibited the highest magnitude of expression changes for the PC3 cells and the ASCs. Gene ontology enrichment indicated ubiquitous down-regulation of genres involved in direction intracellular protein traffic fora ll cell types. Differences seen in proteomics were equally stark -- an average of 400 extra proteins were detected in proteomics of 3D-EVs that were not found in 2D-EVs of the same cell type. Comparing these lists with public databases revealed numerous proteins found in 3D-EVs that have not previously been reported as cargo in EVs. Analysing these lists has the potential to yield novel biomarkers that could be used as targets for liquid biopsy tests to detect and monitor prostate cancer. Above all, the data presented here speaks to the importance of the in vitro model used for characterisation of EVs, especially for clinical applications.

a91690

80-SYIS CS-6 BIO-ENGINEERING TRANSPLANTABLE VASCULARISED HUMAN LIVER ORGANOIDS

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INTRODUCTION

Liver tissue engineering offers a regenerative alternative to donor-derived organ transplantation, and a platform for drug testing and disease modelling. Transplantable liver organoids with intrinsic micro-vasculature were developed using human liver progenitor cells (LPC) as a parenchymal cell source, liver sinusoidal endothelial cells (LSEC) to generate liver-specific vasculature, and adipose-derived mesenchymal stem cells (ASC) as support cells. A hydrogel derived from human liver extracellular matrix and a porous scaffold facilitated tissue assembly.

METHODS & RESULTS

In vitro: Human LPC/LSEC/ASC ($1x10^6$ cells total) mixed in liver gel was seeded into NovoSorb® polyurethane scaffolds (3mm diameter, 0.8mm thickness, 300-500µm wide interconnected pores, bio-absorbed within 12 months and FDA-approved for clinical use). Between day 1 and 3 organoids upregulated key liver genes (HNF4a, Albumin, HGF, CYP3A4). Functional assays demonstrated albumin secretion, urea production, bile acid excretion, and cytochrome P450 CYP3A4 activity, which all increased over time. Capillary and bile canaliculi formation was also observed.

Concurrent organoids were developed by replacing liver gel with the industry's gold standard - Matrigel® (commercial hydrogel derived from mouse sarcoma extracellular matrix). In all gene and functional parameters, liver gel organoids outperformed Matrigel organoids.

In vivo: Organoids with liver gel were transplanted into a vascularised "*in vivo* bioreactor" created in the groin of immuno-deficient SCID mice (a closed protective chamber surgically placed around an artery/vein to artificially induce a capillary bed, providing a fertile angiogenic environment *in vivo*). At 14 days, functional human blood vessels perfused by mouse blood and clusters of differentiating human hepatocytes were present. Measurable levels of human albumin were secreted into the mouse circulation by transplanted organoids (n=4, human albumin level in mouse serum 25.3±9 µg/mL).

CONCLUSION

Functional vascularised human liver organoids can be engineered using a combination of LPC/LSEC/ASC, enhanced by using liver-specific extracellular matrix. Notably, all components were human-derived. Proof-of-concept transplantation studies indicate organoid survival/function in immuno-deficient mice. Longer time-points and transplantation in a humanised mouse model of liver disease are currently underway to assess the therapeutic efficacy of this organoid approach.

a92082

80-SYIS CS-7 A Novel Platform for Screening Hematopoietic Stem Cell Niche Factors in Bioengineered Bone Marrows *In Vivo*

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The major limitation of bone marrow transplants is the availability of hematopoietic stem cells (HSCs). HSCs grown *in vitro* rapidly lose their regenerative capacity likely due to the lack of niche-derived signals comprising molecular and cellular components[1]. Identification of critical hematopoietic niche components necessitates the generation of both more tractable *in vivo* models and heightened throughput screening approaches. Here, synthetic polyethylene glycol-based hydrogels[2] were modified by addition of cells and/or growth factors to simulate bone marrow-like structures when subcutaneously implanted in mouse. Using PEG, a blank material, enabled a systematic approach to evaluate potential niche candidate cells such as human mesenchymal stem cells (hMSCs) in a defined non-inductive 3D microenvironment. We first tuned material characteristics, the needed concentration of bone morphogenetic protein-2 (BMP-2) and hMSCs *in vivo*. Finding a high hMSC concentration that without any conductive or inductive components could remodel PEG gels into ossicles. MicroCT analysis showed presence of mineralization within the constructs, a characteristic further corroborated by histology. Moreover, these implants not only contained still hMSCs, but were also highly infiltrated by murine osteogenic and endothelial cells. While FACS analysis of the cell-laden implants showed a decay in the human population over time, a rising murine hematopoietic cell population enriched with long-term HSCs was observed. These results indicate the formation of a functional ectopic niche in the cell-laden, synthetic hydrogels. Initial results using hMSCs were promising, but in order to screen the many candidate niche cells and factors for their potential participation in bone marrow formation, an implantable screening device was developed. These PDMS devices of 10mm length contained separated wells where individual gels could be polymerized, enabling up to 32 unique conditions to be evaluated per mouse. This approach allows optimization of hydrogel conditions, cell type, and soluble factors that support bone marrow niche formation *in vivo*. Results indicated that at a high cell density, hMSCs alone can direct and participate in both bone formation and murine HSCs recruitment. The PDMS screening device is a powerful new tool for heightened *in vivo* screening of tissue engineering constructs with a broad range of applications.

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a92278

80-SYIS CS-8 Influence of Cell Types and Sources on Angiogenesis *in vitro*

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For tissue engineering of larger substitutes, angiogenesis is crucial. To pre-vascularize the constructs, endothelial cells and supporting cells are embedded to ensure adequate nutrient and oxygen supply after transplantation. This technique is already widely applied to tissue engineering of various organs. So far, structured studies on the use of different cell types or cells from different source are scarce. The growing use of bio-printers increases the need for new angiogenesis techniques in printable scaffolds.

In this study, the focus is based on the quantification of angiogenesis and thus on the formation of vascular structures by comparing different supporting cells of various tissue sources. As scaffolds, 5 % fibrin gel and a printable hydrogel consisting of 0.5 % agarose and 0.5 % collagen were used. As supporting cells, we used primary human dermal fibroblasts (HDFs), human nasal fibroblasts (HNFs), mesenchymal stem cells from human umbilical cord (WJ MSCs), human adipose tissue (AD MSCs), and human femoral bone marrow cells (BM MSCs). Together with endothelial cells from the human umbilical cord (HUVECs) they were cultured in the hydrogels with a concentration of $1{,}0 \times 10^6$ cells/cell type/350 µl hydrogel for 14 days at 37 °C, 5 % CO_2 under water vapour saturated atmosphere. As culture medium, EGM-2 was used. The developed vascular structures were detected by CD31/PECAM antibody staining with a two-photon laser-scanning microscope. In addition, the parameters volume, area, length and number of branches of the structures were statistically evaluated.

We successfully established angiogenesis with all supporting cells. In both fibrin and agarose-collagen hydrogels, branched networks of tubular vascular structures were formed. While AD MSCs showed comparable values for volume (29,100 μm^3), area (8,916 μm^2) and length (211 µm) to our positive control HDFs in agarose-collagen hydrogels. BM MSCs form a homogeneous, area-wide network of structures in both hydrogels and showed an up to 10 times higher branching number than the other supporting cell types. In cultivation, both hydrogels also demonstrated dimensional stability, which is crucial for the subsequent 3D printing process. This study describes the first step to an autologous, pre-vascularized scaffold of endothelial and supporting cells which can be bio-printed.

a93129

80-SYIS CS-9 Different Tenogenic Capacities of Human Mesenchymal Stem Cells from Bone Marrow, Adipose, and Tonsil Tissues

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Because of the limited and unsatisfactory outcomes of clinical tendon repair, tissue engineering approaches using mesenchymal stem cells (MSCs) are being considered as a promising alternative strategy to heal tendon injuries. The objective of this study was to investigate the comparative ability of multi-lineage differentiation of Bone marrow (BM), adipose (AD), and tonsil tissue (T)-MSCs, and to determine if tenogenesis in these cell types can be preferentially augmented by the addition of TGF-β3. Therefore, we suggest the possibility of clinical application for tendon regeneration using T-MSCs. T-MSC were obtained using discarded waste tissue after tonsillectomy. BM-MSCs and AD-MSCs were obtained from patients for total shoulder joint replacement surgery. Immunophenotypic analysis at passage 2 of BM, AD and T-MSCs by flow cytometry revealed MSCs with high expression of CD73, CD90 and CD105 markers and low or absent expression of CD11b, CD19, CD34, CD45, and HLA-DR markers. We performed quantitative real-time PCR to multi-lineage differentiation specific marker gene and analyzed terminal differentiation used by functional assay and staining. Western blot was also performed for tenogenic proteins. T-MSC has a very low adipogenic, osteogenic, and chondrogenic differentiation potential compared to BM-MSC and AD-MSC. When BM-MSC and T-MSC was treated with 5 ng / ml or 10 ng / ml of TGF-β3, the expression of tenogenic specific mRNAs was increased and there was no difference in the expression between the different concentrations. We used 10ng/ml of TGF-β3 for inducing tenogenic differentiation *Scleraxis* mRNA expression level of T-MSC was lower than that of BM-MSC and AD-MSC, whereas *tenomodulin* and *tenascin-C* mRNA expression were increased only at T-MSC on day 14. Although the total amount of collagen production was highest in AD-MSC, T-MSC showed the highest ratio of collagen I/III mRNA expression, which is the quality of collagen production. The expression of Tenomodulin and Tenasin-C protein were also expressed most rapidly at the early stage of T-MSC, and the expression of these proteins was observed in T-MSC without tenogenic differentiation. In conclusion, T-MSC has low fat, bone and cartilage differentiation potential and has excellent tendon-specific differentiation potential, thus being highly useful as a tendon-tailored cell therapy agent.

a90095

90-SYIS CS-1 Engineering Anisotropic Muscle Tissue using Ultrasound Cell Patterning

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In vitro bioengineering strategies must seek to reproduce native cellular organization, to ensure synchronous function, mechanical anisotropy and correct tissue maturation. Here we demonstrate, for the first time, that acoustic fields can be used to produce aligned cell fibers in an engineered tissue. We show that a brief application of ultrasound standing waves (<30 min, 2.0-2.1 MHz) can be used to pattern myoblasts into collagen-based hydrogels for the engineering of muscle tissue with dense, aligned fibers. This strategy offered great flexibility across different bioengineering protocols, addressing several key limitations facing *in vitro* muscle formation. Myoblasts patterned in a type I collagen hydrogel contracted the surrounding matrix to produce high-density muscle fibers, anisotropic tensile mechanics and a remodeled extracellular matrix. Indeed, the cell-mediated contraction was used in combination with collagen clamping to generate, to the best of our knowledge, the first instance of a tissue construct exhibiting cell alignment on both an individual cell and population-wide level.

A third protocol, utilizing photo-crosslinked GelMA hydrogels, was used to demonstrate that acoustic patterning can significantly enhance myofibrilliogenesis, compared to unpatterned controls. Negligible myoblast fusion was observed in the unpatterned tissue after seven days, however, the acoustically-patterned muscle exhibited upregulated *MRF4*, a key marker of myotube maturation, and the formation of large, multinucleated myotubes expressing the muscle-specific proteins α-myosin skeletal fast and tropomyosin. The myotubes were aligned within the acoustically-patterned muscle fibers, with the patterning extending across entire tissue constructs (*cf.* 350 cell widths / 7 mm). This ability to fabricate anisotropic tissue constructs with ordering over large length scales will be a critical factor in the engineering of functional tissue grafts and physiologically-relevant disease models. Indeed, the ability to rapidly and dynamically align label-free cells *en masse* using generic hydrogels and cell culture apparatus makes this platform technology highly advantageous for a host of next-generation bioengineering strategies.

a90805

90-SYIS CS-2 Magnetic bead-based *in touch* immunosensor for monitoring cell biomarkers of neuromuscular disorders simulated by organ-on-a-chip technology

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The pharmaceutical industry relies heavily on *in vivo* animal models and *in vitro* two-dimensional (2D) cell cultures to develop therapeutic strategies. There are many ethical issues surrounding animal studies and serious concerns also exist regarding their biological relevance to humans[1]. However, current *in vitro* tissue models are 2D and do not consider the complexity of physiological microenvironment in which the cells grow. Particularly, current 2D tissue models often do not simulate complex cell-cell and cell-matrix interactions, which are crucial for regulating cell behaviours *in vivo*[2]. In the present work, we have integrated skeletal muscle engineered tissue in a 3D platform by encapsulating C2C12 cells in a Gelatin based hydrogel scaffold with an immunosensing system to obtain a magnetic bead (MB)-based electrochemical platform for the multiplexed detection of different cell stress biomarkers related with neuromuscular diseases**.**

The sandwich immunoassay is based on the use of superparamagnetic microbeads functionalized with a specific antibody to selectively capture the target biomarkers, during the different regimes of stimulation (electrical and/or chemical) of the tissue. In contrast with other works[3-4], the methodology developed here combines the large effective surface area of the beads with the capability of the bioconjugate to capture very low amounts of the corresponding biomarker due to the continuous contact with the 3D skeletal tissue through a transwell permeable support. Afterwards, the antigen captured by the microbeads, is exposed to a biotinylated antibody, and Streptavidin-Horseradish Peroxidase (Sav-HRP) bioconjugate. Finally, after capturing the MB-complex onto the Screen-Printed Carbon Electrodes (SPCEs) surface, the electrochemical signal is achieved by adding the corresponding substrate. The limits of detection for IL-6 and TNF α were 0.028 and 0.035 ng mL^{-1}, respectively.

The device described in this work, let us to extend the knowledge of different clinical scenarios such as metabolic but also, neurodegenerative diseases, due to the possibility of monitoring cell biomarkers continuously under different stimuli.

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a90824

90-SYIS CS-3 Engineering extracellular matrix-rich supramolecularly assembled living substitutes

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INTRODUCTION

Tissue engineering by self-assembly is a technology that offers the possibility to fabricate contiguous cell sheets that are stabilised by intact cell-cell contacts and endogenously produced extracellular matrix (ECM) [1]. However, these systems lack the possibility to introduce topographical cues, that are fundamental for the organisation of many types of tissues. Moreover, the fabrication of ECM-rich cell sheets would be highly desirable. This limitation could be overcome by inducing macromolecular crowding (MMC) conditions in the fabrication process [2]. Herein we venture to fabricate aligned electrospun thermoresponsive nanofibres to sustain growth and detachment of ECM-rich living substitutes in the presence of a MMC microenvironment.

EXPERIMENTAL METHODS

A copolymer of 85% poly-N-isopropylacrylamide and 15% N-tert-butylacrylamide (pNIPAAm/NTBA) were used for all experiments. To create aligned nanofibers, the polymer was electrospun and collected on a mandrel rotating at 2000 rpm. Human adipose derived stem cells (hADSC) were treated with media containing macromolecular crowders to enhance matrix deposition. Cell viability and morphology were assessed, and immunocytochemistry was conducted in order to estimate matrix deposition and composition. Non-invasive cell detachment was enabled by decreasing the temperature of culture to 10 ° C for 20 minutes.

RESULTS AND DISCUSSION

The electrospinning process resulted in the production of pNIPAm/NTBA fibres in the diameter range from 1 to 2 µm and an overall alignment of 80%. Cell viability revealed that hADSCs were able to grow on the thermoresponsive scaffold. The cells were able to align on the fibers after 3 days and they were able to detach as intact cell sheets in presence of MMC. Moreover, it was demonstrated that MMC, by a volume extrusion effect, enhances collagen type I deposition, one of the main components of the ECM.

CONCLUSIONS

The pNIPAm/NTBA fibres were able to successfully sustain growth and detachment of ECM-rich cell sheets. We believe that replacement, repair and restoration of tissue function can be accomplished best using cells that create their own tissue-specific extracellular matrix with a precision and stoichiometric efficiency still unmatched by man-made devices.

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a91211

90-SYIS CS-4 Graphene Oxide-Based Microfluidic System for Efficient Capturing of Circulating Tumor Cells and Microemboli

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Abstract

Circulating Tumor Microemboli (CTMs) are clusters of Circulating Tumor Cells (CTCs), their multicellular aggregation ability made them played greater role in cancer metastasis. Here, we presented an effective graphene oxide (GO)-based microfluidic system to capture viable CTCs and CTMs immediately from peripheral blood samples without any centrifugation. Aiming to diagnose metastasis at early stage, this research presents a non-invasive, real-time observable microfluidic system for highly sensitive capturing of both CTCs and CTMs. We used dual antibodies (Epcam, Vimentin), which recognized both mesenchymal and epithelial tumor cell surface markers, were immobilized on the inner surface of GO-functionalized PDMS microchannel through click chemistry, in which GO was used as a bio-nano interface to enhance antibodies conjugation due to its affluence functional groups and high biocompatibility. Extremely thin channel with large surface area allowed cancer cells to be captured by the corresponding antibodies. Hence, CTCs and CTMs were expected to be detected and captured at early stage, so as to achieve early prediction of metastasis and assist in selecting effective treatment for each patient.

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a91453

90-SYIS CS-5 Identifying the key influencers on the inductive potential of platelet lysate on BMSC proliferation and migration using novel live-cell imaging technique

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Introduction: Autologous platelet rich plasma (PRP) and platelet lysate (PL) are used clinically in the treatment of bone defects following trauma or osteoarthritis (1). PRP can be administered in its whole or lysed form (PL) to the defect site or loaded onto a scaffold. The goal of using PL is to locally enrich growth factors to induce migration of bone marrow (BM) multipotential stromal cells (BMSCs) to the defect site and trigger their proliferation, forming a provisional tissue (2). However, there is still a lack of consensus in the literature of the 'ideal' composition of PL for bone repair and a demand to standardise PL as a product.

Aims: To investigate the impact different PL configurations, with regards to absolute numbers of platelets and lymphocytes, have on the proliferative and migratory capacity of BMSCs.

Methods: Primary BMSCs (n=4) cultured from BM were used for proliferation and migration assays in the presence of PL (n=11), leukocyte-rich PL (LPL) (n=11), and filtered PL (fPL) (n=3). The PL preparations were processed as PRP and freeze-thawed to make their PL counterparts: LPL was made using the operating room-compatible Biomet device whilst PL was generated using a two-spin centrifugation protocol, PL was then filtered to produce fPL devoid of leukocytes. XTT assays were used to quantify proliferation whilst the IncuCyte device was used to image and quantify cell migration across a transwell.

Results: In LPL, significantly fewer total platelets/ml ($P < 0.05$) (4 fold less) and more leukocytes were lysed than in PL ($P < 0.01$) (9 fold more) whilst fPL was generated from the same number of platelets as PL but was devoid of leukocytes. LPL induced significantly less BMSC proliferation than PL but the complete removal of leukocytes in fPL did not further improve proliferation. With regards to BMSC migration, all three PL products induced the same total migration although there was a trend of LPL or fPL inducing less migration than PL.

Discussion: The complete depletion of leukocytes from platelet products used for bone repair may not be necessary. Higher concentrations of platelets are beneficial for BMSC proliferation but not migration. Autologous platelet products made with Biomet device may be used for loading scaffolds intended to be colonised by resident BMSCs for large bone defect repair.

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a92293

90-SYIS CS-6 Inkjet Printing of a 3D Microstructured Human Skin Equivalent

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Needs for highly mimetic human skin models are becoming larger than before these days as animal testing in the cosmetics field is gradually being banned in many countries starting from European Union since 2013. Fabrication of artificial human skin equivalents with the increased representativeness is required to substitute the gap of the conventional animal testing. The skin is the largest organ of the body with the structural and functional complexity. Microstructures of dermis which help sustaining dermal-epidermal junction are the one of the key features among the existing complexities in skin such as appendages, vasculatures, immune cells, and pigment. However, conventional skin equivalents are made simply by stacking dermis and epidermis layers, lacking the detailed microstructures of human skin. Here, we present a 3D human skin model with microstructured dermis. The piezo-type inkjet printer enabled the controlled displacement and density of Type I collagen, dermal fibroblasts, and epidermal keratinocytes with high accuracy. We found that the degree of collagen contraction varies with the density of printed dermal fibroblasts. We used this phenomena to form papillary microstructures in the dermal layer. Various forms of microstructures were fabricated by changing local density of dermal fibroblasts or shapes of patterns. Epidermal keratinocytes were also inkjet printed as a densely packed monolayer within a controlled region preventing slipping away from the surface of the dermal collagen area. We believe that the inkjet-bioprinted 3D human skin equivalents with microstructures will play a critical role as a bridge between *in vitro* human models and *in vivo* tissues by replacing animal testing.

a92453

90-SYIS CS-7 Multi-layer vascularized cell sheets for tissue regeneration

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One of the major challenges in tissue engineering is to produce functional vascularized 3D transplants *in vitro*, able to recapitulate the complex and physiological structure of natural tissues. In fact, the lack of vascularization causes necrosis, nutrient insufficiency and waste accumulation. Cell sheet (CS) engineering techniques provide an excellent microenvironment for vascularization since the technique can maintain the intact cell matrix, crucial for angiogenesis. In an attempt to develop hierarchical cellular 3D vascularized constructs, we herein propose the construction of magnetic responsive multi-layer cell sheets able to be harvest as a whole by applying a magnetic force.

For the purpose, rhodamine B-labeled supermagnetic iron oxide nanoparticles (10 nm) were synthetized by the co-precipitation method and characterized by FTIR and TEM. The developed nanoparticles were then incubated with human adipose derived stem cells (hASCs) and human umbilical vein endothelial cells (HUVECs) for viability assessment studies. After confirming their successful mitochondrial activity, magnetic-responsive cells were used to develop vascularized microtissues through cell sheet technology. Previously to CS formation, hASCs were stained with DIO and HUVECs with Cell Tracker Orange CMTMR Dye.

After 7 days of incubation, microtissues could be macroscopically visualized and easily detached by applying a magnetic-force. Histological evaluation of the developed multi-layer cell sheets was performed, and the angiogenic potential of such microtissues was also evaluated using the chick embryo CAM assay.

This proof-of concept work is expected to open new insights for the development of vascularized 3D cell structures with different cell phenotype combinations, circumventing the current problematics associated with poorly vascularized 3D transplants. Such type of vascularized CS may be suitable for successful transplantations in highly vascularized tissues such as bone, and high metabolic-rate organs like kidney and heart.

Acknowledgements

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a93157

90-SYIS CS-8 Strategy to fabricate human cardiac tubes *in vitro* for circulatory support

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Introduction:
We have attempted to fabricate a tubular cardiac tissue in vitro with pump function using cell sheet engineering. When 3D tissue is fabricated, a vascular network in the tissue is necessary so that nutrients and oxygen are provided. Our laboratory succeeded in building a vascular network in the tissue using a vascular bed. In this study, a small intestine was used as a tubular vascular bed in order to fabricate a tubular cardiac tissue in vitro. Cell sheets were layered on the inner wall of a tubular vascular bed to create a tubular cardiac tissue. We have developed a novel fabrication method in which a silicone balloon was used under negative pressure condition.
Method:
A porcine intestine was harvested and connected to a bioreactor within a completely closed chamber, then, it was decellarized with DCA and DNAse. Next, a cell sheet was harvested from a temperature-responsive culture dish and was wrapped around the periphery of a silicone balloon. Next, a tubular vascular bed was expanded under negative pressure condition and the balloon was inserted into the tubular vascular bed. The balloon was inflated with phosphate buffered saline, then the balloon was removed. The tissue was observed with H.E. staining and immunostaining for cardiac troponin-T.
Result:
When the balloon was inflated, the cell sheet was also expanded. A tubular vascular bed was able to be expanded under negative pressure condition, that is, negative 2 kilopascals or less. H.E. stains indicated the cell sheet existed on the inner wall of a tubular vascular bed. These results show that cell sheets can be layered on the inner wall of the vascular bed by this method. To fabricate a thick tissue with perfusion culture and multi-step procedures is ongoing.
Conclusion:
The method, in which a silicone balloon was used under negative pressure condition, is a promising method to fabricate tubular cardiac tissue with pump function.
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a91530

100-SYIS CS-1 Injectable Body Heat-Activated Tough Biodegradable Nanocomposites for Regeneration

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Orthopedic reconstructive surgery aims to stabilize fractures, replace or repair missing bones, and reattach soft tissue to bone to promote tissue regeneration at the site of injury. While currently used materials, such as poly(methyl methacrylate) (PMMA) or calcium phosphate-based cements offer effective mechanical properties, they have poor handling characteristics, are not conducive to bone regeneration, and are not degradable in the body.[1,2] The objective of this study was to develop and characterize an injectable, shape conforming polymer-ceramic composite that cured in the body to become a tough resorbable bone-like matrix. This material is based on the antioxidant polymer poly(1,8-octamethylene citrate) (POC), which has been shown to form osteoinductive composites when mixed with ceramic micro and nanoparticles.[3] We investigated the use of the initiator 2-2'-azobis(4-methoxy-2,4-dimethyl valeronitrile) (V70) as a biocompatible component to trigger phase-change of the composite within 15 minutes at body temperature. Methacrylation and incorporation of hydroxyapatite nanoparticles lowered the total heat evolved during free radical polymerization to 52.5°C. The compressive strength of mPOC was 220 ± 27.6MPa with Young's Modulus and strain at failure ranging 0.791 ±0.102GPa and 49.7 ± 1.68% respectively. Over a 7-day period, 42.1 ± 3.46% of mPOC mass was lost when submerged in 0.5M NaOH with mPOC-HA compositions exhibiting lower amounts of mass lost. In vitro and in vivo studies utilizing cell culture and subcutaneous injections resulted in cell and tissue compatibility. These results indicate that methacrylated POC retains its biocompatible and mechanical characteristics and would be an effective means for tissue engineering applications.

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a91988

100-SYIS CS-2 Impact of Chitin Nanoforms Impregnated Chitosan Composite Matrices on Stem Cell Survival and Growth

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A key challenge for biomaterials design and development, intended for applications in Tissue Engineering and Regenerative Medicine is the presentation of tuneable bioactive modalities on the material. The function of such modalities (chemical or physical) is to provide cues to cells and tissues to recognize, regenerate and replace the artificial scaffold with natural extra cellular matrix (ECM). In many pathologies, the innate capacity of cells to survive, produce ECM proteins and regenerate tissue is hindered. This gives rise to the need for niche 'smart' materials that can guide regeneration of damaged tissue through the action of their bioactive modalities. Chitin and chitosan are marine-based polysaccharides and have been used in Tissue Engineering applications for several years due their amenability for chemical modifications, solubility, biodegradability and very low toxicity profile towards human tissue. In this study, we present a new engineering approach based on our earlier studies[1,2], whereby the amalgamation of chitin nanoforms (nanocrystals and nanofibres) with chitosan 2D films and 3D porous matrices. The chitosan matrix is cross-linked with the nanoforms through the action of Genipin, a natural cross-linker. The resultant materials exhibit highly desirable chemical properties such as chemical stability, water uptake, mechanical robustness and diverse topography. In addition, to suit various tissue-engineering needs, these scaffolds are biocompatible and offer sustenance of stemness in human mesenchymal stem cells. Another highlight of these materials is the use of green and environmentally sustainable approach for the development of the bionanocomposite materials. It is envisaged that this approach will present a new vision for the development of sustainable biomaterials for bioengineering and biomaterials.

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a92042

100-SYIS CS-3 3D *Ex Vivo* Model of a Methylcellulose Hydrogel and Antimicrobials for Oral Treatment

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Objectives: Management of oral candidiasis, most frequently caused by *Candida albicans*, is limited due to the relatively low number of antifungal drugs and the emergence of antifungal tolerance. Essential oils might provide an alternative treatment. This project aims to i) develop a 3D *ex vivo* rodent mandible model that mimics oral candidiasis to investigate the anticandidal potential of essential oils and to ii) evaluate a methylcellulose hydrogel loaded with essential oil as an antimicrobial delivery vehicle, that could be used in the *ex vivo* model.
Methods: The *ex vivo* rodent mandible model was obtained by dissecting the mandible of 28-day-old male Wistar rats and infecting it with *C. albicans*. *Candida* growth was monitored through histological examination and image analysis, after incubation for 24 and 48 hours with and without 1% (v/v) *Melissa Officinalis* oil. Cytokine expression, using RT-PCR, was used to assess the host tissue response. In addition, a 10% methylcellulose hydrogel loaded with 1% (v/v) and 2% (v/v) *Melissa Officinalis* oil was synthesised, then the rheological properties, oil leaching and the antimicrobial potential against two *C. albicans* strains were evaluated.
Results: The infection of the rodent mandible showed *C. albicans* invasion of the gingiva and the release of pro-inflammatory cytokines (TNF, IL1, IL6, IL12, IL18 and IL23), while the application of *Melissa Officinalis* oil significantly decreased the Colony Forming Units (CFU)/ml and the pro-inflammatory response. 1% (v/v) and 2% (v/v) *Melissa Officinalis* oil were successfully incorporated into 10% methylcellulose hydrogel. Rheology revealed that the hydrogel was injectable and liquid at room temperature and jellified in three minutes at 37 °C. The drug release was a function of the *Melissa Officinalis* concentration, and 60% of the antimicrobial was released in 8 hours. In addition, the loaded hydrogel successfully inhibited *Candida* growth *in vitro.*
Conclusions: A 3D *ex vivo* rodent mandible model to mimic oral candidiasis was developed and used to test the antifungal properties of *Melissa Officinalis* oil in a system that better mimics the *in-vivo* conditions. Moreover, a potential injectable methylcellulose hydrogel loaded with *Melissa Officinalis* oil that showed *in vitro* antifungal properties was synthesised. This shows promise for a future application of the hydrogel in the *ex vivo* mandible model.

a92263

100-SYIS CS-4 Hydrophobic cholesteryl moieties trigger substrate cell-membrane interaction of elastin-mimetic protein coatings in vitro

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INTRODUCTION

The majority of hydrogels or polymer substrates focus on integrin binding sites, in particular on RGD binding site, to generate cell binding, or infiltration. Synthetic materials with covalently bound RGD residues nevertheless struggle to meet the results of native ECM protein materials. In this approach, a cellular coating based on hydrophobic interactions with the cell membrane is presented. To reduce the batch to batch and other host specific variations of organism-derived proteins, an elastin-like recombinamer (ELR) is used as the tissue inductive coating[1]. The main objective lays in the creation of a well distributed initial layer of ELR onto cells. Therefore, the potential of hydrophobic groups as intra-membranous anchors for cellular coatings is estimated.

MATERIALS AND METHODS

ELRs were derived by recombinant bioproduction and further modified at free lysine amines, with functional cyclooctyne groups (BCN). [2] The active ELRs were reacted with either triethylenglycol-cholesterol azide (CTA; Sigma) and/or eterneon azide (Jena BS). ELRs containing 0, 1, 3 or 5 Cholesteryl groups were generated (CTA0 – 5). Samples were further modified by insertion of a fluorophore (CTA0-5*) for microscopic investigation.

The cellular probes were characterized by NMR, DLS, FACS and CA.

RESULTS

The cellular probes were successfully synthetized and characterized. For the quantification of membrane interactions human foreskin fibroblasts and human umbilical vein endothelium-derived cells, and human aortic smooth muscle cells were incubated with the probes at variying concentrations (Figure 1). The probes with higher amounts of CTA showed a significant increase in ELR to cell-embrane binding and enhanced distribution of protein through out the cell membrane.

CONCLUSIONS

The resulting materials showed enhanced cellular recognition and matrix distribution, in line with the amount of CTA groups incorporated. These novel materials have a high potential as cellular marker, drug carrier, or to function as tissue inductive protein coatings. The cell specificity of the process leaves place for further optimization of the process, by varying concentrations, incubation times and the amount of cholesteryl groups might also help to understand the mechanisms by which cholesterol homeostasis is maintained.

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a92358

100-SYIS CS-5 Enhanced surface roughness of additive manufactured scaffolds via foaming agents

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Additive Manufacturing (AM) techniques keep showing a great potential in scaffold production thanks to their versatility, ease of use, and the possibility of having full control over the product morphology. Among the several methodologies available nowadays, melt-extrusion-based techniques are probably the most widely used. This is mainly due to the fact that generally no toxic precursors or binders are needed during the shaping process as in stereolithography (SLA). However, scaffolds produced via melt-extrusion-based techniques show rather smooth filaments originated from the smearing action of the nozzle internal walls on the melt surface. Some researchers have shown that rough surfaces can have a positive influence not only on cell attachment and spreading[1], but also on the differentiation of human mesenchymal stromal cells[2]. Developing methods that combine the potential of AM techniques with controlling cell fate via surface topography is an appealing avenue to fabricate smart scaffolds. The aim of this work is to produce scaffolds with enhanced surface roughness directly through a melt-extrusion-based technique. Poly(lactic acid) (PLA) was foamed with a foaming agent during extrusion. The filaments were successfully assembled to form 3D scaffolds by precisely controlling the temperature profile over the flow path. Depositing the material at different flow rates showed to have an influence on the voids distribution over the cross section. By optimizing processing conditions, the surface roughness of the 3D printed scaffolds could be optimized. Cells cultured on monolayers of foamed filaments with a rough surface, showed higher metabolic and proliferation activities when compared to the controls (monolayers of non-foamed PLA). These results indicate that scaffolds with a controlled rough surface can be successfully fabricated via a melt—extrusion–based technique.

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a92398

100-SYIS CS-6 Specific Detection of Proteases using novel-protein substrates for Zymographic Methods

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The present work provides recombinantly produced proteins, containing a specific series of building blocks for the optimal detection of proteolytic enzymes for medical diagnostic and the estimation of enzyme levels, as well as for scientific research targeted on enzyme expression. The material serves as a substitute for the active compounds that are widely used in in-gel zymography, like gelatin, fibrin, casein, plasminogen, or fluorescently labeled peptides. The tailored arrangement of the protein allows for specific detection. The material was further functionalized with cross-linking groups (acrylates, methacrylates, cyclooctynes, acetylynes, vinyls, etc.) for better integrity with the matrix it is cross-linked to, in particular acrylamide gels. With current advances in genetic modeling this methods opens librarys of proteolytic sequences incorporated into a proteins sequence, that serve as a substrate of proteolytic activity, facilitating the determination and screening of cleavage specifity of peptidases. With the current system matrix metalloproteases, and serine proteases were successfully detected, giving the additional information about the cleaved peptide sequence. This information further was used, to implement it in a novel screening method with a binary read out, without the necessity of electrophoretic denaturation of sample. This method has potential for the peptidase screening of clinical relevant samples, or R&D samples allowing for the determination of increased or abnormal protease levels, which are hallmarks of cancers.

a92949

100-SYIS CS-7 Interfacial Mechanics Controls Cell Adhesion and Expansion on Low-Viscosity Liquids

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Substrate mechanics and topography play an important role in regulating biochemical signals such as integrin-mediated matrix anchorage and cell spreading[1]. Such physical cues have a striking impact on cell phenotype, such as the differentiation of stem cells and the preservation of their potency[2,3], as well as in pathologies such as cancer[4]. These phenomena are mediated by focal adhesions and the associated coupling to microfilaments[5]. Hence the control of the matrix's mechanical properties is important for the design of biomaterials for stem cell expansion and for *in vitro* models and tissue engineering platforms. Recently, we proposed that the nanoscale mechanics of the interface may dominate over bulk cues to regulate cell phenotype[6]. Indeed stem cells did not respond to changes in the bulk modulus of silicones, over a very wide range (0.1 kPa to 2.3 MPa), in contrast to their behaviour at the surface of hydrogels. In addition, we found that the softest silicones used (100 Pa) did not display any elasticity in stress relaxation experiments, suggesting that cells may spread and proliferate on liquid substrates. Here we show that cell spreading, proliferation and control of fate decision occur at the surface of low-viscous liquids and are enabled by the self-assembly of mechanically strong nanoscale protein layers at such interfaces. These findings have important implications for our understanding of cellular mechanosensing, but also call for a shift in paradigm in the design of biomaterials used for regenerative medicine as they demonstrate that bulk and nanoscale mechanical properties may be designed independently to regulate cell adhesion and phenotype. We propose that this may find direct application for the development of 3D bioreactors and in cell sheet engineering.

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a93172

100-SYIS CS-8 Development of Polyelectrolyte Based Composite Artificial Extracellular Matrices For Bone Tissue Engineering Application

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The increasing incidence of bone tissue related defects/diseases and lack of appropriate effective therapies leads toward development of highly effective and enduring alternative therapies through bone tissue engineering approaches. The present work deals with the fabrication of chitosan and chondroitin sulfate based polyelectrolyte complex containing 58S sol-gel derived bioglass. The developed composite biomimetic porous scaffold shows improved physico-chemical and biological property, which further facilitate nucleation of apatite layer over the scaffold. The developed scaffold was further characterized by FESEM, TEM, FTIR and XRD to analyze the morphology of apatite particle and its deposition. Furthermore, MG63 cells were used to study osteogenic potential of developed scaffold. After culturing of cell-scaffold construct for weeks, cells shows significantly higher biomineralization and expression of osteogenic marker. Hence, developed polyelectrolyte based composite biomimetic scaffold may be considered for bone tissue engineering application.

Keyword: Chitosan; Composite scaffold; Bone tissue engineering; Biomimetic

a90153

100-SYIS CS-9 Superparamagnetic Nanoparticle in Gradient Tissue Engineering

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Various tissues, such as bone, tendon and cartilage, possess an inhomogeneous structure due to biochemical gradients that guide tissue formation during embryonic development. A robust and relevant engineered graft must recapture the complex hierarchical microstructure of the physiological tissue, however, most of tissue engineering systems are designed homogeneously. To address this, we introduce a novel platform to rapidly produce smooth biochemical gradients across different biomaterial systems.

The proposed platform is based on glycosylated superparamagnetic nanoparticle (SPIONs) with magnetic field alignment that can be used to produce the biochemical gradient. This proposed approach can provide a smooth transition of bioactive molecule compared to conventional approach such as stacking biphasic layers. To demonstrate our system, human mesenchymal stem cells were used to produce a gradient osteochondral tissue. While the chondrogenic differentiation was triggered universally with the designed osteochondral medium, SPION-mediated patterning of a growth factor gradient provided spatially-directed osteogenesis.

Our strategy successfully generated an osteochondral tissue construct with a mineral transition from cartilage to bone. After 28 days of culture, we produce gradient tissues possessing distinct character on either end. The construct exhibited distinct cell types (chondrocyte and osteoblast), protein expression (alkaline phosphatase activity, distribution of osteopontin) and tissue mineralization (presence of HAP/ β -TCP mineral). This technology offers great versatility and could be tailored to other systems, offering new opportunities for a range of interfacial tissue engineering challenges.