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Engineering Electroconductive Scaffolds for Cardiac Tissue Regeneration

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Oral Abstracts

Scientific Session 1: Advances in Cardiac Tissue Engineering & Bioengineered Valves for Vascular Repair

Monday, December 4, 2017, 10:00 AM - 11:30 AM

1

Tissue-engineered Cardiobundles for *In Vitro* Development of Heart Regeneration Therapies

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Cardiomyocytes (CM) in the adult mammalian heart have limited capacity to proliferate following injury. Thus, therapies to induce endogenous regeneration of the heart would significantly improve cardiac function in the setting of heart disease. We have previously engineered 3-dimensional cylindrical tissues (''cardiobundles'') made from neonatal rat CM embedded in fibrin-based hydrogel (~16 cardiobundles derived from 1 neonatal heart), which accurately mimic the dense and anisotropic cellular structure of native myocardium while also displaying tissue-level functional properties comparable to the adult or adolescent heart [1]. Here, we show that CM in cardiobundles stop proliferating (<1% turnover per day) after 2 weeks in standard culture conditions, representing a high-fidelity *in vitro* model to test potential therapies for re-activation of the cell cycle in post-mitotic myocardium. Furthermore, we establish various injury methods (e.g., cardiotoxin, hydrogen peroxide, calcium ionophores) that result in CM death and a 60-80% decline in the cardiobundles' active contractile force, enabling studies of CM regeneration and functional recovery after cardiac injury. Electrical stimulation of injured cardiobundles results in partial yet incomplete regeneration and recovery of contractile force. The cardiobundle injury model is thus a realistic 3-D system to test new small molecule and gene therapies targeting CM regeneration in the terminally differentiated heart, with higher throughput than *in vivo* studies on the intact heart.

Acknowledgments: NIH grant HL134764, Foundation Leducq grant, NSF GRF # 2013126035. [1] Jackman CP, Carlson AL, Bursac N. Biomaterials 111:66-79 (2016).

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Engineering Electroconductive Scaffolds for Cardiac Tissue Regeneration

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Heart failure following myocardial infarction (MI) is a leading cause of death in the United States. This issue is due to the inability of myocardium to regenerate, leaving behind a fibrous scar tissue that is unable to contract or propagate electrical impulses. Tissue engineering (TE) approaches utilize different natural and synthetic materials to develop scaffolds for cardiac tissue regeneration. Here, we engineered highly biocompatible and conductive hydrogels for cardiac tissue regeneration by combining gelatin methacryloyl (GelMA) prepolymer and a choline-based bio-ionic liquid (Bio-IL). GelMA, a naturally derived photocrosslinkable biopolymer, was selected due to its tunable mechanical properties, favorable biodegradation, and the presence of cell binding sites, which promote cell adhesion and proliferation. Bio-IL, an organic salt high water solubility and ionic conductivity, and electrochemical stability, was also used due to its biocompatibility and conductivity. In this study, the electroconductive scaffolds were fabricated using two techniques: i) bulk fabrication through photopolymerization and ii) electrospinning. Our results exhibited that the scaffolds possessed high conductivity *in vitro* and *ex vivo* as well as remarkable mechanical stability and strength. The *in vitro* 2D and 3D culture of primary rat cardiomyocytes (CMs) showed that the engineered hydrogels were highly biocompatible and could promote CM growth and function. The subcutaneous implantation of the engineered scaffolds in rats exhibited high biocompatibility and biodegradation *in vivo*. Taken together, this study showed that GelMA/Bio-IL scaffolds could be readily tailored to different tissue engineering applications particularly cardiac tissue regeneration.

Scientific Session 1: Biomaterial Scaffolds 1

Monday, December 4, 2017, 10:00 AM - 11:30 AM

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Engineering Silk Biomaterials for Cancer Therapy

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2D models are failing to predict the accuracy and efficiency of cancer therapeutics. Thus 3D culture systems are adapted to unwind true structural and functional cancerous microenvironment. *In situ*, cancer microenvironment contains extracellular matrix (ECM) and diverse cells (stem and immune cells). The most abundant cancerous ECM is collagen, playing critical role in cancer metastasis. Silk protein fibroin is structurally homologous with collagen. Structural homology with naïve disease ECM and ability to adapt different morphologies as per prerequisites of application, enhances its prospect to be explored for cancer modelling and therapy. Porous silk sponges are able to serve as ECM support for breast and liver carcinoma, leading to development of functional solid hypoxic tumors with drug sensitivity. This improves the understanding of cell-ECM interaction in disease progression and therapeutic response. Stem cells are further incorporated within this hypoxic model to unwind the role of cellular cross-talk in cancer metastasis to secondary sites such as bone. Further, the structural diversity of silk biomaterials allows to deliver anti-cancer therapeutics by means of nanoparticle with target specificity imparted by folate conjugation. However, the exploitation of potential biopolymer like silk in cancer is at neonatal stage. Foreseen applications include mimicking of mechano-transducing cancerous ECM using flexibility and mechanical robustness of silk, designing of organ-on-a-chip and micro-tumors using silk. (Supported by Department of Biotechnology [BT/PR10941/MED/32/333/2014], Indian Council of Medical Research [5/13/12/2010/NCD-III], Govt. of India. SCK currently holds ERA Chair under EU Framework Programme for Research and Innovation Horizon 2020 FoReCaST - agreement number n° 668983)

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Advances in Biomaterials for Tissue Regeneration: Functionalization, Processing and Applications

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Scientists can now envision a wide range of possibilites when addressing tissue and organ regeneration due to the great developments

in the biomaterials field. At the 3B's Research Group, we have been developing several natural-based biomaterials (e.g. chitosan, gellan gum, silk fibroin, alginates, and kefiran) with the required functionalities for further processing as micro- and nano-particles, hydrogels, membranes, capsules, scaffolds, and bioinks. Herein, the most relevant advances of biomaterials functionalization and processing methods for further application in musculoskeletal, neurosciences and cancer research will be presented.

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Self-healing and Adhesive Chitosan/Graphene Oxide Composite Hydrogel based on Mussel-inspired Chemistry for Electroactive Tissue Engineering

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Hydrogels currently used in electroactive tissues, such as skin and cardiac tissues, have certain shortcomings, such as a lack of electrical conductivity and adhesiveness, which play a key role in the success of hydrogels in biomedical applications. In this study, chitosan (CS)/graphene oxide (GO) composite hydrogels that are self-adhesive, self-healing, and conductive were prepared by the incorporation of mussel-inspired protein polydopamine (PDA). During the oxidizing process of dopamine (DA), graphene oxide was reduced by PDA and dispersed into the hydrogel networks to form electronic pathways. The covalent bonds, supramolecular interactions, hydrogen bonding, and π-π stacking enabled the chitosan/GO composite hydrogels to have better stability, stronger mechanical behavior, and good adhesive properties, as well as self-healing and fast recoverability characteristics. The self-healed test samples, which were recovered from dissected pieces and reassembled into a whole, healed piece, exhibited almost the same performance and properties as the original whole samples. The electrical conductivity of the CS/GO reached 1.22 mS/cm and the adhesive strength of the composite hydrogel was increased by 300% compared to CS-DA hydrogels. HEF1 and cardiomyocyte cells derived from human embryonic stem cells showed better biological behavior, such as cell viability and proliferation, compared to the CS-DA hydrogels. Moreover, cardiomyocytes showed a faster spontaneous beating rate than those on the control groups. Our work pointed to a cost-effective and scalable approach to fabricating polydopamine-based hydrogels that are adhesive, conductive, self-healing, and have fast recovery rates. These hydrogels have potential applications in electroactive tissue engineering.

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Gellan Gum Spongy-like Hydrogels Reinforced with Hydroxyapatite for Bone Tissue Engineering Applications

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Bone tissue engineering with cell-laden-hydrogels has been attracting attention worldwide. In fact, they can resemble in some aspects the extracellular matrix, as high water content. In a forward thinking manner, our group engineered gellan gum (GG) spongy-like hydrogels where cells adhered and proliferated without the need of any modification. Moreover, we took advantage of these characteristics and combined it with hydroxyapatite to reinforce the GG (GG-HAp) spongy-like hydrogels. Since there is limited knowledge about the interaction of osteoclasts with biomaterials, we assessed the ability of developed matrices to support osteoclastogenesis. After mechanical and morphological characterization, bone marrow cells were seeded and treated with 1,25-dihydroxyvitamin D3 (Vitamin D3) to promote osteoclastogenesis. After 7 days, cell viability and differentiation was assessed. It was shown that the addition of HAp to GG spongy-like hydrogels enable the formation of lager pores and thicker walls and the improvement of the mechanical properties (stiffness). Additionally, cells were viable and metabolically active. Finally, it was observed that the GG-HAp spongy-like hydrogels supported cell differentiation into pre-osteoclasts, as suggested by the presence of aggregates of TRAP-stained cells, and the expression of DC-Stamp and Cathepsin K, necessary for cell fusion and resorption capacity, respectively. Overall, GG-HAp spongy-like hydrogels showed to support osteoclastogenesis, an important feature when establishing new biomaterials for bone tissue applications.

Acknowledgments: The authors thank the funds provided by FROnTHERA (NORTE-01-0145-FEDER-0000232). FRM acknowledges FCT (SFRH/BPD/117492/2016) and ERC-2012-ADG 20120216-321266 (ComplexiTE), LPS thanks FCT (SFRH/BD/78025/2011), JMO and VMC thanks FCT Investigator program (IF/01285/2015 and IF/01214/2014).

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Nanomaterial-Based Electrochemical Stem Cell Differentiations

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Tunable Nano-Bio-Scaffolds (NB-Scaffolds) that direct the Stem Cell differentiation is critical for stem cell-based regeneration. The impact of the chemical composition, surface topography, and stiffness on the stem cell behavior was highly explored and their importance is notable. However, the impact of the NB-Scaffolds-dynamic stimuli to stem cell fate is still unclear. Herein, we will present the synthesis of various scaffold compositions and show/compare that their electrochemical stimuli are inducing the surface adhesion and differentiation of various stem cell types to neuronal and cardiac progenitors. These electrochemical induced NB-scaffolds provide an alternative to the classical culture substrates for a faster and more efficient way of regulating stem cell behavior.

Scientific Session 1: CNS Neural Tissue Repair, Regeneration, Derivation, and Engineering of *In Vitro* Models

Monday, December 4, 2017, 10:00 AM - 11:30 AM

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Regional Patterning and Engraftment of hPSC-Derived Posterior Central Nervous System Tissue

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In vitro modeling of the human central nervous system (CNS) is complicated by the diversity of constituent cell phenotypes. This diversity arises from neural stem cells (NSCs) that are regionally patterned along both the rostrocaudal (R/C) and dorsoventral (D/V) axes during early neural tube development. Recapitulating this patterning with chemically defined, scalable protocols using human pluripotent stem cells (hPSCs) is necessary to optimally model CNS disorders, screen potential drug therapies, and develop cell replacement strategies for regeneration. We have previously reported methods to efficiently differentiate hPSCs to $Pax6^{+}/Sox2^{+}$ NSCs and to deterministically pattern their regional phenotype, generating discrete R/C *HOX* profiles that span the hindbrain through spinal cord. Here, we optimize D/V patterning conditions by modulating Shh, Wnt/β-catenin, BMP, and TGF-β signaling pathways. qRT-PCR of differentiated hESCs demonstrates a shift in expression of dorsally expressed transcription factors in response to Wnt/β-catenin, BMP, and TGF-β signaling to ventrally expressed transcription factors in response to Shh signaling. Immunocytochemistry

and RNA-seq confirm enrichment of NSC and neuronal populations reflective of appropriate phenotypes. To investigate whether regional specificity impacts integration into circuits *in vivo*, hESC-derived motor neurons (MNs) were transplanted into developing chick embryos. Quantification shows cervical MNs are able to selectively engraft into the appropriate region of the developing chick neural tube about the cervical/thoracic border. Optimization and characterization of mature neuronal populations are ongoing. Having access to this hCNS model with organotypic cell phenotype diversity and a comprehensive transcriptomic time-course will be a valuable resource for hindbrain and spinal cord regenerative medicine.

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Nanofiber Scaffold-mediated Non-viral Delivery of microRNA-219/-338 Protects Survival and Stimulates Oligodendrocyte Precursor Cell Maturation After Spinal Cord Injury

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Following spinal cord injury (SCI), oligodendrocytes (OL) adjacent to the lesion site die and cause axonal demyelination. The aim of this study was to use a scaffold-mediated non-viral microRNA delivery approach to enhance remyelination by promoting the differentiation and maturation of endogenous oligodendrocyte precursor cells (OPC) after SCI. We developed a biodegradable electrospun nanofiber-hydrogel scaffold that delivers miR-219/-338 in a sustained manner for up to 20 days *in vitro*. After 1 and 2 weeks post-implantation (WPI), the total number of cells per peri-lesional area was similar with and without miR-219/-338 treatment (miR-219/-338). MiR-219/-338 retained the number of oligodendroglial lineage ($Olig2^+$) cells at 1 and 2 WPI. Conversely, scrambled miR (Neg-miR) showed a significant decrease in cell number ($p < 0.05$). $PDGFR\alpha^+$ OPCs showed a larger cell number decrease in miR-219/-338 (0.39-fold change) compared to Neg-miR (0.37-fold change). Correspondingly, by 2 WPI, miR-219/-338 showed a significant increase in mature OL ($CC1^+$) numbers as compared to Neg-miR (1.47 ± 0.27 $CC1^+$ cells/area in miR-219/-338 vs 1.1 ± 0.21 $CC1^+$ cells/area in Neg-miR, $p < 0.05$). These results indicate that scaffold-mediated non-viral delivery of miR-219/-338 protected the survival of OPC/OL and significantly increased the number of mature OL after SCI.

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Neuron-Glia Interactions Recapitulated in a 3D Organotypic Human Alzheimer's Disease Brain Model

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Alzheimer's disease (AD) is characterized by amyloid beta (A-beta) accumulation, hyper-activation of glial cells, tau tangle formation, and neuronal loss, which eventually leads to neurodegeneration. The underlying mechanisms of AD pathogenesis, however, remain poorly understood partially due to the absence of relevant models that can comprehensively recapitulate pathogenic cascades of human AD brains. Here, we present a new 3D organotypic human cellular AD brain model by tri-culturing human AD neurons, astrocytes, and adult microglia in a 3D microfluidic platform (3D NeuroGliAD). Our model provided representative AD signatures of pathological accumulation of A-beta, phosphorylated tau (pTau), neurofibrillary tangle (NFT)-like structure formation inside neurons, and interferon-gamma (IFN-γ) production from astrocytes. In addition, the model reconstituted microglial inflammatory activities and elicited neuronal loss mediated by nitric oxide synthase (iNOS) expression and cytotoxic NO release via TLR4 and IFN-γ receptors, respectively. We envision that our 3D organotypic human multicellular brain model can represent critical features in human neuroinflammatory diseases and compromise the limitation of animal models for the better understanding of pathogenic neuron-glia interactions in neurodegenerative diseases and serve as a valid drug-screening platform at a high throughput.

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Tissue Engineered Bioelectronics for Brain-Machine Interfaces

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Communication across neural interfaces typically relies on conventional metal electrodes and their *in vivo* electrical and biological performance is restricted by fibrosis. The hypothesis driving this research was that neural cell loaded coatings provide a more physiological interface, able to integrate electrodes with the neural tissue and improve performance. The study aimed to develop tissue-engineered, living electrode (LE) coatings for brain-machine interfaces and evaluate *in vitro* and *in vivo* function. LE fabrication was achieved by coating the electrode with conductive hydrogel (CH) and overlaying the CH with primary neural progenitor cells encapsulated in 3D biosynthetic hydrogels. *In vitro* studies confirmed that charge storage capacity (CSC) and frequency dependent electrochemical impedance were similar in LE and CH. Mass loss and swelling of the LE and CH were also similar, suggesting that material performance of the LE was comparable to the CH alone. Implantation of LE coated and uncoated platinum control electrodes in a rat brain model confirmed that the LE did not cause any adverse events over 8 week implantation. LE had significantly improved signal to noise ratio and further research will examine the tissue response to understand the mechanisms for this observation. These results demonstrate the potential for a tissue-engineered electrode to support development of more robust neural interfaces *in vivo.*

Acknowledgments: The research was supported by the Defense Advanced Research Projects Agency (DARPA) under contract number HR0011-16-C-0032. Experiments were approved by UNSW Animal Care and Ethics Committee approval (15/145B).

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Treatment of Focal Neurological Deficits Using Strategic Generation of Stem Cell-Derived Neurons

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Restoring function to localized neural tissue provides an attractive target for cell-based regenerative medicine therapies estimated to benefit millions of patients annually (WHO, CDC). However, controllably generating specific neuron subtypes to replace damaged cells remains a substantial technical and translational challenge. To address this objective, we used a two-pronged approach. First, we systematically reviewed primary literature to identify promising paths and rate limiting factors in clinical and commercial adoption of stem cell-based neuronal therapies. Second, we designed and applied a paired novel computational model with an *in vitro* embryonic stem cell culture platform to iteratively improve design rationale for excitatory and inhibitory neuron production from stem cells. Results from this work (1) prioritize target patient populations most likely to benefit from options for excitatory and inhibitory neuron therapeutic cells, (2) provide a consensus and evidence-based assessment of the potential use of cell therapies to treat focal neural disorders, (3) yield a quantitative, tuneable system for tracking stem cell commitment to neural and neuronal subtype cells, and (4) demonstrates the relative kinetics of excitatory and inhibitory neuron differentiation, including identification of novel biomarkers for cell fate choice that are >99% predictive of GABAergic or glutamatergic cell fate choice at

significantly earlier time points than currently used markers of fate choice. This work advances technical and quantitative science about on-demand neuron subtypes for regenerative therapies, and provides insight into design choices that may promote faster and more feasible clinical translation and commercialization of regenerative medicine stem cell therapies for focal neural deficits.

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Modeling Neurogenesis and Vascularization in 3D

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Understanding how neural and vascular networks spontaneously regenerate in small injuries is critical to enhance material-based interventions to regenerate large traumatic injuries; which are limited by the development of these networks. During embryogenesis, neural and vascular tissues form interconnected, complex networks as a result of signaling between these tissues. In this study, we report on our efforts to recreate signaling conditions found in development to generate a model tissue-like system to study the co-development of vascular and neural networks in order to understand and exploit this crosstalk towards improved regenerative medicine outcomes. Specifically, we investigated how human umbilical vein endothelial cells (HUVECs) and chicken dorsal root ganglia (DRG) interacted in a series of neighboring channels within a collagen gel. HUVECs seeded in channels maintained CD31 expression for at least 7 days. Axons grew towards and along HUVEC-seeded channels, regardless of whether or not the DRGs were seeded near these channels, demonstrating that this system was able to direct axonal growth towards specific stimuli. These data suggest that HUVEC-seeded constructs direct axonal growth, and support our hypothesis that hollow channel collagen gels provide a facile 3D model to study the interactions between neural and vascular systems. Current work is focused on seeding explants prior to HUVEC incorporation into the model to simulate the co-development of these networks. This co-culture tissue model will allow for the study of neurogenesis and angiogenesis and will be used to inform targeted innervation strategies and ultimately improve remodeling of large tissue defects.

Scientific Session 1: Skin, Inflammation and Wound Healing General Session

Monday, December 4, 2017, 10:00 AM - 11:30 AM

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Autologous Mesenchymal Stem Cells (MSCs) at the Early Passages Accelerate Diabetic Wounds Re-epithelialization: Clinical Case Study

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Diabetic foot ulcers (DFUs) are major complication of diabetes and the common cause of non-traumatic amputation. A pathologically low rate of wound closure is a hallmark of non-healing DFUs. Mesenchymal stem cells (MSCs) ($\geq 1\times10^6$ cells/cm^2) have emerged as a promising tool for the treatment of diabetic wounds, while optimal regime of MSCs use is yet to be established. In the present three-case clinical study, we explored effect of the early passage MSCs with a purpose to select optimal dosage regimen prior to the large scale investigation. We found that the topical application of autologous early passage MSCs ($p \leq 2$) at dose of $4x10^4$ cells per cm^2 of wound surface resulted in a significant 2.2-fold increase of average rates of re-epithelialization until complete neuropathic wound closure as compared to rate values at baseline (0.20 ± 0.04 vs. 0.09 ± 0.01 cm^2/day respectively, $p = 0.0044$, $n = 3$). A maximum 8-fold increase in rates of wound closure was observed within the first week after MSCs applications ($p = 0.037$). Then re-epithelialization rates began to slow, while were still significantly higher than at baseline at any time point. All three wounds were closed successfully. Together, these study provides the first evidence of clinical efficacy of the early passage MSCs ($p \leq 2$) in the treatment of diabetic wounds. Furthermore, a long-term cultivation *in vitro* to yield large amounts of MSCs is not required and clinical effectiveness in DFUs treatment can be achieved at much less doses than those reported previously with the early passage MSCs.

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Hollow Microgels for Enhanced Wound Repair

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Wound repair initiates with the formation of a provisional fibrin mesh to stop bleeding. Subsequent platelet-mediated clot contraction increases fibrin matrix density/stability which promotes healing. Synthetic platelet-like particles (PLPs)[1], which are comprised of pNIPAm microgels coupled to fibrin-binding antibodies, induce clot collapse and indicate potential for enhancing wound healing outcomes. To facilitate fine control over particle-mediated fibrin network collapse/stiffening, we have constructed hollow microgel architectures with varying degrees of deformability (a critical feature for PLP-clot collapse) by varying crosslinking density. Microgels are then coupled to a fibrin-binding antibody with EDC/NHS chemistry. We hypothesized that increased particle deformability would correlate with increased clot collapse and improved wound healing outcomes. Microgel size was assessed with dynamic light scattering and microgel deformability (i.e particle spreading) was determined with atomic force microscopy (AFM). *In vitro* clot collapse in the presence of fibrin-binding particles was visualized with confocal microscopy, and wound healing and inflammatory responses were assessed *in vivo* through analysis of wound size and histology after topical application of microgels in a murine dermal wound model. Particle deformability and particle-mediated collapse were inversely correlated with hollow particle crosslinking. *In vivo* results displayed enhanced wound repair in the presence of deformable microgel treatments as compared to controls. These results indicate that hollow, fibrin-binding microgels induce fibrin network collapse and enhance wound repair.

References: 1. Brown, A. *et al*. Nat. Mater. 13, 1108-1114 (2014).

Acknowledgments: AHA Scientist Development Grant 16SDG29870005Abrams Undergraduate Research Award.

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Novel Porcine Model of Delayed Wound Healing Aimed at Modulating the Rate of Wound Closure

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Background: Previously[1], we demonstrated a model to simulate healing impairment in chronic wounds such as diabetic ulcers caused by glycosylation of collagen resulting from extended exposure to hyperglycemia[2]. In this follow-up study, we evaluated (i) if the intensity of healing impairment could be modulated by varying the concentration of the chemical crosslinker and (ii) if surgical debridement of crosslinked wounds would impact healing time.

Methods: Twenty full-thickness wounds (2 cm diameter) were created on the dorsum of three Yorkshire pigs. To rapidly simulate glycosylation of collagen, wounds were immediately treated with 0.1 to 1% (v/v) glutaraldehyde for 5 minutes to crosslink collagen. Wounds were surgically debrided on Day 3.

Results: In the glutaraldehyde-treated wounds, there was a dose dependent response with respect to wound closure—higher concentrations of glutaraldehyde correlated with delayed wound closure. At day 14, 90% closure or better was attained by 18 of 20 control (untreated) wounds, 14 of 20 of 0.1% (v/v) glutaraldehyde-treated wounds and zero of 20 of the 1% (v/v) glutaraldehyde-treated

wounds. Healing was significantly delayed in all glutaraldehyde-treated wounds at days 1, 3, 7, 11 and 14 (t-test vs. control, $p < 0.001$). Further, all glutaraldehyde-treated wounds regardless of debridement experienced delayed healing compared to the control.

Conclusion: Glutaraldehyde-treatment of dermal tissue can significantly delay wound healing *in vivo* and the degree of wound closure can be modulated by varying the concentration of glutaraldehyde.

References:

1. Kharge A *et al*. Abstract#1390, TERMIS-AM, Dec 2016.
2. Hennessey PJ *et al*. J Pediatr Surg. 25(1):75-8, Jan 1990.

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Targeted and Sustained Delivery of Polyphosphate Suppresses Bacterial Collagenase for Promotion of Post-Surgical Intestinal Healing

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Wound healing and repair of the intestinal tract is a complex process due to the presence of intestinal microbiota which can impair or enhance the healing process. Previous studies indicate that under phosphate depleted conditions certain bacteria (*E. faecalis, S. marcescens, P. aeruginosa*) secrete high levels of collagenase, a key phenotype involved in healing impairment. Drug delivery approaches focused on sustained release of compounds that could suppress collagenase production by pathogens while allowing commensal bacteria to proliferate normally offer a major advantage for prevention and treatment of these problems. We have previously developed a drug delivery approach involving the use of hydrogel nanoparticles that provide prolonged polyphosphate release, and which suppress the *in vitro* virulence of intestinal pathogens while maintaining bacterial survival. Polyphosphate (PPi), was encapsulated in poly(ethylene) glycol hydrogel nanoparticles (NP-PPi) formed using inverse miniemulsion polymerization. Free PPi and NP-PPi reduce *in vitro* collagenase levels in gram-negative pathogens (*P. aeruginosa* and *S. marcescens*) while maintaining bacterial survival. Mice subjected to colonic anastomosis exhibited normal healing ($n = 5$) whereas mice subjected to both anastomosis and intestinal bacterial inoculation exhibited adhesions and inflammation ($n = 4$). Furthermore, mice subjected to anastomosis, bacterial inoculation, and antibiotic treatment exhibited severe inflammation and in some cases, gross leaks ($n = 10$). Mice subjected to colonic anastomosis and bacterial inoculation treated with PPi dosed drinking water exhibited attenuation in *S. marcescens* colonization and prevention of healing complications ($n = 10$). Current studies are being performed to test the effectiveness of NP-PPi in preventing healing complications in mice subjected to intestinal injury.

Scientific Session 1: Biofabrication and Bioreactors 1

Monday, December 4, 2017, 10:00 AM - 11:30 AM

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Laser-based Biofabrication Methods

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North Carolina State University, Raleigh, NC.

Laser-fabricated structures, such as tissue engineering scaffolds, implantable sensors, and drug delivery devices, will become important tools for medical diagnosis and treatment over the coming decades. Over the past decade, we have examined use of several laser technologies, including pulsed laser deposition, matrix assisted pulsed laser evaporation, matrix assisted pulsed laser evaporation-direct write, laser micromachining, and two photon polymerization, to process microstructured and nanostructured materials for medical applications. For example, we have shown that a laser-based approach known as two photon polymerization may be used to process a variety of photosensitive materials, including acrylate-based polymers, hydrogels, and organically-modified ceramic materials, into medically-relevant structures. Two photon polymerization involves an application of the multi-photon absorption phenomenon for 3D printing at sub-micrometer length scales. This technique involves nearly simultaneous absorption of two photons by a small volume of a photosensitive material, leading to highly localized polymerization and hardening of the material. We have prepared structures with complex microscale geometries out of photosensitive materials using two photon polymerization; cell interactions with these structures were examined using a variety of *in vitro* studies. Micromolding can be used to replicate two photon polymerization-prepared patterns. Current efforts to improve the biocompatibility of laser biomaterials and modify laser methods for clinical translation will also be considered.

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Lab-on-a-Printer™ - a Multi-Material 3D Bioprinting Platform for Building Living Tissues on Demand

S. Wadsworth, S. Beyer, K. Walus, K. Thain, S. Pan, T. Mohamed;

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Recreating the complex heterogeneity and microstructure of biological tissues is a challenge. To address this, we have developed a novel 3D bioprinting system based on a paradigm called Lab-on-a-Printer™ (LOP). LOP™ printheads integrate microfluidics and filament deposition technology enabling rapid switching between different cell-containing bioink compositions in a single printhead. Custom software tools precisely coordinate material switching with printer toolpaths to enable rapid fabrication of heterogenous living 3D tissues. In generating the cell-laden hydrogel fiber, we utilize co-axial flow focusing, a method that protects printed cells from damaging shear stress within sub-100 μm hydrogel fibres which are generated with very high cell density (from 0 to $>100 \times 10^6$ cells/mL), printed at high speeds (100mm/sec) and maintain high cell viability (>95%). The LOP™ system can print fibres containing multiple extracellular matrix factors, growth factors, and other bioactive compounds. Selective customisation of the cellular microenvironment enables us to print a variety of cell types and produce functional tissues: The *3DBioRing™ Airway* tissue contains primary human airway smooth muscle cells printed in a proprietary biological matrix. The cells are viable for over 2 weeks post-printing, form interconnected networks and are functionally contractile, exhibiting a physiological bronchoconstriction-like response to histamine ($EC_{50} = 0.60\mu M$) and a pharmacologically-appropriate relaxation to salbutamol ($EC_{50} = 39nM$). The LOP™ system is broadly applicable for printing a variety of cell types including, adherent primary cells (eg. dermal fibroblasts), non-adherent immune cells (eg. cord-blood derived mast cells), and multiple cell lines (eg. HepG2 hepatocytes).

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A 3D Printed Microfluidic Bioreactor to Engineering Biphasic Construct

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Introduction: Tissue engineered constructs coupled with high-throughput bioreactors can be used as innovative *in vitro* preclinical models of tissue functions and disease pathogenesis for drug screening and toxicity assessment [1]. Here, we present an innovative 3D printed bioreactor for biphasic constructs, enabled with optical access for direct monitoring of cellular responses.

Materials and Methods: Two different fluid circuits for the bioreactor were tested using food coloring and the degree of mixing

in the outlets was quantified with a plate reader. Bone marrow derived human mesenchymal stem cells (BM-hMSCs) were suspended (1 million cells/ml) in 8% methacrylated gelatin (GelMA), UV crosslinked *in situ* in the bioreactors [2], and perfused with an inlet flowrate of 1 ml/day. A Dynamic Live Assays was performed adding Calcein-AM to the medium stream and imaging constructs in 3D. A Dynamic Metabolic Assay was similarly performed using Alamar-Blue and analyzing the output media every 42h.

Results and Discussion: Cells were successfully stained and imaged by the dynamic live assay within the bioreactors where they maintained viability and active metabolism, confirming the possibility of non-destructive monitoring within the system. For the biphasic bioreactor, experimental tests confirmed modelling predictions [3], showing minimal mixing in the media stream for each tissue component.

Conclusions: The successful dynamic staining of the constructs validates the use of the bioreactors to perform a continuous, non-destructive, real-time monitoring of cellular responses without breaking sterility. This is especially relevant for biphasic constructs that require specific media for each component [4].

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A Fluidic Bioreactor Platform for Spatially Patterned Cell Growth, Differentiation, and Cocultures

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Perfusion bioreactors have been used widely in the past decade to enhance *in vitro* culture of stem cells. We develop a pillar-based fluidic bioreactor chamber for dynamic stem cell culture, with emphasis on controlling cellular interactions in a three-dimensional environment via flow and substrate curvature, which are physiologically relevant to natural tissues. We utilize shear stress of up to 15 mPa on cell surfaces to enhance growth of human mesenchymal stem cells (hMSCs), resulting in 5-fold expansion within 7 days, exceeding the 3-fold expansion in static two-dimensional culture plates. The pillar geometry in the bioreactors dictates cell spatial patterning through preferential initial cell localization within $\sim$300 μm radius from the pillars, potentially due to cell affinity for local negative curvatures. Fluid flow overcomes this localization, creating homogeneous cell distribution throughout the bioreactor within 24 hours. Flow-enhanced osteogenic differentiation is evidenced by 10-fold enhancement of alkaline phosphatase (ALP) expression within 7 days, where local ALP expression positively correlates with local shear stress profiles caused by the flow past the pillars. Additionally, cell localization in the bioreactor enables patterning of hMSCs and endothelial cells in cocultures, potentially modulating the signaling between the two essential cell types in the bone marrow microenvironment. Overall, we have developed a fluidic bioreactor platform for dynamic stem cell culture, with demonstrated capabilities to create spatially patterned cell growth and osteogenic differentiation through flow patterning. The platform's scalability, adjustability, and capability of forming three-dimensional tissue structures allow for integration into large-scale perfusion bioreactor systems for tissue engineering applications.

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Effects of 3D Printing on Flow-Induced Shear Distributions

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As 3D printing gains more exposure as the future of scaffold fabrication for tissue and tumor engineering, the ability to preemptively model the constructs microenvironment becomes of vital importance. Particularly when using perfusion based bioreactor systems, the most important properties to obtain are the fluid flow and wall shear fields that potential cells will experience during and after seeding. Traditionally, 3D printing has given users the impression that the scaffold obtained after printing will exhibit the same, or closely similar, architecture as the designed model. While this may be the case in terms of the macrostructure, we hypothesized that the local fluid dynamic environment would differ greatly. The intention of this study was to 3D print scaffolds of various pore size gradients, image these constructs utilizing micro-computed tomography, and perform computational fluid dynamic simulations on the resulting reconstructions and the initial designs in order to compare the average shear on the fibers, where the cells would be adhered. As a determining factor, the probability density function (p.d.f.) of each was compared to not only highlight the major differences, but also to show the reproducibility of the printing process. The results presented in this manuscript give users the knowledge of how the 3D printing process may alter the intended fluid environment they designed, and how to, in spite of that fact, produce repeatable results.

Scientific Session 2: Strategies for Respiratory, Urologic and Gastrointestinal Engineering

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Engineered diaphragma

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Background: In repair of congenital diaphragmatic hernia with large defects, prosthetic patches can cause infection, recurrence, and thoracic deformity. Biosheets (collagenous connective tissue membranes) are reportedly useful in regenerative medicine. We evaluated the efficacy of biosheets in regeneration and repair of diaphragm in a rabbit model.

Methods: Biosheets were prepared by embedding silicone plates in dorsal subcutaneous pouches of rabbits for 4 weeks. In group 1 ($n=11$), Gore-Tex® sheets (1.8×1.8 cm) were implanted into the diaphragmatic defects. In group 2 ($n=11$), SEAMDURA®, a bioabsorbable artificial dural substitute, was implanted in the same manner. In group 3 ($n=14$), biosheets were autologously transplanted into the diaphragmatic defects. All rabbits were euthanized 3 months after transplantation to evaluate their graft status.

Principal results: Herniation of liver was observed in 5 rabbits (45%) in group 1, 8 (73%) in group 2, and 3 (21%) in group 3. A significant difference was noted between groups 2 and 3 ($p=0.03$). Biosheets had equivalent burst strength and modulus of elasticity as native diaphragm. Muscular tissue regeneration in transplanted biosheets in group 3 was confirmed histologically.

Conclusion: Biosheets may be applied to diaphragmatic repair and engineering of diaphragmatic muscular tissue

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Assessment of Injected vs. Native Cells in Urinary Sphincter Regeneration of Nonhuman Primates with Urinary Sphincter Deficiency

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Objective: A major question remaining in the development of tissue engineering medicine approaches to tissue and organ replacement is the role of injected vs. native cells in tissue regeneration. The goal of this study was to develop a method to label and then identify injected autologous muscle precursor cells (skMPC) vs. native bone marrow cells (BMCs), labeled with different colored lentivirus promoters.

Methods: Ten female cynomolgus monkeys had surgically-induced urinary sphincter muscle deficiency. A muscle biopsy was taken to isolate and label (using a lentivirus-delivered M-cherry

promoter) skMPCs. Five weeks later, BMCs were collected by biopsy and adherent stromal cells labeled with a lentivirus GFP promoter. 20 million BMCs were injected intravenously 4 days following the monkeys receiving 3 intravenous injections of the bone marrow suppressant busulfan (1 mg/kg/day). One week later, 5 million M-cherry labeled skMPCs were injected directly into the urinary sphincter.

Results: Transduction produced 40% m-cherry positive skMPCs and 60% GFP positive BMCs. Fluorescent antibody cell sorting indicated that injected skMPCs were strongly skeletal muscle actin and MyoD positive and the BMCs were strongly CD133, CD117 and CD34 positive. Once injected, GFP-labeled cells constituted 40% of the bone marrow cells and could be readily found in the muscle layer and lamina propria of the sphincter complex. M-cherry labeled skMPC were more scarce (5% of cells) and more commonly found in the muscle layers.

Conclusion: It is concluded that this is an effective method to assess the relative contribution of injected vs. mobilized cells in tissue regeneration.

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Intestine Re-endothelialization For Whole Organ Bioengineering

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Short-bowel-syndrome remains an unmet clinical need. To address this issue, transplantation with engineered whole organs would an ideal solution. However, regeneration of the vascular network is a major limitation to engineered tissue survival and scaling-up for clinical use. The aim of this study is to regenerate a functional vascular network composed of endothelial and perivascular cells within decellularised rat intestine. Small intestine segments were harvested from rats preserving the mesenteric vascular plexus via cannulation, obtaining a segment suitable for orthotopic transplantation and decellularised according to a detergent-enzymatic protocol previously developed that preserves the integrity of the vascular network. Adult and iPSC-derived endothelial and perivascular cells were perfusion-seeded in the vasculature and cultured in specific growth condition, in order to promote the proliferation and stabilisation of the regenerated endothelium. Culture was performed inside a custom-made bioreactor specifically designed to provide perfusion through the blood vessels and the lumen of the intestinal scaffold. After 7 days in culture, the blood vessels patency was confirmed with fluorescent dextran assay. Immunofluorescence staining performed on whole mounts showed that endothelial cells engrafted till the very distal capillaries of the intestinal scaffold with considerable lumen coverage, expressing functional markers and showing development of a mature barrier function by formation of tight junctions. These findings represent a crucial step in the engineering and scale-up of a whole intestine. The functional vascular network will be essential for the survival of the graft *in-vivo*.

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An Acellular Regenerative Medicine Approach for Ulcerative Colitis

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Ulcerative colitis (UC) is the most common form of inflammatory bowel disease, affecting approximately 900,000 patients in the US. Although the exact etiology of UC is unknown, a damaged epithelial layer and an aberrant pro-inflammatory immune response to commensal microflora is characteristic of the disease. In a pre-clinical study of dextran sulfate sodium (DSS)-induced colitis in rats, extracellular matrix (ECM) hydrogel treatment rapidly resolved clinical signs of the disease, restored the epithelial barrier, and mitigated the pro-inflammatory state of resident tissue macrophages. Matrix bound nanovesicles (MBV) containing microRNA (miR) and protein cargo have been isolated from the ECM and are thought to be a potent vector of bioactivity. MiR-143/145 are highly expressed in MBV, down-regulated in UC patients, and essential for epithelial regeneration following DSS injury. MiR-143/145 knockout mice were used as a tool to identify the specific role of miR-143/145 in ECM-based UC therapy. ECM was isolated from the intestine of wild-type and knockout mice to produce ECM bioscaffolds that contain either physiologic levels or complete lack of expression of miR-143/145, respectively. *In vitro*, macrophages treated with wild-type and knockout ECM displayed differential activation states. Colitic mice treated with knockout mouse-derived ECM showed a prolonged pro-inflammatory state and impaired epithelial carrier as compared to wild-type ECM treated animals, suggesting the importance of miR-143/145 in ECM-mediated therapeutic effects. In conclusion, an acellular regenerative medicine approach based upon inductive signals delivered by MBV within ECM can restore homeostatic macrophage function and facilitate mucosal healing in a rodent model of ulcerative colitis.

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Restoration of Fecal Continence in a Large Animal Model: Long Term Follow-up

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Introduction: Fecal continence is primarily maintained by the internal anal sphincter (IAS). Loss of IAS integrity or functionality leads to passive fecal incontinence. Our previous studies have shown restoration of fecal continence in rabbits following implantation of engineered autologous IAS Biosphincters for up to 3 months.

Objective: Here, we studied a longer follow up (12 months) of restoration of fecal continence using IAS Biosphincters.

Methods: Fecal incontinence was induced in rabbits as described before. Rabbits were assigned to 3 groups: (i) non-treated group ($n=4$), (ii) treated group ($n=7$) that received implantation of Biosphincters and (iii) sham group ($n=5$). Anorectal manometry (resting pressure and RectoAnal Inhibitory Reflex, RAIR) was followed regularly for up to 12 months. Histological and pathological analyses were performed.

Results: Incontinence in rabbits was evident by their lack of fecal hygiene and by significant reduction in resting pressure and RAIR (26 ± 7 mmHg and $19\pm6\%$) compared to baseline (38 ± 2 mmHg and $62\pm5\%$). Resting pressures and RAIR were restored and maintained in the treated group (32 ± 3 mmHg and $63\pm6\%$) and were significantly higher than those in the non-treated group at 12 months. Resting pressures and RAIR were not significantly different between non-treated and sham groups.

Conclusion: This study provides a promising tissue engineering and regenerative medicine approach to treat passive fecal incontinence using engineered Biosphincters. Our long term follow up study (12 months) indicated the sustained long-term effect of Biosphincters implantation on restoration of continence.

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Cell Therapy for Neo-Innervation and Restoration of Neural Function in the Gut

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Background: Neuro-degenerative diseases can result in loss of innervation of the pyloric sphincter. Replenishing of neurons in the pylorus via direct injection of neural-progenitor cells (NPCs) provides a possible approach for long-term treatment.

Aim: The objective of this study was to inject gut-derived NPCs into denervated pylorus explants for re-innervation and reinstatement of function.

Methods: Pylorus tissue was harvested from rats and prepared for ex-vivo model of denervated pylorus. Explants were then either injected with fluorescently tagged NPCs or with PBS. Non-treated explants served as control. Neo-innervation and restoration of function were assessed by immunohistochemistry, qPCR and physiological analysis.

Results: Fluorescence microscopy confirmed presence of red fluorescing neurons in the submucosa and muscularis mucosae of the explants injected with NPCs, indicating the success of the injection process. Explants injected with tagged NPCs stained positive for nitric-oxide-synthase neurons (nNOS), indicating the differentiation of tagged NPCs. qPCR studies confirmed the presence of differentiated neurons in the explants injected with NPCs. Electrical field stimulation showed 87% regain of relaxation in the explants injected with NPCs compared to control and PBS-injected explants. With nNOS inhibitor, relaxation diminished ($\sim$ 51%) similar to control, which further validated differentiation of injected NPCs into nNOS neurons.

Conclusion: This study demonstrated that injection of NPCs into denervated pylorus tissues ex vivo resulted in neo-innervation of the tissue. Injected NPCs integrated into the pylorus, differentiated into functional neurons and reinstated its function. These findings suggest that NPCs cell therapy can be used to treat neurodegenerative diseases of the gut.

Scientific Session 2: Tissue Engineering Approaches for Musculoskeletal Defects Session 1

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Skeletal Muscle-Macrophage Platform for Modeling Tissue Regeneration

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In response to injury, adult skeletal muscle shows robust capacity for self-repair enabled by an intricate interplay between muscle stem cells and the immune system. Generating high-fidelity *in vitro* models of self-regenerative muscle could facilitate basic studies of muscle regeneration and enable screening and discovery of factors to enhance muscle repair and function in various pathologies. Here, we show that functional muscle tissues engineered from adult rat myogenic progenitor cells fail to properly self-repair *in vitro* following injury with cardiotoxin despite the presence of $Pax7^+$ muscle satellite cell (MuSC) pool and treatment with known pro-regenerative cytokines (IGF-1, CTGF, IL10). Remarkably, incorporation of bone marrow-derived macrophages (BMDMs) into the engineered muscle enabled near-complete structural and functional repair after injury. Notably, the BMDMs proved capable of significantly attenuating injury-induced myofiber apoptosis which enabled MuSCs within the engineered muscle to activate, proliferate, and differentiate to rebuild muscle mass. Shared-media experiments, immunostaining, and cytokine analysis further suggested a role of the immune cells in stimulating the transition from a pro- to an anti-inflammatory environment post-injury supported by the reduction of muscle death with direct TNFα inhibition. *In vivo*, BMDMs within implanted engineered tissues augment blood vessel ingrowth, cell survival, muscle regeneration and contractile function. Our studies reveal requisite roles of macrophages in engineering adult-derived muscle tissues with capacity for self-regeneration and inspire future development of biomimetic *in vitro* platforms for modeling and treatment of degenerative muscle injury and disease. This study was supported by grant AR070543 from NIAMS to N. Bursac.

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In situ 3D Bioprinting for cartilage regeneration

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Objectives: *In-situ* 3D printing is an exciting bio-fabrication technology to deliver tissue-engineering techniques by the surgeon at the time and location of need. We have created a hand-held extrusion ink-jet printing device (Biopen) that allows the simultaneous and co-axial extrusion of Bioscaffold and cultured cells directly into the defect that needs to be repaired. This pilot study aimed at assessing the use of the Biopen *in vivo* to repair a full thickness chondral defect in a large animal model.

Methodology: An 8-mm circular critical sized full thickness chondral defect has been created in the weight-bearing surface of the lateral and medial condyles of both femurs of 8 sheep. Each defect has been treated with either (i) hand-held *in situ* 3D printed bioscaffold using the Biopen (HH group), (ii) pre-constructed bench-based printed bioscaffolds (BB group), (iii) micro-fractures (Clinical Group) or (iv) left untreated (Negative Control Group). Histology and IHC have been performed in the retrieved condyles (O'Driscoll score) and biomechanical indentation tests have been performed to assess the physical properties of the regenerated cartilage tissue.

Results: The HH printed scaffolds (i) remained in place for the duration of the experiment (8 weeks), (ii) do not induce inflammatory reaction, (iii) allow early cartilage formation which shows superior macroscopic and microscopic score when compared to micro fractures.

Significance: This pilot study shows that direct in-situ bioprinting can be used to regenerate articular cartilage with superior results when compared to the clinically available surgical techniques.

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Repair Of Segmental Bone Defect Using Totally Vitalized Tissue Engineered Bone Graft By A Combined Perfusion Seeding & Culture System

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In this study, we proposed the ''Totally Vitalized TEBG'' (TV-TEBG) which was characterized by abundant and homogenously distributed cells with enhanced cell proliferation and differentiation and further investigated its biological performance in repairing segmental bone defect.

Methods: In this study, we constructed the TV-TEBG with the combination of customized flow perfusion seeding/culture system and beta-tricalcium phosphate (beta-TCP) scaffold fabricated by Rapid Prototyping (RP) technique. We systemically compared three kinds of TEBG constructed by perfusion seeding and perfusion culture (PSPC) method, static seeding and perfusion culture (SSPC) method, and static seeding and static culture (SSSC) method for their *in vitro* performance and bone defect healing efficacy with a rabbit model.

Results: Our study has demonstrated that TEBG constructed by PSPC method exhibited better biological properties with higher daily D-glucose consumption, increased cell proliferation and differentiation, and better cell distribution, indicating the successful construction of TV-TEBG. After implanted into rabbit radius defects for 12 weeks, PSPC group exerted higher X-ray score close to autograft, much greater mechanical property evidenced by the biomechanical testing and significantly higher new bone formation as shown by histological analysis compared with the other two groups, and eventually obtained favorable healing efficacy of the segmental bone defect that was the closest to autograft transplantation.

Conclusion: This study demonstrated the feasibility of TV-TEBG construction with combination of perfusion seeding, perfusion culture

and RP technique which exerted excellent biological properties. The application of TV-TEBG may become a preferred candidate for segmental bone defect repair in orthopedic and maxillofacial fields.

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Novel Coupled Framework of In Situ and In Silico Analysis to Accelerate Development of Muscle Tissue Engineering Therapeutics

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Regenerative medicine approaches for volumetric muscle loss (VML) injuries have generated partial recovery of muscle force in biologically relevant rodent models. However, the mechanisms responsible are poorly understood. We present a novel coupled framework of *in-silico* and *in-situ* methods to address knowledge gaps and identify opportunities for improved therapeutics.

A 3D finite element model of the rat latissimus dorsi (LD) was created *in-silico*. The muscle was modeled as a transversely isotropic, hyperelastic and quasi-incompressible material. Six distinct VML injuries were simulated in the model, and used to predict LD contractile forces. From these results, two different VML injuries were created in the LD of rats and muscle function was immediately assessed *in-situ*. The distal tendon of the LD was attached to a lever arm and peak isometric contractions were measured via electrical stimulation of the motor nerve.

In-silico predictions were confirmed experimentally using *in-situ* testing. In the intact computational model with *in-situ* conditions, the pennate portion of the LD generated two times more force than the longitudinal portion. With only the longitudinal portion anchored, force was just 60% smaller indicative of lateral force transmission.

Functional deficits from VML injuries vary widely. Thus, regenerative technologies would be more effectively targeted if they were injury-based and muscle-specific. To this end, we illustrate how understanding of VML injuries may be improved using a coupled framework of computational and experimental methods to identify relevant biomechanical mechanisms of muscle function. This approach should accelerate development of more effective technologies for functional recovery from VML injury.

Scientific Session 2: Ophthalmology & Soft Tissue

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Magnetic Nanoparticles For Targeted Corneal Endothelial Transplantation

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Purpose: The corneal endothelial cell layer poses great challenges due to its lack of regenerative potential and reducing cell population with age. This study investigates human corneal endothelial cells (HCEC), loaded with iron-based nanoparticles, being magnetically-directed to injured regions of the cornea.

Methods: HCECs were grown and exposed to 50nm dextran-coated biotin conjugated super paramagnetic iron oxide nanoparticles (SPIONP) at 37°C for up to 72 hrs. SPIONP uptake was evaluated via Atomic Emission Spectroscopy (ICP). Mathematical modeling based upon stokes law, gravity, and magnetic field strength was 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 then evaluated by injecting SPIONP loaded HCECs onto a denuded human corneal endothelium in the presence of an applied magnetic field.

Results: HCEC were cultured and maintained their *in-vivo* cell-specific marker expression of CD200 and Glypican-4, confirming their endothelial lineage. ICP analysis revealed that SPIONP internalization by HCEC was increased by magnetic exposure during cell-MNP loading. 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 through an aqueous solution to a predetermined area when a magnetic field is applied. Results of this study may lead to the development of a non-surgical technique to replenish this vital cell layer.

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Local Affinity Release to the Retina

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Introduction: Blindness and visual impairment are largely caused by irreversible loss of photoreceptors and retinal pigment epithelium cells in the retina. Pro-survival therapeutic proteins can improve retinal cell survival in damaged retina, but require controlled delivery systems due to their short half-lives and rapid clearance. To achieve local controlled release, we designed an affinity-based system that sustains the release of proteins from an injectable, hyaluronan-based hydrogel.

Materials and methods: In this strategy, we exploit the interaction of an intracellular protein, Src homology 3 (SH3), with its binding peptides. By expressing fusion proteins of SH3 with the protein of interest and modifying a hyaluronan–methylcellulose (HAMC) hydrogel with the corresponding binding peptides, the release can be adjusted. The rate of release can be tuned by varying either the binding strength of the SH3-protein/peptide pair or the peptide concentration. Two fusion proteins, SH3-IGF-1 and SH3-CNTF, were successfully expressed. Their controlled release from modified HAMC were investigated *in vitro* as potential candidates for local affinity release to the retina.

Results and discussion: Reflecting the difference in binding equilibrium between the binding peptides, we showed that the strongest binder released SH3-IGF-1 more slowly. Furthermore, RPEs in the HAMC/SH3-IGF-1 release system had higher cell viability than cells cultured in the absence of the slow release IGF-1 system. Moreover, we validated the prediction of our mathematical model for the controlled release of SH3-CNTF from weak-binding HAMC, and demonstrated bioactivity of released SH3-CNTF. This system lays the foundation for future *in vivo* studies combining cell and biomolecule delivery.

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Neuroprotective Factors Secreted By Retinal Progenitors Derived From Induced Pluripotent Stem Cells

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Purpose: We hypothesize that secreted factors by retinal progenitors derived from induced pluripotent stem (iPS) cells offer neuroprotective and neuroregenerative functions. In this study, we investigated the secreted factors by retinal progenitors derived from iPS cells.

Methods: 3D1 iPS cells were differentiated toward retinal lineage. Embryoid bodies (EBs) of 9000 cells were generated using Aggrewells. EBs were transitioned to neurons by adding neural induction medium and maintained in a three-dimension (3D) culture. On Day17, neuro-rosettes were selected. Fifty rosettes were in each well of ultralow attachment plate with 2 ml media. Conditioned media was harvested every 72 hours and replaced with fresh media. Stepwise differentiation was confirmed by immunofluorescence analysis of neural (OTX 2, SOX1), eye field (LHX2, SIX6), and retinal progenitor markers (CHX10, PAX6). Multiplex luminex Immunoassays were performed to simultaneously measure multiple factors.

Results: EBs derived from 3D1 cells expressed neural, eye field and retinal progenitor markers at different time points constant with previous

studies. Expressions of retinal progenitor markers started on Day21 and peaked on Day30 of differentiations. Neuroprotective factors detected from retinal progenitors included Osteopontin, Hepatocyte growth factor (HGF), Stromal cell-derived factor 1 (SDF-1), Insulin-like growth factor 1 (IGF-1), Monocyte chemoattractant protein-1 (MCP-1) and Mesencephalic astrocyte-derived neurotrophic factor (MANF).

Conclusions: We observed that retinal progenitors derived from iPS cells secret several neuroprotective factors. This study suggests that conditioned media from retinal progenitors derived from iPS cells may be a novel treatment for retinal degeneration and retinal injury.

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3D Bioprinting of IPSCs to Generate Cartilage Like Tissue Using Laminin Bioconjugated Cell-instructive Bioink

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Cartilage lesions can develop into osteoarthritis (OA) and are a worldwide burden. As a prospective treatment for such lesions, we show that human-derived induced pluripotent stem cells (iPSCs) can be 3D bioprinted and directed to form cartilage-mimics (1). The benefits by using an established iPSC line are unlimited cell source with regeneration capacity. The objective of this study is to develop a cartilage prototype by 3D bioprinting of human chondrocyte derived iPSCs using novel cell-instructive bioink composed of laminin-conjugated nanocellulose fibrils. We bioprinted iPSCs in nanocellulose/alginate bioink and compared with nanocellulose/alginate bioink which was bioconjugated with laminin, to resemble the microenvironment found in cartilage tissue. Designing protocols that generates hyaline cartilage from pluripotent cells *in vitro* is still a challenge, due to that joint formation are late in development and far from the pluripotent state. We used a protocol for hyaline-like cartilage generation from iPSCs using combinations of growth factors or co-culture with irradiated chondrocytes. The 2-photon-fluorescence-microscopy results showed an increased cell density within the cartilaginous-like tissue, indicating the importance for good cell survival during bioprinting. Collagen type 2 could be detected and the pluripotency marker Oct4 was lost in the cartilage mimics. Improved cartilage generation was achieved in the cell-instructive bioink. 1. D. Nguyen *et al*. Scientific Reports, 2017, 7: *658* | DOI:10.1038/s41598-017-00690-y

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Platelet-Like-Particles Mediate Matrix Properties to Promote Cell Migration in a Hemophilia Wound Healing Model

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Coagulation is the initial step in the wound repair process.[1] In situations in which coagulation is impaired, such as hemophilia, subsequent wound healing has been shown to be significantly impaired. To that end, we have created synthetic platelets capable of augmenting healing and improving wound repair via application of these platelet-like-particles (PLPs) to enhance clot stability and stiffness.[2] Increased clot stabilization and stiffness subsequently leads to improved cell migration into and within the wound environment. We characterized these clot stabilization effects via cryogenic SEM and fibrinolysis assays, which indicated that PLP-laden clots have decreased porosity and increased stability relative to standard clots. We then used our PLPs in *in vitro* cell migration assays modeling hemophilia and normal wound repair conditions. PLPs were shown to enhance cell migration in hemophilia conditions. Additionally, application of PLPs *in vivo* in a mouse model of wound repair demonstrated a dose dependent increase in wound closure rates.

References:

1. Nandi, S. & Brown, A. C. *Exp. Biol. Med.* **241,** 1138-1148 (2016).
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Characterization of a Newly Developed Dermal Sponge for Soft Tissue Replacement

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Introduction: A macroporous, moldable acellular dermal sponge was developed as a soft tissue filler to address clinical needs for breast reconstruction following lumpectomy or for deep and tunneling wounds.

Methods: Dermal sponges were produced from decellularized human dermis from donors with research authorization. Sponges were evaluated for adipoconductivity by seeding adipose derived stem cells (ASC) and inducing adipogenic differentiation. Sponges were also assessed for their regenerative and degenerative properties in a subcutaneous athymic mouse implantation model in comparison with an acellular dermis and Helistat® collagen sponge at 6, 12, and 24 weeks. Cellular infiltration, neovascularization and inflammatory response were evaluated histologically. Adipogenic remodeling and implant size were quantified from their cross-sectional area.

Results: Dermal sponges supported ASC attachment, proliferation and differentiation to adipocytes demonstrated by perilipin-A staining and enhanced adiponectin levels in culture media. Dermal sponges showed significantly enhanced fibroblast infiltration and neovascularization over acellular dermis and Helistat at week 6 (p&lt0.05). No significant inflammatory cell infiltration or fibrous capsule formation was observed. Sponges showed three times the level of adipogenic remodeling over acellular dermis and Helistat by week 12. From week 6 to week 24, neither dermal sponges nor acellular dermis showed a significant decrease in cross-sectional area, while the cross-sectional area of Helistat sponges was reduced significantly (p&lt0.01).

Significance: The shapeable, space-filling dermal sponge may be an effective matrix for breast reconstruction and wound healing as it allows for rapid cellular infiltration and angiogenesis while maintaining its volume over 18 weeks in the subcutaneous pocket.

Scientific Session 2: Stem Cells and Microenvironments for Dental and Craniofacial Tissue Regeneration

Monday, December 4, 2017, 12:45 PM - 2:15 PM

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Microrna-200a Inhibitor Promotes Bone Regeneration at Calvarial Defects Of Rats

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MicroRNA (miR)-200a suppresses β-catenin/Wnt signaling and TGF-β2 /Smad pathway and inhibits BMP-2-induced differentiation by targeting Dlx-5.

Objectives: This project is to test whether we can promote osteogenic differentiation and bone regeneration by inhibiting miR-200a.

Methods: Plasmid-based miR-200a inhibitor system (PMIS-miR-200a) was transfected into human bone marrow MSCs. The cells with miR-200a inhibition were subsequently exposed to osteogenic differentiation media for up to 4 weeks. The biomarkers of osteogenic differentiation were quantitatively measured at different time points. In addition, PMIS-miR-200a at different doses were loaded into collagen sponge and implanted into calvarial defects at a rat model.

The bone regeneration with different treatments were analyzed using μCT and histology.

Results: The expression of PMIS-miR-200a in human bone marrow MSCs was confirmed using real-time PCR after the PMIS-miR-200a were treated. The osteogenic differentiation were up-regulated in the cells treated with PMIS-miR-200a, compared to the cell without treatment or the cells treated with empty vectors. The bone regeneration measured using μCT in the defects treated with PMIS-miR-200a is twice higher than controls.

Conclusions: These data demonstrated that inhibition of miR-200a potently improves osteogenic differentiation of human MSCs and promote bone formation, which indicating the potential of PMIS-miR-200a to be used for oral and craniofacial bone regeneration.

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Development of a PDGF-BB Delivery System to Enhance Adipose-Derived Stem Cell-Mediated Cranial Bone Regeneration

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Critical-sized bone defects require treatment with bone grafts to facilitate complete healing. Adipose-derived stem cells (ASCs) are a promising cell source for engineering biologically-functional bone grafts. Recently, our group demonstrated that physiological concentrations of platelet-derived growth factor-BB (PDGF) significantly enhanced osteogenesis of ASCs. To capitalize on this unique feature of ASCs, we developed a novel heparin-conjugated, decellularized bone matrix (HC-DCB) particle system to deliver PDGF to ASCs within bone grafts. Here, we assessed the loading efficiency of 100 ng of PDGF and release kinetics over 21 days from non-conjugated DCB and HC-DCB. Using ELISA, we found that HC-DCB particles had a higher loading efficiency (97.5% vs. 83.5%), and released less PDGF over 21 days (3.25 vs. 14.48 ng) compared to DCB particles. We evaluated the osteoinductive effects of PDGF on ASCs using DCB and HC-DCB with and without PDGF. Particles were incubated with PDGF in fibrinogen overnight, combined with ASCs and thrombin, and infused into porous polycaprolactone scaffolds. Grafts were cultured *in vitro* for 21 days in osteogenic medium or directly implanted into critical-sized murine calvarial defects. Consistent with our prior findings, grafts with PDGF had roughly five times higher calcium deposition, while there was no difference in calcium content between HC-DCB-PDGF and DCB-PDGF *in vitro*. However, osteocalcin expression was three times higher in HC-DCB-PDGF versus DCB-PDGF. *In vivo*, HC-DCB-PDGF regenerated 150% greater bone volume at Week 4 than DCB-PDGF ($p = 0.17$). Together these data demonstrate a significant potential of delivering PDGF using HC-DCB for ASC-mediated cranial bone regeneration.

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Bioengineered Tooth Bud Model Recapitulates Significant Features of Natural Tooth Buds

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Our long-term goal is to enhance the quality of dental and oral health for those suffering from tooth loss by creating bioengineered functional, living replacement teeth as a superior alternative to currently used synthetic dental implants. For this study, we co-encapsulated postnatal dental cells and endothelial cells within GelMA hydrogel scaffolds to fabricate tooth bud constructs. After 2 weeks of subcutaneous implantation, bioengineered tooth bud constructs exhibited proliferating dental epithelial (DE) rosette structures that expressed Sox2 and LEF-1, markers of the DE stem cell (DESC) niche. The putative bioengineered DESC also exhibited canonical Wnt signaling activation as demonstrated by robust Beta-Catenin and Sonic Hedgehog (Shh) expression and a co-expressed Shh and FGF3 signaling center resembling the natural enamel knot. The putative DESC niche was surrounded by a distinct basement membrane, and condensed dental mesenchymal cells that expressed Fibroblast Growth Factor 3 (FGF3) and de novo Fibrillin-2 expression, all hallmarks of naturally developing tooth buds. After 4W of *in vivo* growth, GelMA tooth bud constructs exhibited robust mineralized tissue formation that adopted the size and shape of the construct. These high impact results are the first to recapitulate the natural DESC niche and other features of natural tooth development using postnatal dental cells. These results are anticipated to improve the quality of dental and oral health for people suffering from tooth loss, and facilitate a variety of regenerative medicine approaches to bioengineer functional organs and tissues.

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3-D Topographical Controls to Angularly Organize Periodontal Ligaments

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Objective: For tooth-supportive functions under occlusal/masticatory loadings, specific orientations of periodontal ligament (PDL) to tooth-root surfaces are important. However, angular orientations in PDL regeneration is currently challenging in periodontal tissue engineering. Here, we investigated 3-D architectures, which can spatiotemporally organize PDL in 3-D printing technology.

Methodology: The programmed slicing with three different angulations is the key step to create oriented microgroove-patterns on 3-D scaffold surfaces. The spatial scaffold for PDL regeneration was designed using CAD and wax molds were manufactured with the 3-D wax printer. The poly-ε-caprolactone was casted into 3-D printed molds and surfaces were analyzed by micro-CT, SEM, and confocal microscope. After *in-vitro* human PDL cell cultures, cell orientations were statistically analyzed using fluorescence staining of cell nuclei. *In-vivo* subcutaneous experiments are performed to evaluate PDL cell/tissue alignments and collagen formation.

Results: Individually different microgroove-patterns on the scaffold were analyzed and each topography had high reproducibility and predictability by 3-D printing technology. Promisingly, microgroove-patterned scaffolds provided more predictable cell alignments and cell orientations were significantly angular-controllable with statistical difference in *in-vitro* and *in-vivo*.

Significance: It is still challenging to spatiotemporally control orientations of PDL for physiological functioning restorations in periodontal complexes. Here, we demonstrated the different angulated microgroove-patterns on scaffolds can spatiotemporally control orientation of ligaments with high reproducibility. This simple strategy provide topographical platforms to precisely form periodontal-mimetic architectures for PDL organizations. Furthermore, ligament-guiding architectures can integrate designed bone compartments for ligament-bone constructs and the multi-compartmentalization can lead to tissue-functioning restoration in biomedical applications.

Scientific Session 3: Rapid Fire Session 1

Monday, December 4, 2017, 4:15 PM - 5:45 PM

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Hyaluronan-Collagen Type-I Hybrid Bioink for 3D Printed Microenvironments

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Current *in vitro* 3D tissue constructs are restricted by the materials in which they are grown and the inability to create physiologically accurate extracellular matrix (ECM) micro-architecture. These restrictions have thus far prevented optimization of true ECM microenvironments that mimic the composition and structure of *in vivo* tissues. Consequently, this artificial nature can affect cellular phenotype and behaviors in *in vitro* model-based studies such as drug screens. Collagen type-1 is the major structural component in the ECM, and is widely used as a 3D hydrogel, but is not easy to use as a bioink for 3D bioprinting due to low viscosity and slow crosslinking properties. We have developed a novel, yet simple bioink formulation that combines thiol-modified hyaluronic acid with methacrylated collagen type-1. The resulting bioink has the properties needed for extrusion bioprinting and cell-driven internal micro-architecture formation. In validation studies, we developed three bioink formulations, verified viscoelastic behavior and printability in extrusion bioprinters, and have employed a panel of cell types to verify biocompatibility and cell-driven reorganization of collagen fibrils into fiber-based micro-architectures. Viscoelastic properties were measured using rheology and collagen reorganization was characterized with picrosirus red and trichrome staining. Cell viability of multiple cell lines was confirmed using MTS proliferation assays and quantification of LIVE/DEAD staining in comparison to current on-the-market 3D hydrogels. Combined, we have been able to create a simple bioink with ideal, tunable elements for 3D cell culture leading to optimized formulation of 3D printing of functional bioengineered liver constructs and primary tumor organoids.

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MRI-informed Biomimetic Design of Artificial Intervertebral Disc Scaffolds using 3D Bioplotting

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Introduction: Damage to intervertebral discs (IVD's) is a major health concern. However, current artificial disk replacement are associated with an inability to absorb compressive forces and debris release. This investigation aims to address this issue using 3D bioplotting of alginate methylcellulose (alg/MC) with primary bovine articular chondrocytes (pBAC), to replicate native disc tissue.

Methods: Magnetic resonance imaging (MRI) IVDs provided the dimensions for the alg/MC scaffolds with pBACs ($5x10^6$). Cell viability was measured by fluorescent imaging (CMFDA-green and ethidium bromide), and phenotype by PCR gene expression analysis of collagen type I & II, and histological imaging of proteoglycan. Scaffolds were assessed using quantitative (qMRI); $T2^*$ for water content, spectroscopy for proteoglycan and MTR for collagen.

Results: The gene expression ratio of collagen type I to II in 14-day growth samples was 4:96, and mimics freshly isolated chondrocytes (100% collagen II). Safranin-O staining of proteoglycans demonstrated significant increasing content with longer culture time (1,7,14 days). qMRI techniques were successfully applied to achieve measurements of water, collagen, and proteoglycan contents in both healthy disc tissues and day 21 scaffolds. High cell survival of 80% was recorded throughout, and was improved by the variation of print parameters and material sterilisation procedures.

Conclusions: 3D bioplotting using alg/MC represents a promising new material capable of supporting appropriate chondrocyte phenotype and extracellular matrix synthesis. Mechanical stimulation and testing during growth is the next stage in experiments, to further encourage proteoglycan production and monitor scaffold physical properties.

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Decellularized Xenograft Bone Scaffold is Superior to Demineralized Bone Matrix in Healing of Critical Bone Defects in Rat Femurs

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Approximately 5.2 million musculoskeletal surgeries are performed every year in the United States, nearly half of these utilizing bone grafts. The economic and clinical implications of bone grafting are enormous, and tissue-engineered bone graft substitutes have garnered considerable attention in recent years. Using novel decellularization protocols developed in our laboratory, a porcine bone scaffold was developed. Previous work demonstrated that this protocol significantly reduced nuclear material within the xenograft suggesting that the scaffold was effectively decellularized compared to the starting material. Furthermore, the native bony architechture was maintained. We hypothesized that the decellularized construct would be superior to demineralized bone matrix (DBM) in repairing a critical size bone defect. The purpose of this study was to compare osteointegration of this novel xenograft to a DBM commercial product in a segmental bone defect using micro-computerized tomography (CT) and histological analysis. An 8 mm segmental bone defect created in 13 week old Sprague-Dawley rats (n = 3/group) beneath a polysulfone plate attached to the femur. This defect was filled with xenograft scaffold or DBM, for 16 weeks. Histology of the explanted femurs demonstrated newly formed collagen within the scaffold. Disorganized fibrous growth was observed within the DBM group. Micro-CT demonstrated host osteointegration into the xenograft while DBM failed to integrate in the defect site. These preliminary results demonstrate superior osteointegration with a xenograft scaffold in a rat femoral critical defect when compared to DBM. Further work must be done with larger group sizes to confirm our findings.

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3D Bioprinting of BMP-2 Gene-Activated and Stem Cell-Encapsulated Gelatin Scaffold: Efficacious in situ Transduction and Robust Osteogenesis for Bone Regeneration

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Objective: Currently, sustained *in vivo* delivery of active bone morphogenetic protein-2 (BMP-2) protein to responsive target cells, such as bone marrow-derived mesenchymal stem cells (BMSCs), remains challenging. *Ex vivo* gene transfer, while efficient, requires additional *in vitro* cell culture. In this study, we incorporated lentiviral vector encoding BMP-2 gene (Lv-BMP, BMP group) or GFP gene (Lv-GFP, GFP group) and human BMSCs into photo-crosslinkable gelatin solution, and encapsulated them into gelatin hydrogel scaffolds with porous infrastructure in a single step, using a visible light-based projection stereolithographic (VL-PSL) 3D printing technology. We hypothesize that the Lv-BMP-activated, BMSC-laden constructs would independently form bony tissue without extra BMP-2 administration.

Methods: hBMSCs were isolated and expanded with IRB approval. *In vivo* bone formation was examined by implanting constructs into SCID mice with IACUC approval.

Results: Upon *in vitro* culture, compared to the GFP group, cells from BMP group showed >1,000-fold higher BMP-2 release, and the majority of them stained intensely for alkaline phosphatase (ALP) activity. Real-time RT-PCR also showed dramatically increased expression of osteogenesis marker genes only in the BMP group. Post implantation into SCID mice, the micro-computed tomography imaging showed bone formation only in the BMP group, which was restricted within the scaffolds locally. Alizarin red staining and

immunohistochemistry of GFP and osteocalcin further indicated that the grafted hBMSCs, not host cells, contributed primarily to the newly formed bone.

Significance: This novel one-step, gene-activated and live cell encapsulated scaffold fabrication procedure developed in this study is potentially applicable for point-of-care treatment of bone defect.

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Involvement of FAK-BMP-2/Smad Pathway in Mediating Osteoblast Adhesion & Differentiation on Nano-Hydroxyapatite/ Chitosan Composite Coated Titanium Implant under Diabetes

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Chitosan (CS)-based hydroxyapatite (HA) composite emerges as a novel strategy for promoting bone regeneration. Here the nanophase HA/CS composite coated porous titanium implant (nCT) was fabricated & its biological behavior under diabetic conditions was investigated. We proposed that focal adhesion kinase (FAK)-mediated BMP-2/Smad pathway played a role in mediating the promotive effect of nCT on osteoblast adhesion and differentiation under diabetes-induced high reactive oxygen species (ROS) condition. To confirm the hypothesis, rat osteoblasts on bare titanium implant (Ti) or nCT were subjected to normal serum (NS), diabetic serum (DS), DS + NAC (a potent ROS inhibitor) & DS + cytochalasin D (a FAK inhibitor). *In vivo* study was performed on diabetic sheep implanted with Ti or nCT with or without NAC administration. Results showed that diabetes-induced ROS overproduction impaired osteoblast adhesion evidenced by immunostaining of F-actin and vinculin, & morphological observation through inhibition of FAK phosphorylation, which contributed to suppressed BMP-2-dependent Smad1/5/8 phosphorylation. nCT substrate reactivated FAK-BMP-2/Smad pathway, thus reversed osteoblast dysfunction, which exerted similar effect to NAC treatment on Ti. These effects were further confirmed by the improved osseointegration within nCT in diabetic sheep evidenced by Micro-CT & histological examinations. Our study demonstrated reactivation of FAK-BMP-2/Smad pathway was involved in improving osteoblast adhesion & differentiation by nano-HA/CS composite coating, hopefully directing biomaterial modification & biofunctionalization under diabetic conditions.

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Quantitative Assessment of Cell and Progenitor Population Resident in Different Zones of Human Cartilage Tissue and its Correlation with Tissue Health

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A rational development of cell-based therapy for cartilage regeneration requires knowledge of the number and functional attributes of the chondrogenic connective tissue progenitors (CTP-Cs) resident in potential source tissues. The concentration, prevalence and biological performance of cells in the superficial (Csp, top 500μm thickness) and deep cartilage (Cdp, remainder of cartilage excluding the calcified cartilage zone) were compared. The lateral femoral condyle of patients undergoing total knee arthroplasty in varus knees with osteoarthritis (OA) was used. Cell and CTP data, histological grade, and patient variables were assessed. Colony formation was assessed using automated quantitative image analysis methods, Colonyze™. Cell concentration (cells/mg) in Csp (Median: 7280; Range: 3440,17600) was significantly higher than Cdp (Median: 5686; Range: 3393,9660), $p = 0.039$. Prevalence of CTPs (CTPs/million cells plated) was similar between Csp (Median: 1274; Range: 0,3898) and Cdp (Median: 1365; Range: 0,6330), $p = 0.42$. The biological potential of CTPs varies widely, within and between patients, manifest by variation in colony size and morphology. Early stages of cartilage degradation were associated with an increase in cell and CTP concentration in Csp ($p < 0.05$), whereas later stages of matrix degradation were associated with a decrease in cell and CTP concentration in Cdp ($p < 0.05$). Overall these findings provide systematic quantification of stem and progenitor cells in different zones of cartilage and their correlation to various histopathological features observed during idiopathic OA progression.

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Mechanically Functional 3D-Printed Bioresorbable Vascular Scaffolds

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Mechanically Functional 3D-Printed Bioresorbable Vascular Scaffolds

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It is estimated that over 15 million people and approximately 8-12 million people in the United States have atherosclerotic coronary artery disease (CAD) and peripheral artery disease (PAD), respectively.[1] Problems (e.g. restenosis, thrombosis, and impaired vasomotion) associated with bare and drug-eluting metal stents have prompted the development of bioresorbable vascular scaffolds (BVS). However, current BVSs do not meet the mechanical properties of metal stents (nitinol) and their increased strut thickness and width increases the risk of thrombosis. The objective of this study is to develop a biomaterial ink (B-Ink™) formulation that will enable 3D printing of a biocompatible, mechanically functional BVS to overcome the above challenges.

In this study, we fabricated BVS with strut width and thickness of 155 μm, which is comparable to the dimensions of a polymer-based BVS approved by the U.S. Food & Drug Administration. Concentrations of B-Ink™ components (e.g. prepolymer: methacrylated poly(1-12 dodecamethylene citrate; co-photoinitiator: Ethyl 4-dimethylamino benzoate) had significant effects on the final mechanical properties of the printed BVSs and allowed the BVS to meet the mechanical properties of nitinol stents. This work is a step forward toward the development of patient-specific 3D-printed BVSs.

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WITHDRAWN

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Design and Synthesis of Cell Instructive Bioinks for Controlling Cell Fate and Functions in 3D Bioprinted Soft Tissue

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3D Bioprinting is an emerging technology which will revolutionize the field of tissue engineering and regenerative medicine. 3D Bioprinting can replicate any tissue or organ by building biological material layer by layer using 3D bioprinter, bioink and cells. The function of bioink is to facilitate viable cell deposition in predetermined pattern and then become the scaffold when cells are cultured *in vitro* or *in vivo*. Among the most important properties of bionks are rheological properties. We have developed bioinks with excellent printability using polysaccharide nanofibrils (such as cellulose, CNF) and these bioinks have been successfully commercialized and are available on the market under trade name CELLINK.

The hydrophilic nature of the CNF surfaces covered by water prevent them from protein adsorption and make them bioinert. Cells do not recognize CNF surfaces which is a big advantage when it comes to biocompatibility. In this presentation we introduce the new generation of cell instructive bioinks for controlling cell fate and functions of cells forming soft tissue. Cellulose nanofibrils were bioconjugated with extracellular matrix components such as collagen, fibronectin, peptide motifs such as RGD or GRGDSP, laminin and growth factors such as TGFBeta and BMP7 and then used as bioinks. We have seen remarkable effects of bioink - cell interactions on cell adhesion, proliferation and also cell differentiation. The results from *in vitro* and *in vivo* studies using human chondrocytes, fibroblasts and adipose derived stem cells as well as iPSC will be presented.

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A Novel Strategy for Vocal Fold Tissue Engineering to Promote Cell Attachments and Migration

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Injectable hydrogels are under investigation for vocal fold (VF) engineering to regenerate extracellular matrix in the VF lamina propria or to restore viscoelastic functions of the damaged tissue. The hydrogels should thus be biocompatible, biodegradable, and offer a tissue-mimetic micro-environment to support cell culture and implantation in terms of cell attachments and migration. In the present study, a novel injectable hydrogel composed of collagen fibrils in a glycol-chitosan matrix was developed and characterized. A recombinant human epidermal growth factor (EGF) with four target concentrations of 125, 250, 375 and 500 ng/mL was encapsulated within the cell-seeded hydrogels. Samples without EGF were used as negative controls. The proposed hydrogel was shown to be biocompatible, biodegradable and possess a fibrillar porous micro-structure. The hydrogel was found to be mechanically stable under phonation-induced stimulations using a previously described phono-mimetic bioreactor (1). The hydrogel also supports cell attachments due to the presence of collagen fibrils. It was also found that the addition of 375 ng/mL EGF promotes VF fibroblasts migration significantly. To investigate the gradual release of EGF, glycol-chitosan micro-particles were fabricated and characterized. The particles were loaded with EGF, and encapsulated in the bulk hydrogels. The two biomaterial models were then compared in terms of cell-biomaterial interactions.

1. Latifi N, *et al*., Tissue Engineering Part C: Methods 22(9), 823, 2016.

Acknowledgments: NIH R01-DC005788 (Mongeau, L.).

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Ovarian ECM-Derived Hydrogels Provide a Bioactive Scaffold for Follicle Maturation

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A large percentage of female cancer survivors lose reproductive function due to the deleterious effects of treatment on ovarian tissues and cells. *In vitro* follicle maturation (IVM) has been suggested as a safer option to help restore fertility by isolating follicles from the stroma to obtain mature oocytes devoid of malignant cells. In an effort to improve current IVM outcomes, our group has developed a bioactive ECM hydrogel derived from decellularized porcine ovarian tissues to mimic the ovarian microenvironment. Ovarian tissues were treated with mild detergents to remove immunogenic components, then lyophilized and milled into a fine ECM powder. The hydrogel scaffolds were formed from solubilized ECM powder at 37°C after neutralizing pH and balancing the salt concentration. Sohlh1-mCherry newborn mouse ovaries were cultured on top of the ovarian scaffolds at increasing ECM concentrations to determine the effects of the hydrogel on primordial follicle populations. A volumetric analysis of confocal images showed no significant difference between the number of Sohlh1^{+} cells in native day 7 and hydrogel-cultured ovaries. This result was confirmed using immunohistochemistry (NOBOX) and immunofluorescence (Sohlh1/2) staining, showing morphologically healthy oocytes throughout the ovaries after seven days. Ovaries cultured on higher ECM concentrated scaffolds showed a decrease in the total follicle population, which may suggest that increasing mechanical stiffness could drive follicle activation. Finally, ovarian follicles were enzymatically isolated from 6-14 day mice and successfully embedded in ovarian ECM scaffolds demonstrating the potential of ovarian hydrogels as an alternative biomaterial for fertility preservation.

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Nanovesicles within ECM Bioscaffolds as Regulators of the Strength of Soft Tissue Repair

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Bioscaffolds derived from mammalian extracellular matrix (ECM) are widely used clinically as templates of inductive tissue remodeling in multiple anatomic sites. Constructive tissue remodeling facilitated by ECM-scaffolds is largely attributed to the degradation of the scaffold material and subsequent release of bioactive components including matrix-bound-nanovesicles (MBV). However, the mechanisms by which MBV regulate the functional and mechanical properties of remodeled tissue is only partially understood. The present study shows that Lysyl Oxidase (LOX) isoforms are functional MBV surface proteins. The heterogeneous family of LOX extracellular enzymes are responsible for collagen cross-link formation and stabilization of collagen fibrils. Although there has been much interest in utilizing LOX proteins in tissue engineering applications, these efforts have been confounded by the inherently low solubility of LOX, which obviates effective clinical translation. Our finding that LOX is associated with MBV provides not only a feasible method for purifying functional LOX proteins for therapeutic applications, but also a conceptual model for how LOX is incorporated into ECM of native tissues as part of the homeostatic process. Moreover, emerging evidence suggests that secreted LOX can re-enter cells and rapidly concentrate in the cell nucleus, resulting in increased promoter activity of the collagen alpha-1(III) gene, and upregulation of elastin mRNA. Stated differently, the *in-vivo* degradation of ECM-bioscaffolds and associated release of MBV can serve as a feedback loop to instruct cells to coordinate the synthesis and deposition of matrix components. MBV represent an attractive potential tissue engineering strategy to enhance the biomechanical properties of injured soft tissue.

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Fabrication of 3D Biomaterial Scaffolds for Bone Tissue Engineering Applications using Additive Manufacturing Technology

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In bone tissue engineering, polymers and ceramics are used as essential materials for the fabrication of scaffolds in recent years. Poly (ε-caprolactone) (PCL) is one of the most flexible among the bioresorbable polymers, which is commonly used as basic material to fabricate three-dimensional (3D) composite scaffolds based on a 3D printing technique. Calcium phosphate-based bio-ceramics are being widely used in bone tissue regeneration due to their excellent biocompatibility and osteoconductivity. In our laboratory, we fabricated 3D hybrid polymers and ceramics scaffolds with designed pore structures using various additive manufacturing technologies. The morphology and characterization of the fabricated scaffolds were evaluated. These results suggest that the 3D scaffolds with

interconnected pores could be a suitable candidate for bone repair and regeneration. In this presentation, self-developed scaffold fabrication techniques will be introduced.

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In Vitro Chondrogenesis in 3D Bioprinted Human Cell-laden Hydrogel Constructs

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A fundamental challenge of cartilage tissue engineering is the de-differentiation of chondrocytes resulting in the loss of chondrogenic phenotype. TGF-β_1 plays a crucial role in initiating and maintaining chondrogenesis of chondrocytes, mesenchymal precursor cells and deposition of cartilage-specific ECM molecules. The aim of the study was to control the chondrogenic differentiation of human chondrocytes (hNC) and human adipose derived stem cells (hASC) by conjugating a bioink with TGF-β_1 and 3D Bioprinting constructs.

For cartilage reconstruction, we evaluated the biological functionality of a bioink consisting of nano-fibrillated cellulose conjugated with TGF-β_1 and supplemented with alginate. We loaded the nano-fibrillated bioink with and without TGF-β_1 with hNC as well as co-cultures of hNCs with hASC (ratio 20:80). Constructs were produced by 3D bioprinting and cultured for up to 21 days. The chondrogenic phenotype of hNCs as well as the effect on chondrogenic differentiation processes in hNC:hASC co-cultures and neo-synthesis of cartilage specific components were examined on protein- and gene-expression level.

3D printing with both bioinks facilitated the production of cell-laden implants with a high cell density and a homogenous cell distribution. Both inks provided an optimal nutrition supply of the embedded hNCs and hNC:hASC, confirmed by high cell vitality during 7d 3D culture. All constructs exhibited excellent shape and size stability. Conjugation with TGF-β_1 did not change 3D stability and viability of cells. Data of histological, immunohistochemical and biochemical analyses for evaluation of redifferentiation and chondrogenesis of hNCs as well as hNC:hASC co-cultures will be presented.

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Effects of 3D Printing on Flow-Induced Shear Stress Distributions

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As 3D printing gains more exposure as the future of scaffold fabrication for tissue and tumor engineering, the ability to preemptively model the constructs microenvironment becomes of vital importance. Particularly when using perfusion based bioreactor systems, the most important properties to obtain are the fluid flow and wall shear fields that potential cells will experience during and after seeding. Traditionally, 3D printing has given users the impression that the scaffold obtained after printing will exhibit the same, or closely similar, architecture as the designed model. While this may be the case in terms of the macrostructure, we hypothesized that the local fluid dynamic environment would differ greatly. The intention of this study was to 3D print scaffolds of various pore size gradients, image these constructs utilizing micro-computed tomography, and perform computational fluid dynamic simulations on the resulting reconstructions and the initial designs in order to compare the average shear on the fibers, where the cells would be adhered. As a determining factor, the probability density function (p.d.f.) of each was compared to not only highlight the major differences, but also to show the reproducibility of the printing process. The results presented in this manuscript give users the knowledge of how the 3D printing process may alter the intended fluid environment they designed, and how to, in spite of that fact, produce repeatable results.

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Low Intensity Ultrasound Prolongs Lifetimes of Mesenchymal Stromal Cell Transplants in Skeletal Muscle

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Mesenchymal stromal cells (MSC) are the most clinically-applicable stem cell for transplantation. MSCs are considered immune-privileged, but typically do not engraft into host tissue, dying within 3-10 days post-transplantation. We have extensively studied image-guided focused ultrasound tissue molecular responses and sought unfocused therapeutic ultrasound (TUS) as a cost-effective mechanism to produce similar results. Daily TUS (10 min; 2W/cm^2; 10% duty cycle) to mouse hamstrings following implantation of 10^6 luciferase-transfected bone marrow MSCs prolonged MSC survival. Luciferase was detectable in treated legs for 6 days compared to 4 days in untreated legs. TUS resulted in mild hypoxia in hamstrings, indicated by pimonidazole accumulation. Furthermore, increased muscle levels of hypoxia-inducible factor-1a (HIF-1a), nuclear factor kB (NFkB), cyclooxygenase-2 (COX2), vascular endothelial growth factor (VEGF), interleukin-10, fibroblast growth factor (FGF), intercellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM) were detected, consistent with mild or transient hypoxia. While hypoxia-driven factors (e.g. VEGF, FGF, IL-10) are pro-growth and could promote cell survival, hypoxia itself is known to enhance MSC survival and potency. Further mechanistic investigations into TUS exposures are necessary to elucidate which specific TUS effects are beneficial (i.e. on host tissue and/or transplants). Prolonging lifetimes with TUS has profound implications for a wide range of MSC therapies because TUS is noninvasive, safe, and inexpensive. Therefore, utilizing a straightforward clinically-approved modality (TUS) toward transplanted MSCs could increase longevity and function of transplanted cells, reduce the number of transplantations/injections required to treat disease, and ultimately improve cellular therapy.

Scientific Session 3: Imaging Tissue Engineering Therapies

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Design and Testing of a Non-Invasive 3D Imaging System for Cell Mapping Within Bioengineered Tissue

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Background: Many tissue engineering laboratories are working to develop bioengineered organs from either printed or decellularized scaffolds. These functionalized platforms could address significant clinical transplant scarcities. Clinical translation of bioengineered organs would be accelerated with more precise feedback and control of the microenvironment within engineered tissues. For instance, it is currently very challenging to acquire noninvasive 3D images of developing organs or quantitatively assess the seeding and growth of cells within developing organs. These parameters can only be evaluated destructively by histology or by rudimentary input/output assays with no spatial sensitivity. This slows down the experimental feedback loop and increases the costs of R&D.

Methods: To address this problem, we have constructed a robotic 3D organ imaging system. The system uses high resolution ultrasound to image tissue (b-mode), vasculature/microvasculature (Acoustic Angiography), and cellular maps within the organ (targeted microbubble

contrast). The system's spatial resolution is ~100 um, can image ~2 cm into tissue, and is sensitive to a single microbubble (smaller than a blood cell).

Results: We have collected 3D images of different species and organs, including mouse (liver), rat (liver), and pig (kidney+lung). Endothelial cell mapping has been achieved using microbubbles targeted against CD-31. An image fusion algorithm has been written to allow seamless blending across an entire field of view as large as 7×7 cm.

Next steps: To allow cells beyond the lumen to be mapped, nanoparticle contrast agents will be explored. Additionally, hardware developments are underway to improve ease of use and maximize study consistency.

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Quantitative nano-mechanical characterization of biomaterialsand living cell samples using atomic force microscopy

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The determination of the biophysical parameters and mechanical properties of biomaterials such as structural and physico-chemical composition, topography, roughness and adhesiveness is gaining significant recognition in biomedical applications. These factors strongly affect cell development, tissue formation and also influence the healing capacity of the human body. Atomic Force Microscopy (AFM) is a versatile tool suitable for measuring all of these characteristics with nanometer scale resolution under physiological conditions. We have developed a multipurpose AFM device which allows the comprehensive characterization of biological samples such as live cells, tissues and biomaterials in the nanoscale. Our Quantitative Imaging (QI™) mode acquires real force distance curves at every pixel of an image in order to simultaneously obtain topographic, nano-mechanical and adhesive sample properties. To get those quantitative data, such as Young's modulus images and topography at different indentation forces, experiments on from living cells, skin tissue and hydrogels were performed. Micro-rheology can be used to characterize the viscoelastic properties of the same sample. Oscillatory measurements (Hz-1000Hz, amplitude 5-20nm) were carried out to calculate the dynamic storage and loss modulus (E', E'') distribution on living fibroblast cells. Furthermore, we have developed a complimentary device which enables tensile stretching with AFM. This comprehensive toolbox allows applying sample deformation in the sample plane, which can then be investigated in detail by the AFM. The potential of the combination of QI™ mode and tensile stretcher is demonstrated with a study of an elastic film during and after the tensile load

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Multi Target Labeling To Monitor Myoblast Differentiation

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Introduction: Better non-invasive assessment tools for tissue maturation and development are necessary to advance the field of regenerative medicine into the clinic. We have previously described a novel fiber based imaging tool capable of real-time non-invasive monitoring. The goal of the current study was to adapt this system to assess skeletal muscle development. Specifically, we have determined the necessary cell labeling parameters to allow high resolution imaging.

Materials and methods: Fluorescently labeled C2C12 myoblast cells were used as a cell model. GFP H2B mCherry cells were labeled via lentiviral infection and sorted for highest expressing cells. The labeling of cells was compared with sacrificial tools of immunostaining and RT-qPCR. Cell fusion was assessed on plastic and electrospun PCL/Collagen scaffold with and without protein coating.

Results: Fluorescent labeling of both the nucleus and cytoplasm in C2C12 enabled a quantitative measure for differentiation level. The live cell fusion was comparable to immunostaining and mRNA levels expression typical of myoblast differentiation. Geltain coating of plastic had no significant impact on myoblast fusion, while collagen film coating of the scaffold improved cell differentiation with higher fusion index.

Discussion and conclusions: The labeling approach developed in this study offers a new way to monitor myoblast differentiation quantitatively in real time. The application of the labeled cells shows the promise in using this approach to assess cell morphology non-invasively. Future studies will measure skeletal muscle cell differentiation on different scaffolding types and in response to drugs.

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Real-time MRI of Intrathecal Transplantation of Hydrogel-Embedded Glial Progenitors in Dogs Suffering from ALS-like Disease

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Stem cell-based regenerative medicine is an attractive approach for the treatment of neurodegenerative diseases. Disorders with global pathology such as ALS require broad cell distribution hence, cerebrospinal fluid-filled spaces are attractive gateways for cell transplantation. To prevent sedimentation, we propose to embed cells in hydrogel for intrathecal injection. The goal of this study was to investigate the applicability of injectable hydrogels for intrathecal transplantation of glial restricted precursors (GRPs) in dogs suffering from ALS-like disease degenerative myelopathy (DM). Canine GRPs ($6\text{-}7\times10^6$/ml) were labeled with superparamagnetic iron oxide nanoparticles (SPIO;Biopal). Immediately prior to injection, hydrogel components were mixed (EsiBio). Dogs suffering from DM (n=4) were subjected to X-ray guided catheter placement within thoracic spinal cord. Next, hydrogel containing SPIO-labeled GRPs was injected under MRI with acquisition of dynamic T2*-weighted images. Tissue from two dogs that succumb to the disease was processed for *ex vivo* MRI and histopathology (Prussian blue). Dynamic imaging during hydrogel infusion revealed hypointensity in the area of catheter tip extending rostrally and caudally. The rostral-caudal distance, covered by hydrogel ranged between 42-168 mm. *Ex vivo* MRI of spinal cord revealed hypointense regions in the thoracic part, that correlated with *in vivo* MRI scans during hydrogel injection. Prussian blue staining detected iron particles within hydrogel confirming presence of SPIO-labeled GRPs. MRI-guided, intrathecal injection of hydrogel-embedded cells in DM dogs is safe and feasible. Embedding cells in the injectable hydrogels prevents sedimentation and facilitates attachment of cells to spinal cord surface tissue setting the stage for their migration and engraftment.

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Labeling Of Hyaluronan-based Hydrogels Using Fluorine Nanoemulsions

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Hydrogels can serve as injectable scaffolds for transplanted stem cells, but the precision of hydrogel injections, as well as monitoring of gel biodegradation, remains challenging. We studied the feasibility of using a fluorine nanoemulsion to label and image hyaluronan-based hydrogels with ^{19}F MRI. We studied three hydrogels with various V-sense (CelSense, Inc.) to HA (Hystem, Inc.) volumetric ratios (1:50, 1:10, and 1:5). Gelation time and elasticity were measured by oscillatory stress at 1h and 7d using an ARES 2 rheometer. Diffusion of fluorine from the hydrogel was evaluated through acquisition of ^{1}H and ^{19}F MRI scans at until 2 months after hydrogel preparation using a Bruker Ascend 750 scanner. The viability of glial restricted precursors (GRPs) embedded within hydrogels was assessed *in vitro* and *in vivo*. We found that increasing concentrations of fluorine gradually elongated the gelation time from 194 s for controls to 304 s for 1/5 V-sense/HA hydrogels, while their elastic properties slightly decreased, from 183 Pa for the control to 95.3 Pa for 1/5 V-sense/HA hydrogel at day 0, and from 870 Pa for the control to 588 Pa for 1/5 V-sense/HA hydrogel for day 7. There was no release of fluorine nanoemulsion from hydrogels. No negative influence of V-sense on the proliferation/viability of GRPs was also observed either *in vitro* or *in vivo*, and, at some time points, the presence of fluorine seemed to have a beneficial effect. Thus, fluorination of hydrogels appears promising to follow the location of scaffolded cells non-invasively with MRI.

Scientific Session 3: Regenerative Pharmacology

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Developing a Novel Small Molecule Based Therapy to Restore Hearing and Balance

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Hair cells in the inner ear are essential for hearing and balance that are lost due to aging, medications or loud sounds. The adult mammalian inner ear fails to regenerate hair cells resulting in permanent deficits. During development, supporting cells within inner ear sensory epithelia can self-renew to give rise to new hair cells; however they become quiescent postnatally. Previously, we have shown that *in vivo* gene delivery of c-Myc (c-MycT58A) into the inner ear stimulated reprogramming of supporting cells in adult mice utricles leading to formation of few new hair cells. Based on this, we have developed a small molecule based therapy to induce in situ reprogramming of supporting cells for proliferation and regeneration of inner ear hair cells. In order to select potential c-Myc activators, we screened several small molecules synthesized in our laboratory using a Luciferase based assay. Few potential c-Myc activators were further tested on adult mice utricle cultures. Supporting cell proliferation and hair cell regeneration were analyzed using EdU (Ethynyl-2'-Deoxyuridine) along with supporting(SOX2) and hair cell(MYO7) specific markers. For *in vivo* studies, the most potential small molecule candidate was injected into the mouse inner ear via Canalostomy along with multiple EdU-IP injections. We have thus identified a novel small molecule drug candidate which can induce supporting cell proliferation and limited hair cell regeneration in *situ* in both cultured and live adult mice inner ear utricles. A preclinical study of this drug candidate is currently under process.

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Development Of A Novel Alginate based Material For Targeted Intestinal Delivery Of Therapeutic Agents

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Alginate is a complex polysaccharide routinely used for various encapsulation purposes including oral drug delivery of therapeutic agents since it can immobilize and protect compounds from the harsh stomach acid. We have chemically modified alginate to enhance the bioavailability of orally-administered therapeutic substances through the generation of alginate microbeads resistant to low pH stomach conditions, but readily disintegrate in the more neutral pH of the small intestine. The alginate modification was achieved by oxidizing the vicinal diol in the alginate chain to an aldehyde, then coupling aminoethyl benzoic acid to the aldehyde. By uniquely combining both oxidizing and condensation chemistries, we have been able to more precisely control the degree of modification of alginate, which results in controlled levels of degradation of alginate hydrogel microbeads under simulated gastrointestinal tract (GIT) regional pH conditions. Under these simulated GIT conditions, our *in vitro* experiments have shown that we can encapsulate specific compounds for oral drug delivery, protect them from low pH conditions and deliver them under neutral-basic pH conditions similar to those in the small intestine where nutrients are absorbed most effectively. Thus, the modification of alginate with aminoethyl benzoic acid would lead to enhanced bioavailability of therapeutic agents after release and absorption in the small intestine and as well as for targeted site delivery of certain substances such as probiotics.

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Tissue Engineering Bio-mimetic Ovarian Constructs With Sustained Hormone-releasing Profile For Cell-based Hormone Replacement Therapy For Ovarian Failure

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We recently developed a cell-based hormone replacement therapy (cHRT) as an alternative to pharmacological HRT (pHRT), which has been associated with some adverse effects. Our previous constructs used calcium as alginate cross-linker to encapsulate ovarian endocrine cells. Although the constructs corrected many of the abnormalities associates with ovarian failure they did not deliver sustained levels of estrogens. Therefore, in order to develop a long-term sustained hormone-secreting ovarian constructs, we have replaced calcium with strontium in the fabrication process. In addition, we have incorporated bone marrow stromal cells (BMSCs) into the constructs for cellular aromatase enzyme supplementation in order to enhance the secretion of estrogens. Rat ovarian cells (granulosa and theca cells) and BMSCs were encapsulated in layer-by-layer constructs, thus compartmentalizing the two ovarian cell types along with the BMSCs in each layer. The *in vivo* functions of the constructs were tested in an ovariectomized (ovx) rat model for a period of 90 days. The improved constructs were able to deliver sustained levels of ovarian sex steroids (17β-estradiol and progesterone) while suppressing the levels of follicle-stimulating hormone and luteinizing hormone, thus restoring the negative feedback loop, which in contrast to pHRT approaches. The end-organ effects induced by ovariectomy such as body weight alterations, body fat changes, uterine morphometry and bone density were corrected by the ovarian constructs. The current study demonstrates that our improved bio-mimetic ovarian constructs fabricated using encapsulation techniques deliver long-term sustained levels of hormones, and could be used as a cHRT for ovarian failure.

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Combined Resveratrol and Nicotinamide Mediated Activation of PARP-1 Restricts the Inflammatory Phenotype of Macrophage

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Inflammation is the response of body to injuries and foreign materials, and plays an important role in the effective management of diseases. The cascade of inflammatory response begins with the recruitment of neutrophils that produces inflammatory cytokines to stimulate the recruitment and differentiation of monocytes into macrophages. The phenotypes of macrophages, generally classified as pro-inflammatory (M1 macrophages) and wound healing (M2 macrophages), are the key targets in the anti-inflammatory drug discovery approaches. In the present work, we studied the combinatorial role of nicotinamide and resveratrol in the phenotypic regulation of inflammatory macrophages. Consistent with previous works, we also observed that both nicotinamide and resveratrol could suppress the expression of pro-inflammatory cytokines including TNFα and IL-6 and could up regulate the expression levels of the anti-inflammatory mediators (IL-10 and MRC1). Strikingly, the effects of nicotinamide were more profound than resveratrol while co-treatment with resveratrol further modulated the effects of nicotinamide. The results suggest that a combination of resveratrol with nicotinamide would trigger the conversion of nicotinamide into NAD^+ through the salvage pathway and would result in a favorable immune response for a longer period of time. Further investigations into the molecular mechanisms of action demonstrated that Poly [ADP-ribose] polymerase 1 (PARP-1)-mediated activation of Bcl-6 was primarily responsible for the observed anti-inflammatory effects. Our work further delineates the potential of pharmacologically active natural compounds to modulate inflammation with broad applicability in regenerative medicine and drug discovery.

Scientific Session 3: Regenerative Medicine Transplantation

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Transplantation of a Human Tissue Engineered Bowel (hTEB) in a Athymic Rat Model

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Background: Intestinal failure is a condition that results from the loss of a large surface area of the small intestine. Patients lose the ability to digest and absorb nutrients.

Objective: The objective of this study was to transplant human-based engineered neo-intestines into athymic rats.

Methods: (1) TEB was developed by engineering innervated human intestinal circular smooth muscle sheets around tubular chitosan scaffolds. (2) TEBs were implanted in the omentum of athymic rats. (3) After 4 weeks, TEBs were anastomosed to the native small intestine of the same rats.

Results: (1) Following omentum implantation, TEB before anastomosis revealed maintenance of cell morphology, phenotype and function. (2) Following anastomosis, the rats initially lost weight then started steadily regaining normal weight over the study period. (3) At time of harvest (6 weeks), TEB was healthy with food digest observed inside the lumen. (4) Organ bath studies revealed functional innervated smooth muscle. (5) H&E demonstrated maintenance of smooth muscle alignment. Neo-epithelialization was also observed with well-defined crypts and villi structures. Alcian blue stain demonstrated the presence of mucin, confirming the presence of Goblet cells.

Conclusion: The results shown in this study demonstrated the successful transplantation of engineered human intestines in rats. Food particles were observed in the lumen of the implanted tissues and rats gained weight over the study period. The bioengineered tissues were viable and have acquired epithelial components with villi structures necessary for digestion and absorption. TEB provides a therapeutic approach to lengthen the gut to treat patients with intestinal failure.

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Generating a New Pediatric Surgical Option to Treat Esophageal Atresia

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Esophageal atresia occurs in 1 in 3000 births. Typically, surgical repair includes reconnection of the esophagus or in cases where the esophagus cannot be reconnected, interposition of a piece of stomach or intestine. These surgical options cause significant morbidity, therefore, a new therapeutic option is needed. Porcine amniotic fluid stem cells were obtained via amniocentesis approximately 70-90 days prior to term. Cells were isolated, expanded and characterized via flow cytometry and qRT-PCR. Cells were over 90% positive for mesenchymal stem cell markers CD73, CD90 and CD105. Approximately 10 million cells were seeded onto the extra luminal surface of a polyurethane scaffold (Biostage™) and allowed to incubate for 7 days in a rotating hollow organ bioreactor (Biostage™). Approximately 4cm of intra-thoracic esophagus was removed in 2 piglets from the same litter as the isolated amniotic fluid stem cells and replaced with the seeded scaffolding in accordance with IACUC approval (HH#2014-0132). The lumen in both piglets were stented open with a 10 mm metal stent. The stents were then removed and changed after 3 weeks. This revealed that the scaffold had been extruded and the lumen regenerated. Piglets were fed via G-tube for 5-6 weeks, and then successfully transitioned to oral feeding. These piglets demonstrated the translational and preemptive application of maternal amniotic fluid stem cells and a bioengineered synthetic scaffold in replacing missing gaps of esophageal tissue in offspring. Further studies are ongoing to delineate the mechanism of esophageal regeneration.

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3D Bioprinting of Human Kidney Construct: A Step Towards Solid Organ Printing

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Current treatments for end-stage renal disease include dialysis or kidney transplants. However, problems such as limited donor organs, graft failure, and other complications remain a concern. Bioengineering of living organ-like structures offers an alternative to the present treatments. Recently, three-dimensional (3D) bioprinting has evolved as a revolutionary technology capable of bioengineering living tissues or organs for transplantation. Here, we used 3D bioprinting strategy to bioengineer a kidney construct containing human primary renal cells. Primary renal cells were isolated from human kidneys, and they were suspended in gelatin methacrylate (GelMa)-based bioink for 3D bioprinting. For *in vitro* study, a solid block design of kidney construct was fabricated using our Integrated Tissue and Organ Printing (ITOP) system. The bioprinted renal constructs were evaluated for cell viability, proliferation, and renal tissue maturation/formation over a period of 14 days in culture. The bioprinted renal tissue constructs showed high cell viability and proliferation and exhibited the structural and functional characteristics of kidney tissue. We demonstrated the potential of the use of 3D bioprinting strategy to fabricate the functional renal constructs for future application.

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Beginning a New Banff Classification of Tissue Engineering Pathology

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The science of regenerative medicine is arguably older than transplantation - the first major textbook was published in 1901 - and a major regenerative medicine meeting took place in 1988, three years before the first Banff meeting. However, the subject of regenerative medicine/tissue engineering pathology has never been given focused attention. Depending on one's vantage point and organ orientation regenerative medicine is already here, is right around the corner, or will occur within our lifetime. Defining and classifying tissue engineering pathology is long overdue. In the next decades, the field of transplantation will enlarge at least tenfold, through tissue engineering approaches. We suggest that transplantation pathologists will become tissue-(re-)engineering pathologists with enhanced skill sets to address many concerns involving the use of bioengineered organs. We outline ways of categorizing abnormalities in tissue-engineered organs through either traditional light microscopy or several other modalities of examination. Based on these analyses we propose creating a new Banff Classification of tissue engineering pathology to standardize and assess de novo bioengineered solid organs transplantable success *in vivo*. We recommend constructing a framework for the creation of a classification of tissue engineering pathology now with interdisciplinary consensus discussions to further develop and finalize the classification at future Banff Transplant Pathology meetings, in collaboration with the human cell atlas project. The future safety and efficacy of bioengineered tissues and organs is crucial to long-term transplantation success. A possible nosology of pathologic abnormalities in tissue-engineered organs is suggested.

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Engineering Chorionic Villus-derived Mesenchymal Stromal Cells For In Utero Treatment Of Spina Bifida

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Spina bifida (SB) is the most common cause of lifelong paralysis. SB is more than just a neurological disorder; it is a complex disease with significant malformation of bone, muscle and connective tissue overlying the spinal cord that results in severe neural tissue damage and neurological deficits. Repairing the damaged spinal cord is a long-standing challenge for the entire field; however, the question of how to protect the spinal cord once repaired has been largely ignored. This study proposes a fetal tissue engineering approach to design a bioengineered construct to generate de novo segments of the bony spine in order to provide support and protect the cord from future damage. In this study, three methods were evaluated for the feasibility of obtaining a significant number of placental first trimester chorionic villus-derived mesenchymal stromal cells (CV-MSCs) within a time frame relevant for autologous in utero fetal transplantation. 1.262×10^9 ($\pm 3.937 \times 10^8$ Standard Deviation, SD, $n = 4$) CV-MSCs were obtained within 3 weeks using the optimal isolation method. The cells exhibited high proangiogenic, while also possessing robust osteogenic potential when seeded on composite hydroxyapatite/poly (lactide-co-glycolide (HA-PLG) scaffolds. In conclusion, this approach develops a bioengineered scaffold that can facilitate new bone regeneration, holds great potential for fetal bone regeneration and provides a path toward a more comprehensive approach for the treatment of SB.

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Protecting Transplanted Cell Therapies Using Immunomodulatory Materials

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Cell and organ transplantation offers the potential to restore organ function, however, a major limitation to this therapy is the requirement for patients to take global immunosuppressive drugs, often daily, for the remainder of their lives, perhaps putting them at risk of serious side effects. This research describes efforts to design new ways to deliver localised and targeted immunosuppression to protect cell transplant therapies from host immune attack. We have developed a targeted microparticle system which can deliver localised immunomodulatory cues to a host transplant niche. These materials can control the behaviour of T cells, and we use them to modulate the host immune system in transplantation by directing T cell phenotype *in vitro* and *in vivo*. By increasing the percentage of Treg cells in the local transplant niche, alongside the draining and non-draining lymph nodes, we have been able to extend transplant graft lifetimes in two *in vivo* models, namely partial organ (skin graft) transplants and organoid transplants in islet therapy. In both systems, comparison of engraftment in systems using repeat administration of immunosuppressive drugs, or a single dose of our targeted and biodegradable microparticle delivery platform was performed. Our results show an increase in functional transplant lifetime in systems using our materials; in some cases functional graft lifetime was increased from 3 weeks to up to 3 months.

Scientific Session 4: Biofabrication and Bioreactors 2

Tuesday, December 5, 2017, 8:00 AM - 9:30 AM

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WITHDRAWN

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3D Bioprinting of Cartilage Tissue Using Canine Mesenchymal Stem Cell Spheroids under the Optimal Growth Factor Condition

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Introduction: 3D bioprinting technology using spheroids of mesenchymal stem cells (MSCs) is expected to offer a new strategy for regenerative therapy. In this study, we investigated the optimal growth factor condition for hyaline cartilage differentiation of canine bone marrow peri-adipocyte cells (BM-PACs) that we have previously reported as newly developed MSCs [1] and tried to fabricate cartilage constructs with a 3D bioprinting device.

Materials and Methods: Spheroids of BM-PACs were cultured in chondrogenic media supplemented with BMP-2, GDF-5 or IGF-1. The optimal chondrogenic condition was determined by the amount of cartilage matrix synthesized and chondrogenic gene expression. Then, spheroids were assembled into a patch-like construct by a Bio-3D printer (Regenova®, Cyfuse Biomedical KK) and cultured under the optimal chondrogenic condition.

Results: Spheroids treated with BMP-2 and GDF-5 showed increased glycosaminoglycan production. The expression of type II collagen tended to increase in GDF-5 treated spheroids. Although there was no change in chondrogenic gene expression, BMP-2 significantly increased the expression of type X collagen. After chondrogenic induction, the assembled spheroids were integrated each other and a transparent construct was obtained. Similar synthesis of cartilage matrix as spheroid was observed in the construct.

Discussion: GDF-5 was effective growth factor for hyaline cartilage differentiation of canine MSCs. 3D bioprinting of cartilage construct using canine MSC spheroids under the optimal condition can offer new insights for cartilage regenerative therapy for dogs.

References: 1. Lin HY *et al*., Stem Cells Dev. 26(6), 431-440, 2017.

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Adapting Commercial Projection SLA system for Printing Live Cell-Laden Hydrogels

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3D Bioprinting applies layer-by-layer based 3D printing technologies to develop precisely designed scaffolds for tissue repair and organ replacement [1]. Projection stereolithography presents a promising method to enhance yield with faster turnaround time due to its fine resolution and high fabrication speed [2]. We report an optimized process for printing live cell-laden hydrogels using a commercial SLA printer. This procedure employs visible light photocuring of poly (ethylene glycol) diacrylate (PEGDA) hydrogels in presence of photoinitiators. Desktop PICO2 (Asiga) SLA printer was modified: (a) Smaller build tray volume for lesser usage of precious materials (b) PMMA build tray base coated with hydrophobic fluorinated ethylene film (FEP) to prevent scaffolds from adhering to the build tray. Cytotoxicity of the water soluble photoinitiator lithium phenyl(2,4,6-trimethylbenzoyl) phosphinate (LAP) on WS-1 fibroblasts was evaluated and 0.3% (w/v) LAP concentration was chosen. Percoll 37.5% SIP solution was found to ensure the highest even suspension of cells in the monomer solution. Printing parameters for hydrogels were optimized: Keeping the light wavelength, intensity and slice thickness constant, burn-in exposure time and layers were varied. Both bright field and fluorescence microscopy indicated live cells inside the scaffold after 72 hours of culture. These optimization studies lay groundwork for higher-resolution, large scale and faster printing of tissues using SLA system. *In vitro* printed tissues could be used for drug testing, regenerative medicine and disease modelling. 1. Murphy SV, Atala A. Nature Biotechnology. 32, 773, 2014. 2. Pereira R, Bártolo P. Computational Methods in Applied Sciences. Springer 31, 149, 2014.

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The Effect Of Varying Duration And Frequency On A Tendon Tissue Engineered Construct

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The proper utilization of mechanical stimulation, as documented in literature, is typically a positive influence on the differentiation of *in vitro* cultured stem cells and on the quality of deposited extracellular matrix. Cyclic mechanical stimulation is the primary mode of inducing tenocytic differentiation, and this study aims to validate and optimize cyclic strain regimes previously used in house studies on mesenchymal stem cell (MSC) seeded human umbilical veins (HUV). We hypothesize that the extended stretching regimens normally utilized cause cellular fatigue, eliciting less of a response than repeated short stimulations. During culture, the HUVs are subjected to cyclical 2% strain for differing durations and frequencies before being removed and examined for cellularity, maximum tensile strength and tendon gene expression. In this study we investigated the effects of changing the duration (0.5, 1, and 2 hours/day) and frequency (0.5, 1, 2 cycles/minute) of stimulation of a human umbilical vein seeded with mesenchymal stem cells cultured for up to 7 days and up to 14 days. The tendon constructs are examined for cellularity, tensile strength, collagen fibril alignment and tendon gene expression compared to static

controls. At 7 days, slower frequencies and shorter durations were best for construct quality. Preliminary results will be compared with day 14 results. Through this optimization, we hope to engineer a more tendon like construct than has previously been obtained.

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Innovative Cartilage Regeneration for in situ Co-axial 3D Bioprinting

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Cartilage injuries can cause pain and loss of function, and if severe may result in osteoarthritis (OA). Surgical procedures have been disappointing because they lead to poor quality regenerated tissue. 3D bioprinting introduces a new strategy to cartilage tissue engineering for the generation of organized 3D scaffolds composed of biomaterials with cells. Our team has developed a handheld 3D printer (Biopen) that extrudes Mesenchymal Stem/Stromal Cells encapsulated within a hydrogel (GELatin-Methacrylamide/Hyaluronic Acid-Methacrylate), with the aim to induce hyaline cartilage regeneration (1). The key feature of the Biopen is a co-axial nozzle that prints an organized Core/Shell structure in which the inner soft Core, containing stem cells, is protected by a robust Shell which hardening is induced by UV photocuring of a selected photoinitiator. The aim of this study is to prove the chondrogenic differentiation of human adipose derived Stem Cells harvested from the infra-patellar fat pad of patients undergoing total knee joint replacements, after 3D bioprinting with the Biopen. We demonstrated that our strategy drives chondrogenic differentiation, as reflected by the production of hyaline-like extracellular matrix and the increase in phenotypic cartilage gene expression markers. The ability to deliver in real time and in situ a chondrogenic bioscaffold is a world-first innovation and has important implications for customizing cartilage repair in patients with cartilage injuries or early OA.

References: 1. O'Connell, C. D. *et al. Biofabrication* **8,** 15019 (2016).

Acknowledgments: Arthritis Australia. Victorian Orthopaedic Research Trust. ARC Centre of Excellence Scheme (# CE140100012). St. Vincent's Hospital (Melbourne) REF grant.

Scientific Session 4: Cancer

Tuesday, December 5, 2017, 8:00 AM - 9:30 AM

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In Vitro Models of Liver Cancer PDXs

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Perfusable Tissue-Engineered Model of Neuroblastoma for Studying Drug Resistance

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Neuroblastoma is a vascularized pediatric tumor derived from neural crest stem cells that express a number of stemness markers, including SOX2, a factor associated with the undifferentiated stem cell phenotype. For high-risk patients, long-term survival is barely 50% despite surgery and induction chemotherapy consolidated by isotretinoin (INN). INN has shown effectiveness in inducing cell differentiation, cell growth arrest and inhibition of angiogenesis in *in vitro* studies and in clinical trials. However, while more than 40% children suffer relapse during or after INN treatment, little is known about the mechanism of drug resistance. Interestingly, expression of SOX2 was related to drug-resistance and tumor relapse in many types of cancer. We engineered a perfusable, vascularized *in vitro* model of three-dimensional human neuroblastoma to study the effects of INN on tumor vasculature and drug-resistance. The bioengineered model recapitulated the clinically observed vasculogenesis and vasculogenic mimicry, and contained subpopulations of stem-like neuroblastoma cells expressing high levels of SOX2. Treatment with INN augmented populations of cells expressing high levels of SOX2 and did not target new blood vessels formed by SOX2+/CD31+ cells. Our results reveal some roles of SOX2 in drug resistance, and suggest that SOX2 could be a therapeutic target in neuroblastoma.

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The Effects of Normal, Metaplastic, and Neoplastic Esophageal Extracellular Matrix upon Macrophage Activation

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Laboratory tools to study the effect of the microenvironment upon cancer cell phenotype have largely remained unchanged; specifically, collagen gels and Matrigel have represented the gold standard for the past several decades. The stimulus for the present study was to develop a new tool to investigate cancer cell biology with disease-specific extracellular matrix (ECM) hydrogels.

ECM hydrogels were prepared from decellularized normal, metaplastic, and neoplastic (adenocarcinoma, EAC) esophageal tissue, and the effect of these hydrogels upon macrophage activation was investigated. Matrix-bound nanovesicles (MBV) were isolated from the ECM materials. MBV have been shown to rapidly and markedly affect cell phenotype. Important unanswered questions are: 1) What is the miRNA profile contained within the ECM-MBV to drive EAC progression? 2) How does diseased ECM activate an important cell type in an inflammatory-driven cancer, the macrophage?

The three ECM types showed distinctive fiber networks by SEM and protein profiles by mass spectroscopy. Metaplastic and neoplastic ECM activate human THP1 macrophages to a dual ''pro-inflammatory''

(TNFalpha+) and ''immunomodulatory'' (IL1RN+) state, with greater expression correlating with advanced disease. Neoplastic ECM modulates the macrophage secretome to increase normal esophageal epithelial cell migration, characteristic of tumorigenesis. Top differentially regulated MBV-miRNA were notably related to epithelial-mesenchymal transition, gastrointestinal cancer, and macrophage activation by small RNA sequencing, suggesting the distinctive role of ECM to influence cell behavior.

In conclusion, ECM hydrogels representative of the normal, metaplastic, and neoplastic microenvironment were developed. A better understanding of ECM-MBV miRNA and disease-specific macrophage activation will help guide regenerative therapies for EAC.

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A Tumor-on-a-Chip Platform for Screening Precision Driven Medicines

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Cancers of the digestive system enclose the largest projected cancer group in new cases reported and the second largest in deaths projected, with one third of both coming from colorectal cancer. New methods of detection and treatment are needed as these cancers can develop from a plethora of mutations requiring different targeted treatments. In order to improve on these limitations, we have developed a 3D colorectal cancer model that can be used to test high throughput drug screening in comparison to 2D testing. The model was tested using several colorectal cancer cells lines with detected metastatic mutations including K-RAS and B-Raf and cells were encased in hydrogel to be tested against known targeted chemotherapy regimens utilized in the clinic today. A two-layer gel structure was also developed to mimic the tumor microenvironment to test how the chemotherapeutics would affect the migration of the cells in the metastatic core to the outer epithelial layer. Additionally, the system was utilized to test novel 5FU analogue chemotherapeutics developed to reduce general toxicity, while maintaining tumor specificity. Finally, patient derived primary colorectal tumors have been procured and after processing, the system has tested both current and novel chemotherapeutics to compare efficacy of the targeted regimens.

Scientific Session 5: Rapid Fire Session 2

Tuesday, December 5, 2017, 10:00 AM - 11:30 AM

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Highly Valuable Endogenous Molecules Incorporated Within Physically Cross-linked Gellan gum Scaffolds for Bone Tissue Regeneration

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Traumatisms, infections and bone disorders are main causes that affect bone homeostasis and produce damage in bone tissue. Their clinical relevance and increase in the elderly population has promoted intensive research in the area of bone regeneration during the last years. Nevertheless, unsatisfactory results have extended the search for the perfect bone regeneration-promoting platform to the present day. This platform should be highly biocompatible and, ideally, benefit from the intrinsic biopotential of endogenous molecules. Nevertheless, endogenous molecules are prone to lose their activity when submitted to harsh conditions. Therefore, their incorporation into a regenerative platform is very challenging. The authors developed hydrogel scaffolds formed by gellan gum and physically cross-linked by the endogenous polyamine spermidine. The mild preparation conditions permitted the incorporation of other interesting endogenous molecules, including Bone Morphogenetic Protein 2 (BMP-2). Indeed, the effective trans-differentiation of C2C12 cells toward osteoblastic lineage confirmed the release of bioactive BMP-2. After *in vivo* implantation in Wistar rats, abundant angiogenesis, mature bone tissue and bone marrow tissue were observed. BMP-2 was also loaded into a commercial scaffold formulation, thus allowing for comparison. Micro-computed tomography and tissue staining confirmed the ability of the studied hydrogel scaffolds to induce the formation of more mature and dense ectopic bone tissue, which remained during a longer period without being reabsorbed when compared with the commercial formulation. These results confirm the potential of the developed hydrogel scaffolds as both innovative growth factor delivery platforms and scaffolds for regenerative medicine applications.

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Response Surface Optimization of Alginate Biomaterial Parameters for Maintenance of Preconditioned MSC Phenotype

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While cell-based tissue engineering remains an attractive alternative to current strategies for repair of tissue defects, directing cell fate post-implantation remains a challenge. Immediate implantation of naïve stem cells, while clinically relevant, cannot provide implanted cells with sufficient bioactive signals to repair tissue. Prolonged *in vitro* cultivation allows control over presentation of appropriate cues, but raises safety and translatability concerns. In this study, we hypothesized that mesenchymal stem cells (MSCs) preconditioned for a brief 1-week period under differentiation conditions can continue to differentiate after removal of differentiation factors, provided they are aggregated and implanted in an instructive biomaterial. Cell-cell cohesion and endogenous matrix production within MSC aggregates enhance MSC viability and function, while interaction with biomaterial substrates greatly influences MSC differentiation. To test this hypothesis, MSCs were cultivated in monolayer under adipogenic, chondrogenic, and osteogenic conditions for 1 week and then aggregated into spheroids of 5,000 to 20,000 MSCs. Spheroids were encapsulated in alginate with storage modulus ranging from 200 to 15,000 *Pa* and arginine-glycine-aspartate (RGD) concentration ranging from 0 to 4 *mM*. Constructs were then cultured under expansion conditions for 2 weeks and assessed for differentiation. Outputs were fit to response surface models with spheroid cell number, storage modulus, and RGD concentration as input parameters. Differentiated phenotype was maintained and response surface analysis showed cell number alone did not affect differentiation, but modulus and RGD content influenced chondrogenesis and osteogenesis. Moreover, the three parameters had significant interactions. This approach may inform future cell-based approaches while minimizing *in vitro* cultivation.

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Development Of A Textured Patch for the Engineering of Heart Tissues

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Ischemic heart failure continues to pose an unequivocal challenge to the health care systems around the world owing to the structural complexity of cardiac tissue, as well as the polymorphism of tissue degradation inherent to the chronic phase of myocardial infarction that are still refractory to conventional treatments. The cardiac tissue rehabilitation concept notably consists in stem cells transplantation and/or tissue engineering (TE) to repair or reshape the highly-damaged tissue However, outcomes extracted from both experimental and early clinical settings have shown poor

therapeutically benefices. Therefore, the development of suitable innovative biomaterials for Cardiac Tissue Engineering (CTE) to enhance the regeneration effect represents an attractive and active field of research. In CTE, different fabrication methods are proposed to develop 3D-scaffolds, in this work an innovative therapeutic functional ''natural'' thin patch with flat geometry and controlled hierarchical surface texture is proposed. Mechanical, physico-chemical and biological characterizations assess the therapeutic potential of these new substitutes

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Hybrid Degradable Scaffold supports Functional Skin Tissue Generation upon Seeding Adipose Derived Mesenchymal Stem Cells committed to Dermal Lineage

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Tissue-engineered skin equivalents are expected to have great potential to treat chronic burn wounds. However, they have several limitations such as immune reaction and entrapped non-degradable epidermal components. A combination product of electrospun terpolymer poly(lactide-glycolide-caprolactone)(PLGC) and biomimetic fibrin composite (PLGC-F) has been found to promote dermal fibroblast growth[1]. Similar grafts with enhanced surface area has been studied in this work to explore their potential to guide skin tissue regeneration. Human adipose derived mesenchymal stem cells (ADMSCs)-induced fibroblasts (IEC & IC-SCRT approved) were used to validate the graft for dermal tissue generation *in vitro* and to study the scaffold degradation after long-term (90 days) culture. Extra cellular matrix (ECM) deposition upon culture suggested formation of skin like tissue with adequate mechanical strength. Parameters studied include cell proliferation studies, ECM quantification, polymer degradation and variation in mechanical strength. Scaffold patches were implanted in subcutaneous tissue of rabbit skin and explanted after 90 days. Both *in vitro* cell grown scaffolds and explants were analyzed for mechanical strength and their solvent extracts to estimate polymer degradation. The wound healing efficiency was analyzed by treating $4cm^2$ size burn wounds in rabbit model for 28 days which suggested that the combination graft (PLGC-F) showed optimal properties for guided tissue regeneration in terms of reduction in wound size, epithelialisation, collagen organization and angiogenesis. Polymer degradation after 90 days implantation was remarkable and no adverse tissue reaction was noted.

References: 1. Renjith PN *et al*., Biores Open Access. 3(5), 217-225, 2014.

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Microribbon-based Hydrogels with Tunable Biochemical Compositions Enhance MSC-based Cartilage Regeneration

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Introduction: Hydrogel scaffolds are often used to deliver cells for cartilage repair but generally have weak mechanical properties and lack the macroporosity to facilitate extracellular matrix deposition. We recently reported a method to fabricate novel microribbon (μRB)-based hydrogels, which are injectable, macroporous, and possess cartilage-mimicking shock-absorbing properties. The objective of this study was to evaluate the effects of varying μRB compositions on enhancing mesenchymal stem cell (MSC)-based cartilage regeneration.

Methods: Microribbons were fabricated by wet-spinning polyethylene glycol (PEG), gelatin, chondroitin sulfate (CS) and hyaluronic acid (HA). All polymers were modified with methacrylate to enable intercrosslinking. Passage 6 human MSCs (15M/mL) were encapsulated in μRB-based hydrogels (7.5% w/v). Eight hydrogel compositions were examined: each composition alone (PEG, GEL, CS, HA) and mixed μRB compositions in a 1:1 ratio (PEG-CS, PEG-HA, GEL-CS, GEL-HA). All samples were cultured in chondrogenic medium for three weeks. Outcomes were evaluated by cell viability, biochemical assays, mechanical testing, and histology.

Results: Gelatin uRBs led to robust neocartilage deposition throughout the scaffold. In contrast, CS and HA uRBs led to minimal cartilage formation. Interestingly, when CS or HA uRBs were mixed with PEG or gelatin uRBs, the resulting scaffolds supported enhanced neocartilage formation, with up to a 45-fold increase in compressive moduli compared to day 1 and extensive sGAG and collagen deposition.

Significance: Our results validate μRBs as macroporous matrices for supporting robust cartilage regeneration by stem cells in 3D. μRBs with tunable biochemical cues could serve as novel injectable building blocks for further enhancing MSC-based cartilage regeneration.

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Alginate/gelatin Hydrogels as Bioprintable Inks Directing Tumor Spheroid Formation

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Physiological tumor microenvironments exhibit varied mechanical and biological properties that influence the formation rate, size, and frequency of multicellular tumor spheroids (MCTS). Here we developed a tunable composite hydrogel composed of alginate and gelatin to create 3D bioprinted models of triple-negative breast cancer cells that develop distinct MCTS differences dependent upon the mechanical and biochemical properties of the gels for greater than 30 days in culture. MDA-MB-231 cells were homogenously dispersed in Alginate (1%, 3%, 5%) and gelatin (5%, 7%, 9%) dissolved in phosphate buffered saline before crosslinked by $CaCl_2$. The crosslinked hydrogels have stiffness varying between $4.6 \sim 28.9$ kPa primarily depending upon the concentration of alginate. Confocal imaging shows cancer cells in the gelatin containing hydrogel formulations start to form MCTS on day 15 while those in alginate-only gels remain as single punctate cells with decreasing number over time. The MCTS continue expanding in hydrogels containing gelatin and 1% alginate until reaching a diameter over 300 μm after 30 days. In gels containing 3% and 5% alginate with stiffnesses of 16.6 and 28.9 kPa, cells remain as small MCTS. Gelatin provides cell-matrix adhesion sites that promote MCTS formation, while the MCTS growth can be suppressed due to the increased stiffness of the matrix. These mechanically and biochemically tunable bioinks are capable of recapitulating the native tumor stroma and provide a novel tool to study how mechanics and cell-matrix interactions result in tumor development.

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Engineering Fibrin Nanoparticles for Enhanced Wound Healing

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The inability of a wound to heal quickly is a key concern in managing wounds in major injuries, especially in conditions, which impair coagulation such as hemophilia, diabetes and Von Willebrand disease. Commercially available fibrin sealants quickly seal tissues and stop bleeding. However, these sealants require supraphysiological concentrations of thrombin and fibrinogen to obtain sufficient polymerization kinetics and mechanics required for clinical use. These formulations can inhibit subsequent wound repair due to the lack of porosity, which limits cell migration into the fibrin network. Our objective is to synthesize pre-polymerized fibrin nanoparticles that will quickly seal tissues while also promoting healing by i) enhancing cell migration due to increased fibrin network porosity while maintaining mechanical robustness and ii) improving clot degradation kinetics through tunable fibrinolytic properties. To that end, we have synthesized Fibrin Nanoparticles (FBN) using a 2-phase microfluidic fibrin droplet generator. We hypothesize that macroscopic gels created from FBN networks will be better suited for wound healing therapies than commercially available bulk fibrin gels and will enhance cell migration. Indeed, our results show that FBNs promote human dermal fibroblast migration in a 3D wound assay mimic compared to bulk fibrin. Additionally, traction force microscopy experiments demonstrate that fibroblasts generate contractile forces on FBNs similar to collagen surfaces.

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Cell-Cell Interactions Enhance Cartilage Formation in 3D Gradient Hydrogels that Mimics Tissue Zonal Organization

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Cartilage tissue is characterized by zonal organization with gradual transitions of biochemical and mechanical cues from superficial to deep zones. Previous tissue engineering strategies mostly employ scaffold with homogeneous cues, which fail to mimic the zonal organization of native cartilage. We have recently developed hydrogels as 3D cell niche with continuous gradient of biochemical and mechanical cues, which induce zonal-specific response of chondrocytes to form cartilage that mimics articular cartilage organization. While the role of cell-ECM interactions have been studied extensively, how cell-cell interactions across different zones influence cartilage zonal development remains unknown. The goal of this study is to examine the role of cell-cell interactions in modulating cartilage formation in 3D using gradient hydrogels. When encapsulated in PEG/CS gradient hydrogels, chondrocyte exhibit zonal-specific responses in cell proliferation as well as cartilage ECM deposition. In contrast, when each zone is cultured separately, the extent of zonal-response of chondrocytes were much lower than intact gradient hydrogel group. Co-culturing of hydrogels cut into 5 zones in the same well led to comparable cellular response across all zones. We further evaluated the memory of cell-cell interactions by first priming chondrocytes in intact gradient hydrogels for one week, then divided into separate zones and cultured separately. Priming enabled memory of cell-cell interactions and restored the zonal response. Together, our results highlight that cell-cell interactions play a critical role in resulting in the cartilage zonal development. Such gradient hydrogels could facilitate employing heterotypic cell interactions to enhance regeneration of cartilage with biomimetic zonal organizations.

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Graphene Oxide Influence the Release of Protein from Layer-by-Layer Self Assembled Electrospun PCL Fiber

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The controlled delivery of growth factors (GFs) at harmed tissues has shown promising results in tissue regeneration applications. Since they can stimulate cell differentiation, growth of new blood vessels and accelerate new tissue regeneration at damaged tissues in human body. However, when large amounts of GFs are released at affected area of the tissue it may trigger abnormal cell responses. To overcome this problem, we propose the production of GFs reservoirs as coating over polymeric membranes using self-assembled Layer-by-Layer (LbL) technique as protein carriers, in order to obtain a device of slow and controlled release of GFs. Electrospun polycaprolactone (PCL) mats were chosen as carrier membranes for this study, while bovine serum albumin labelled with fluorescein isothiocyanate (BSA-FITC) and lysozyme labelled with fluorescein isothiocyanate (Lyso-FITC) were taken as model proteins. Graphene oxide (GO) particles and amine terminated GO particles were coated over the electrospun PCL fiber mat using LbL technique in order to enhance protein load and stability. Using heparin/chitosan polyelectrolyte solutions and the protein co-solutions, a series of LbL reservoirs containing either BSA-FITC/GO-BSA-FITC or Lyso-FITC/GONH_2-Lyso-FITC were assembled in 10 layer pairs (10 LP) with different architectures. To confirm the protein loading on LbL grown fibers, they were subjected to different studies such as functional, thermal, morphology and fluorescence analysis. Further, the protein release from fiber was examined in PBS at 37°C and the release profile showed controlled and extended protein release in the presence of GO when compared to amine terminated GO particles.

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Emulsion-free Chitosan Microgels for Growth Plate Cartilage Tissue Engineering

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While chitosan has been used extensively for articular cartilage regeneration, few studies have investigated chitosan for growth plate cartilage regeneration. Further, chitosan microspheres are commonly fabricated using an emulsion method, which requires cytotoxic solvent washes that can negatively affect tissue growth. In this work, degradation, release, and growth plate regeneration properties of emulsion-free chitosan microgels were investigated. All animal procedures were approved by the University of Colorado IACUC. 6% chitosan was crosslinked with 50μM genipin, and was manually pressed through a 106 μm sieve producing irregularly-shaped particles ≤500 μm in diameter. Microgels 125-250 μm in diameter were selected by filtering and used for subsequent experiments. Microgels loaded with the chemokine CXCL12 displayed a linear release up to 37 days as measured by ELISA. Incubation in 0.5 mg/ml lysozyme showed degradation after one week *in vitro*. Further, injection of these microgels in a rat growth plate injury model showed full microgel degradation, and increased cartilaginous repair tissue by 28 days. Thus, these microgels demonstrate both *in vitro* and *in vivo* degradation, and can release chemokines in a linear, time-dependent manner. This fabrication method provides a novel, emulsion-free chitosan-genipin biomaterial system that may be used in tissue engineering applications, and is particularly promising for treating growth plate injuries.

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Hyaluronic Acid conduits filled with polylactic fibres and Ependymal Progenitor Cells create a new biohybrid concept for cell transplantation in Spinal Cord Injury

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SCI results in the loss of sensory and motor functions due to the death of neurons and degeneration of axons. Transplant of ependymal

progenitor/stem cells (epSPC) may improve functional recovery of the injured spinal cord by delivering neurons and their support cells through differentiation. We have employed a combinatory therapeutic strategy (hybrid scaffold with stem cells) to induce differentiation towards neurons rather than glial cells and to enhance axonal growth. In this study, epSPC were seeded in a tubular conduit of hyaluronic acid (HA) containing aligned poly(lactic acid) fibers (PLLA) and we evaluated the efficacy of neuronal differentiation and axonal growth along of the PLLA fibers. To evaluate the security of the biohybrid the construct was implanted into a rat spinal cord injury model. The biohybrid showed minimal inflammatory reaction and the epSPC were distributed and spread in and around the lesioned area. This approach of combining biomaterials with different structures and stem cells offers a promising treatment for the injured spinal cord.

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Vessel Formation from Modular Vascularized Microbeads

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Significant morbidity and mortality due to limb ischemia can be attributed both to macrovascular and microvascular inadequacy. We developed pre-vascularized fibrin microbeads that can be delivered in a minimally invasive manner, jump-start inosculation with host vasculature, and quickly enhance local microvascular networks with the potential to improve blood flow to ischemic areas. Microbeads containing endothelial cells and stromal fibroblasts pre-cultured for 3 days formed more extensive microvascular networks when embedded and cultured in model tissues, compared to microbeads pre-cultured for other time periods. *In vivo* implants containing pre-cultured microbeads, fresh microbeads with no pre-culture period, acellular microbeads, or cells without microbeads were injected within a fibrin matrix into subcutaneous pockets on the dorsal flanks of SCID mice. Implants containing pre-cultured microbeads formed functional connections to host microvasculature within 3 days, exhibiting more extensive vessel coverage, higher vessel numbers, and less implant compaction after injection compared to other groups. They also maintained a more broadly distributed microvessel network at day 7, which we attributed to a better preservation of the implant volume compared to other groups and confirmed with ultrasound imaging of model implants. Surprisingly, constructs containing pre-cultured microbeads had a lower bulk elastic moduli compared to controls, but higher local stiffness near the microbeads, suggesting the initial cell distribution influences construct compaction and the subsequent distribution of perfused microvasculature.

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Fabrication of hollow beads With Precision Pore Structures For Cell Transplantation

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Scaffold materials find important applications in tissue engineering and cell transplantation. Although various techniques have been used to create porous scaffolds for construction of cell-material systems, precisional control over the internal nano-/micro-pores in scaffolds are still hard to achieve. Here, an immersion-precipitation phase transformation (IPPT) process was established for fabricating the hollow bead-based scaffolds using polyethersulfone (PES). Hollow beads with the outer diameter in 1.5-4 mm and the wall thickness in about 450 μm were obrained. In particular, the wall contained parallel channels, with the diameter decreasing outward from 50μm to reach a dense layer on the surface. By controlling the designing time in dimethylacetamide, two types of beads, with highly monodisperse pore sizes on the surfaces, 0.401 ± 0.187 μm (bead with nanopores, BNP) and 9.22 ± 3.83 μm (Bead with micropores, BMP), were prepared. When loaded with mesenchymal stem cells (MSCs), the cells were found to distribute inside channels and form clusters. Through *in vivo* noninvasive imaging studies in the murine host, it was found that both BNP and BMP scaffolds significantly extended the residence time of MSCs transplanted in the subcutaneous space, in comparison with the directly injected MSCs. Specially, the BNP scaffold supported a higher percentage of cell survival at all time points compared to BMP, and the cellular residence time reached approximately two weeks. Our study shows the IPPT process can generate hollow spherical membranes with oriented channels with precision pore sizes and structures. The hollow beads may be further investigated as cell scaffolding/delivery materials for cell-based therapy.

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Novel Supercritical Co_2 Processed Composites For Bone Applications

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Tissue engineered grafts are still used significantly less in contrast to autologous or allogenic bone grafts. However, with bone transplants there are problems like the morbidity of the harvesting site and limited availability. Therefore, new bone substitutes are needed in the treatment of large bone defects. Composites comprising poly(L-lactide-co-ε-caprolactone) with 50wt-% of β-tricalciumphosphate were foamed with supercritical CO_2 and cut into discs. Porosity and pore size of the composites were analyzed by μ-CT. *In vitro* testing was done by culturing human adipose stem cells (hASCs) in the composites up to 21 days. Cell viability was assessed by Live/Dead staining and osteogenic differentiation was analyzed by alizarin red and immunocytochemical staining of osteogenic marker proteins. *In vitro* analyses were conducted at 7-, 14- and 21d. Thereafter, composites were implanted in rabbit distal femur defects. Empty defects were used as control. Histological staining and μ-CT analysis were conducted 4-, 12- and 24 weeks after implantation. 6 rabbits were sacrificed per time point. Porosity of the composites was 63-65% with average pore size of 380-440μm. The novel composites supported the viability of hASCs. Furthermore, hASCs differentiated towards osteogenic lineage when cultured in the composites. In the rabbit model, host tissue was able to grow into the composites already in 4 weeks and histological staining showed that at 24 weeks calcified bone had formed inside the porous composites in defects. To conclude, these flexible and user-friendly supercritical CO_2 -processed composite scaffolds show great potential as a new bone substitute material for treating bone defects.

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Gellan Gum: a Multifunctional Tool to Modulate Cell Microenvironment

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Cell encapsulation is an alternative to the use of immunosuppressant drugs after cell transplantation. It shields cells from the host immune system, allowing the diffusion of nutrients and oxygen[1]. The alginate - poly-L-lysine - alginate system is the most well-studied method[2], but biocompatibility issues were reported[3]. This work aims to use methacrylated gellan gum (GG-MA), an anionic heteropolysaccharide, to engineer the microenvironment provided in cell encapsulation strategies. Capsules were formed by gravitational dripping, extruding GG-MA into a poly-L-Lysine (PLL) bath[4]. Due to the interaction between the carboxylic groups of the GG-MA and the charged PLL amines, a capsule is formed. Morphology was assessed using scanning electron microscopy and micrographs, revealing a diameter of 2.3 ± 0.145 mm. Drug release capacity was quantified using albumin-fluorescein isothiocyanate conjugate (BSA-FITC, 66 kDa) as a model of large glomerular molecules; methylene blue (MB, 319.85

Da) as a small molecule model; and Dextran-FITC with 4, 20 and 70kDa. While small molecules (MB and 4kDa Dextran-FITC) were rapidly released, the larger molecules had a hampered flow. *In vitro* tests, using hASC, have shown that cells remain viable after 7 days of culture. *In vivo* results, using CD1 mice, have shown that GG/PLL complexes do not elicit fibroblast deposition and can tune the microenvironment, from bioactive to biotolerable. Briefly, the results herein presented show the potential of GG-MA/PLL capsules for cell encapsulation as they are: (i) easy to produce, using one-step only; (ii) have selective permeability; (iii) hASC maintained their viability after encapsulation; and (iv) biocompatible.

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Development of Thermally-Responsive Fibrin Gels for On-Demand Delivery

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One of the goals of personalized medicine is to deliver the right dosage at the right time, which requires innovative delivery systems capable of customization. On-demand delivery systems are capable of exerting explicit control over where, when and how much of a therapeutic is released by making release stimuli-dependent. This lab has developed a strategy for light-actuated delivery that uses the photothermal response of biocompatible chromophores to trigger release from thermally-responsive delivery vehicles. However, there is a lack of biocompatible thermally-responsive biomaterials. Fibrin is a traditionally non-stimuli-responsive biomaterial with a long history of use in FDA approved products, and has previously been functionalized with exogenous peptides by enzymatic incorporation via Factor XIII for tissue engineering applications. This same approach can be used to functionalize fibrin with thermally-responsive oligonucleotide tethers for on-demand growth factor delivery. Specifically, a peptide sequence from a Factor XIIIa substrates, α_2-plasmin inhibitor, has been conjugated to fluorescently-tagged oligonucleotides using the heterobifunctional crosslinker (succinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (SMCC) and enzymatically incorporated into fibrin hydrogels during polymerization. Both the incorporation efficiency of double-stranded oligonucleotides and the thermally-triggered release of single-stranded oligonucleotides from the fibrin hydrogels was measured via fluorescence. Triggered release data shows that heat labile oligonucleotide tethers remain annealed at room temperature and thermal release of compliment oligonucleotide strands from the parent strands is achievable with 5 minute heating intervals at 45°C. These results suggest have the potential to aid in future studies aiming to functionalize other traditionally non-stimuli-sensitive biomaterials with a long history of use in FDA approved products.

Scientific Session 5: Tissue Chips and Tissue Organoid Models 1

Tuesday, December 5, 2017, 10:00 AM - 11:30 AM

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Tumor-on-a-chip Microfluidic Devices for Personalized Cancer Drug Testing

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Traditional chemotherapeutic treatments show variability in patient response even between similar cancer types. Recent advances have helped address this variability by aiding chemotherapy design based on genetic profile through precision medicine. However, despite this individualized data, clinicians are still left with multiple drug options to which patient response can vary unpredictably. As a result, there is a need for model systems to help predict the personalized response to chemotherapeutics. Here, we address this need by engineering 3D tumor organoids directly from patient tumor biopsies that provide a patient-specific module in a microfluidic device for *in vitro* treatment optimization. Initially, we implement this tumor-on-a-chip platform using a patient-derived mesothelioma tumor biospecimen. We demonstrate the ability to biofabricate and maintain viable 3D patient tumor constructs within a microfluidic device for 14 days. The tumor constructs were exposed to one of two different doses (0.1 μM, 10 μM) of chemotherapeutic mixtures carboplatin/premetrexed or cisplatin/premetrexed for 7 days and LIVE/DEAD assays were performed. Quantification performed on segmented confocal images of constructs showed that constructs treated with cisplatin/premetrexed resulted in significantly increased tumor cell death, which was notable as retrospective comparisons to clinical outcomes revealed that the corresponding patient responded exceptionally well to cisplatin-based treatment. These data demonstrate the power of correlation and predictive potential for these patient-specific tumor models. This platform is ideally positioned to test personalized response to drug combinations in a parallel format to predict treatment effectiveness.

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Primary Patient Mesothelioma Organoids for Genetic Mutation-Driven Experimental 3-Deazaneplanocin a Treatment

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Many cancers continue to go untreated as their mutations or combination of mutations have no effective treatment. However, many novel chemotherapy drugs are being tested both *in vivo* and *in vitro* to show potential efficacy in cancers with actionable mutations that have previously gone untreated. One such compound is 3-deazaneplanocin A (DZNep), which is both an S-adenosylhomocysteine synthesis inhibitor and a histone methyltransferase EZH2 inhibitor. The recent advance of precision medicine has allowed for genetic testing to determine actionable mutations in tumors and thus predict potential best treatments. We have had the unique opportunity to treat two separate sets of patient tumor-derived 3D organoids with various concentrations of DZNep to determine efficacy. Omentum samples of mesothelioma origin were obtained from two separate patients, both of which received genetic testing and one of which had an EZH2 mutation (*BAP1*), which we hypothesized could be targeted with DZNep. Organoids were treated with DZNep and it was found that the tumor with the mutation was responsive to the drug in comparison to the control environment whereas the other was not. Additionally, we used HCT116 cells as a negative control cell line that is unaffected by the treatment of DZNep. Results included quantification of live/dead staining, MTS proliferation assay quantification, and IHC antibody staining. This data is promising in that it shows we have been able to advance organoid development to allow for precision medicine-driven testing of experimental drugs not clinically approved and match genetic testing to appropriate treatments for patients.

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Three-dimensional (3D) High Throughput Organophosphate Toxicity Screening and its Correlation with Pharmacokinetic/pharmacodynamic Model

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High-content, high-throughput platforms are constructed to screen a test set of OP agents for concentration-dependent effects on: 1) cell viability/toxicity, 2) penetration of OP across the blood brain barrier (BBB), and 3) inhibition of acetylcholinesterase (AChE) activity in target cells following OP exposure. We,

further explore a pharmacokinetic/pharmacodynamics (PKPD)-based computational model, based on experimental data, to identify the most critical pathways that mediate OP acute neurotoxicity. We estimated parameters from the experimentation and existing literature, which is used to develop 3D finite element method (FEM)-based models incorporating 3D *in vitro* assay geometry, diffusion kinetics, chemical reaction dynamics, and ionic conductance. We correlate the results generated from pharmacokinetic/ pharmacodynamics (PKPD) computational simulation model with the data from *in vitro* brain on a chip platform, allowing *in silico* modeling of various OP compounds under various conditions (sensitivity analysis), which provide key knowledge for OP screening to acute neurotoxicity.

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Biofabrication of 3D Neural Microphysiological Systems

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Neural system-on-a-chip platforms have demonstrated great potential as *in vitro* tools for modeling neuronal injury and disease and as high-throughput preclinical drug screening devices. Current designs based on microfabricated platforms and 3D neural organoids have limitations in their abilities to recapitulate cell-cell interactions, morphology, and functionality seen *in vivo*. Microfabricated platforms can provide specified tissue organization, but typically restrict cell biology to 2D configurations, which can alter cellular physiology. Conversely, self-assembling 3D neural organoids provide a more physiologically-relevant structure but fail to represent macroscale tissue organization. There is a need for neural system-on-a-chip platforms that articulate the complexity and robustness of *in vivo* tissue at all levels to accurately inform scientific discovery. We describe the fabrication and characterization of rat spinal cord-derived and human iPSC-derived neuronal organoids along with their incorporation into a micropatterned 3D dual hydrogel culture system. The neural organoids can be fabricated with a consistent size, are highly viable, and contain a variety of cells present in the spinal cord. Sensory neural cell aggregates were found to extend neurites towards spinal cord organoids when co-cultured. The micropatterned 3D dual hydrogel culture system, consisting of growth restrictive and growth permissive components, provides a 3D environment for constraining neurite growth to form anatomically-relevant connections between cell populations. The combination of organoid and micropatterned hydrogel system results in the creation of an *in vitro* CNS nerve-on-a-chip device that controls for the multiscale organization by providing 3D cellular assembly along with directed guidance for neurite outgrowth into physiologically relevant neural tracts.

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Creating 3-Dimensional Human Testis Organoid System from Immature Testicular Cells for *in vitro* Spermatogenesis and Evaluation of Environmental Agents

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Introduction: 3-dimensional (3D) organ-like structures mimicking the function of native organs is eagerly desired. Recently we developed *in vitro* 3D organoids from mature human testicular cells capable to differentiate spermatogonial stem cells (SSC) and produce androgen. Our objective is to show the feasibility of the same 3D system using immature cells.

Methods: Isolated cells from prepubertal testicular tissue were cultured in 2-dimensional (2D) condition. Specific genes expression assay and Flowcytometry were used to prove the presence and quantify SSCs, Sertoli, Leydig and peritubular cells. 2D cultured cells integrated into 3D culture using 10000 cells per organoid. The system was evaluated with live/dead cell staining, ATP production assay, genes expression and androgen production. Undifferentiated organoids were exposed to environmental agents including Mercury, Thallium and Lead in different concentrations for 48 hours.

Results: Specific markers for spermatogonia including ZBTB16, PGP9.5, THY1, CD9, FGFR3 and SSEA4; GATA4, SOX9, Clusterin and CD49f for Sertoli cells; STAR, TSPO and Cyp11A1 for Leydig cells; and CD34 for peritubular cells; all proved the presences of different cell types. The organoids maintained their structure, viability, metabolic activity, and produced androgen. Digital PCR for PRM1 and Acrosin showed 0.4% early and 0.2% late post meiotic germ cells respectively. All three environmental agents decreased Leydig cells population but only Mercury and Thallium affected spermatogonial cells.

Conclusion: Human 3D testicular organoid system was generated successfully using isolated immature human testicular cells and maintained long term in 3D culture, produced androgen, differentiated SSCs and was used as a novel testicular toxicity model.

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Controlling and Monitoring Oxygen Levels in 3D Gut-on-Chip

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In the small intestine, a strict oxygen gradient is necessary to support a diverse cell environment, including aerobic intestinal epithelial stem cells and anaerobic bacteria. *In vivo*, oxygen supplied to intestinal cells comes from microvasculature surrounding intestinal crypts. The intestinal lumen remains anoxic, maintaining microbial homeostasis. Recapitulating this environment *in vitro* to simultaneously study anaerobic bacteria and aerobic epithelial cells remains a technical challenge. We hypothesize that a 3D organ-on-chip model of the intestinal epithelium can recapitulate the steep oxygen gradient within intestinal crypts. Using conventional soft lithography techniques, a microdevice has been created with key components that establish a steep oxygen gradient. The microdevice includes a biomimetic scaffold composed of gelatin and collagen with embedded microvessels that provide oxygen to primary intestinal epithelial stem cells and an anaerobic chamber to facilitate bacterial growth. Using finite element analysis, a COMSOL model demonstrates oxygen diffusion through the scaffold to an epithelial cell layer. The model informs parameters such as optimal microvessel diameter and scaffold thickness necessary for epithelial cell growth, as well as influx of nitrogen and outflow of oxygen required to create the targeted oxygen gradient. The COMSOL model will be experimentally validated using a microdevice with implanted photonic oxygen sensors to monitor an oxygen gradient in real-time. A phosphorescent lifetime detector will be used to monitor oxygen tension during controlled perfusion experiments. Optimization of a 3D organ-on-chip model that contains a physiologically relevant oxygen gradient will inform studies concerning intestinal stem cell differentiation and epithelial-endothelial cell dynamics.

Scientific Session 5: Matrix Biology

Tuesday, December 5, 2017, 10:00 AM - 11:30 AM

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Antimicrobial Peptides with Biologic and Biosynthetic Scaffolds for Regenerative Medicine

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Naturally occurring antimicrobial peptides(AMP) are an important endogenous component of the innate immune response, providing protection against pathogens(1). The AMP cathelicidin-LL37 has a broad-spectrum bactericidal activity, promotes cell infiltration and re-epithelialization(2,3), and has been recognized as a pro-angiogenic factor(4), all of which are important in tissue repair and potential regenerative medicine applications. The degradation products (i.e.

matricryptic peptides) of biologic (extracellular matrix(ECM)-derived) and biosynthetic (byproducts of microorganisms) scaffolds modulate macrophage response, promote constructive tissue remodeling(5,6) and provide resistance to persistent bacterial contamination(7). The present study evaluated the expression of AMP by macrophages exposed to ECM-scaffolds or biosynthetic materials, and the molecular mechanisms promoting the cathelicidin-LL37 expression. Murine bone marrow-derived macrophages were exposed *in-vitro* to degradation products of ECM-bioscaffolds from different tissue sources, or the biosynthetic material poly-4-hydroxybutyrate. In a separate *in-vivo* study, a rat bilateral partial thickness abdominal wall defect model was used to evaluate the host response to the implanted scaffold materials at 3, 7, 14, and 35 days. The expression of AMP by macrophages was determined by qPCR and immunolabeling. Without exception, biologic and biosynthetic scaffolds significantly promoted the expression of cathelicidin-LL37 in exposed macrophages by mechanisms that involve activation of the MAP-kinase pathway. The results expand the understanding of the role of cathelicidin-LL37 in regenerative medicine. The already recognized ability of biologic and biosynthetic scaffolds can be expanded to include the induction of AMP expression by infiltrating cells. These findings provide a plausible explanation for the relative infection resistance of biologic and biosynthetic scaffolds.

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Il-33 As a Key Signaling Molecule for the Therapeutic Effects of ECM Bioscaffolds for Cardiac Repair

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ECM-scaffolds are FDA approved for numerous clinical applications including cardiac repair. Although a Phase I study investigating the use of an intracardiac injection of ECM hydrogel following myocardial infarction is currently in progress, the mechanisms by which ECM directs cardiac tissue remodeling are only partially understood. A new perspective on the bioactivity of ECM-scaffolds is presented herein; specifically, that matrix bound nanovesicles (MBV) embedded within ECM-scaffolds are a rich source of extra-nuclear interleukin-33 (IL-33). IL-33 is typically found in the nucleus of stromal cells and generally regarded as an alarmin to alert the immune system to cell injury, resulting in production of pro-inflammatory mediators via poorly defined mechanisms involving the IL-33 receptor, ST2. However, emerging evidence suggests that IL-33 may function as a promoter of tissue repair especially in models of cardiovascular disease where IL-33 induction following cardiac stress has been correlated with improved outcomes. We show that IL-33 is stably stored within ECM and protected from inactivation by incorporation into MBV. Results of the present study show that MBV from $IL33^{+/+}$, but not $IL33^{-/-}$ mouse tissues, directs $ST2^{-/-}$ macrophage differentiation into the reparative, pro-remodeling M2 phenotype, and further suggest that MBV-associated IL-33 modulates macrophage activation through a non-canonical ST2-independent pathway. The discovery of IL-33 as an integral component of ECM-MBV provides mechanistic insights into the regulation of immune-driven pathological fibrosis. Furthermore, the use of ECM-scaffolds for cardiac repair can now be examined with new insights that will help guide the design of next generation products, diagnostics and therapeutic applications.

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SMAD2/3 & MAN1 Interactions are Negatively Regulated by Substrate Stiffness

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Activation of matrix-bound TGFβ and its effects on fibroblasts are mediated by extracellular matrix (ECM) stiffness. TGFβ is of paramount importance in diseases of altered mechanics like cancer and fibrosis, but the underlying mechanisms of TGFβ's mechano-sensitivity are not completely understood.

MAN1, an integral, inner nuclear envelope protein sequesters SMAD2/3, TGFβ's signaling partners, inhibiting cytokine signaling. We hypothesized that the contractility stress of cells on stiffer, fibrotic substrates (~20 kPa for fibrotic lung tissue vs. ~2kPa for healthy lung tissue) is transmitted to the nucleus and ultimately to MAN1 through the actin-nuclear LINC complex and MAN1's LEM domain. We hypothesize that MAN1's conformation is disrupted under strain, disrupting interaction with SMAD2/3.

We tested this premise by stably transfecting a luciferase reporter for SMAD expression in human foreskin fibroblasts (HFF). Those were plated on fibronectin coated polyacrylamide gels of different stiffness and exposed to varying concentrations of TGFβ. Luciferase signal increased with substrate stiffness. Moreover, interaction frequency of MAN1 and SMAD, measured by proximity ligation, increases with TGFβ dosing while decreasing with substrate stiffness. shRNA knock down of MAN1 recapitulates the fibrotic phenotype for HFF on soft substrates.

Current studies are investigating isolation of MAN1 from cytoskeleton forces by stably expressing a dominant negative version of KASH and overexpressing the LEM domain.

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Use of Osteogenically Enhanced Mesenchymal Stem Cells and Their Cell-Derived Matrices for Bone Tissue Engineering

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Of the 13 million bone fractures that occur annually in the United States, about 10% fail to repair (Marsh *et al*. 1998). The canonical wingless (cWnt) signaling pathway is critical for healing of bone fractures (Silkstone *et al*. 2008). We have demonstrated that inhibiting peroxisome proliferator-activated receptor gamma activity with GW9662 reduces negative cross-talk of the cWnt pathway, resulting in an osteogenically enhanced human mesenchymal stem cell (OEhMSC) phenotype (Zeitouni *et al*. 2012). OEhMSCs secrete an extracellular matrix (OEhMatrix) that mimics the composition of anabolic bone tissue and strongly enhances human mesenchymal stem cell retention and subsequent bone repair *in vivo* (Zeitouni *et al*. 2012). Proteomic analysis indicated high amounts of collagen types VI and XII in OEhMatrix deposited by GW-treated OEhMSCs in conventional monolayer culture. Thus, we assessed OEhMatrix deposition onto gelatin methacrylate (GelMA) spheres by immunostaining against collagens VI and XII. Fresh OEhMSCs seeded onto decellularized OEhMatrix-coated spheres showed enhanced osteoprotegerin secretion compared to OEhMSCs seeded on uncoated spheres. Combining OEhMSCs with OEhMatrix-coated GelMA spheres resulted in enhanced healing of murine critical-sized calvarial defects, which was comparable to that achieved with a recombinant human bone morphogenetic protein 2 (rhBMP2) gelatin sponge clinical control. We submit that these injectable microspheres accelerate bone repair, indicating their potential as a bone graft for use in minimally invasive surgery for non-loading conditions such as cranial repair and intervertebral cages for spinal fusion.

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In Situ Imaging of Tissue Remodeling with Collagen Hybridizing Peptides

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Collagen, the major structural component of nearly all mammalian tissues, undergoes extensive proteolytic remodeling during developmental states and a variety of life-threatening diseases such as cancer, myocardial infarction, and fibrosis. While degraded collagen could be an important marker of tissue damage, it is difficult to detect and target using conventional tools. Here, we show that a designed peptide (collagen hybridizing peptide: CHP) which specifically hybridizes to the degraded, unfolded collagen chains, can be used to image degraded collagen and inform tissue remodeling activity in various tissues: labeled with 5-FAM and biotin, CHPs enabled direct localization and quantification of collagen degradation in isolated tissues within pathologic states ranging from osteoarthritis and myocardial infarction to glomerulonephritis and pulmonary fibrosis, as well as in normal tissues during developmental programs associated with embryonic bone formation and skin aging. The results indicate the general correlation between the level of collagen remodeling and the amount of denatured collagen in tissue, and show that the CHP probes can be used across species and collagen types, providing a versatile tool for not only pathology and developmental biology research but also histology-based disease diagnosis, staging and therapeutic screening. This study reveals the potential of CHP as a promising targeting moiety for theranostic delivery in many diseases.

[The manuscript of this work is currently under review of *ACS Nano.*]

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Soluble Extracellular Matrix Derived from Cartilage and Stem Cells: Application for Inflammation Suppression and Cartilage Regeneration

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Introduction: Osteoarthritis (OA) often begins with pathologic activation of resident chondrocytes, followed by production of pro-inflammatory factors and other degradative enzymes. Additionally, macrophages migrate and undergo polarization to the proinflammatory state (M1) in OA joint, further aggravating cartilage damage. We have previously shown that water-soluble extracellular matrix from cartilage (cECM) upregulated chondrogenesis of human mesenchymal stem cells (hMSCs), and that from hMSCs (sECM) maintained chondrocyte phenotype during expansion. In this study, we further explored the potential application of cECM and sECM for OA treatment. We hypothesized that cECM and/or sECM would promote cartilage regeneration and suppress inflammation, via modulating M1 macrophage polarization, thus mitigating OA cartilage degeneration.

Methods: Soluble, urea-extracted ECMs from bovine cartilage and hMSCs were prepared according to our published procedure. Interferon-γ and lipopolysaccharide were used to active macrophage (RAW264.7) to M1 state. Cartilage explants were harvested from adult bovine knee joint.

Results: Upon treatment with either cECM or sECM, nitric oxide and pro-inflammatory cytokine production from M1 macrophage significantly decreased. Moreover, with cECM addition, matrix was retained in cartilage explant, indicated by stronger Safranin O staining, suggesting the positive effect of cECM on cartilage regeneration. Co-cultures of M1 macrophages with cartilage explant are being examined for induction of cartilage degeneration, to assess the efficacy of cECM and sECM in improving cartilage health.

Significance: These findings demonstrate that the positive effect of cECM or sECM in suppressing inflammation and promoting the regeneration of cartilage, which may be applicable for OA treatment.

Scientific Session 6: Research Challenges in Stem Cell Culture and Differentiation on Biomaterials targeting for Stem Cell Therapy

Tuesday, December 5, 2017, 1:00 PM - 2:30 PM

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Tissue Development-Mimicking Extracellular Matrices and Scaffolds for Stem Cells Culture

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Extracellular matrices (ECM) surround cells *in vivo* and play important roles in controlling cell functions. Extracellular matrices are tissue-specific and dynamically remodelled during the development of each tissue and organ. To mimic the dynamic change of ECM composition, we have developed a method to prepare development-mimicking ECM by controlling stepwise differentiation of human bone marrow-derived mesenchymal stem cells (MSCs). Matrices derived from undifferentiated cells and cells at early and late stages of osteogenesis and adipogenesis of MSCs were termed as stem cell matrices, osteogenesis early stage matrices, osteogenesis late stage matrices, adipogenesis early stage matrices and adipogenesis late stage matrices, respectively. The stepwise osteogenesis- and adipogenesis-mimicking matrices had different compositions and showed different effects on osteogenic and adipogenic differentiation of MSCs. Osteogenesis early stage matrices were more favorable to osteogenic differentiation, while adipogenesis early stage matrices were more favorable to adipogenic differentiation of MSCs than other matrices. Furthermore, 3D stepwise chondrogenesis-mimicking ECM scaffolds were prepared from MSCs by controlling the stages of chondrogenic differentiation. ECM scaffolds mimicking early stage chondrogenesis facilitated chondrogenic differentiation, while ECM scaffolds mimicking late stage chondrogenesis remarkably inhibited chondrogenic differentiation of MSCs. The biomimetic matrices and scaffolds should be useful for stem cell culture and tissue engineering.

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Proliferation and Differentiation of Human Pluripotent Stem Cells Cultured on Biomaterials having Nanosegments

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Human embryonic stem (ES) and induced stem (iPS) cells have the potential to differentiate into any kind of cell and produce an unlimited cell source for cell therapy. Establishing cultures of human ES and iPS cells in xeno-free conditions is essential for producing clinical-grade cells. We designed oligopeptide-grafted hydrogels with several structures: single chains, single chains with joint segment, dual chains with joint segment, and branched-type chains. Furthermore, oligopeptide sequences used for oligopeptide-grafted hydrogels were selected from the integrin-binding domain of ECM and the glycosaminoglycan-binding domain. The goal of this study was to investigate which oligopeptide-grafted hydrogel molecular design and elasticity favorably supports long-term culture of human ES and iPS cells while maintaining their pluripotency in xeno-free cell culture conditions. We found that the hydrogels grafted with vitronectin-derived oligopeptides having a joint segment or a dual chain, which has a storage modulus of 25 kPa, supported the long-term culture of human ES and iPS cells for over 10 passages. The dual chain and/or joint segment with cell adhesion molecules on the hydrogels facilitated the proliferation and pluripotency of human ES and iPS cells. Our results suggest that hydrogel design and surface density of cell-binding domains are important for facilitating the proliferation of human ES and iPS cells and for maintaining the pluripotency of these cells in xeno-free culture conditions for a long-term. We also discuss the efficiency of hESC differentiation

into cardiomyocytes cultured on biomaterials grafted with several nanosegments.

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Designing an Artificial Pancreas

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Objective: Type 1 Diabetes (T1D) is an autoimmune disease characterized by the destruction of pancreatic β-cells. No protocol has yet been established for a retrievable device that contains pancreatic β-cells differentiated from induced pluripotent stem cells (iPSCs) to treat T1D. We are investigating methods to increase the yield of pancreatic β-cells differentiated from iPSCs and researching the use of hydrophilized, expanded polytetrafluoroethylene (ePTFE) for use in an implantable, retrievable, and replaceable containment device that mitigates the effects of T1D.

Methodology: Human iPSCs were generated from peripheral blood monocytes. Differentiation to the pancreatic progenitor stage was accomplished by using the STEMdiff™ Pancreatic Progenitor kit. Correctly differentiated cells were then tested by performing fluorescence-activated cell sorting (FACS), using a Becton, Dickinson FACSAria™ Fusion system. FACS analysis was performed with FlowJo™ software. The ePTFE membranes were treated with cross-linked polyvinyl alcohol to create a hydrophilic surface modification.

Results and Significance: We successfully completed the STEMdiff™ Pancreatic Progenitor kit protocol and our analysis suggests that approximately 23% of differentiated cells expressed CXCR-4, a key definitive endoderm marker, compared to only 2% of the undifferentiated iPSCs. This demonstrated that iPSCs can be differentiated to an early pancreatic lineage with moderate yield. Improved yields are the focus of future work. After testing three different densities of ePTFE, we found that the highest porosity allowed for the best diffusion of D-glucose, greater than 96%. This demonstrated that glucose can diffuse through ePTFE. These results increase the feasibility of encapsulating differentiated pancreatic β-cells in an ePTFE containment device.

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iPSC Differentiation to Various Lung Cells and their Progenitors and Subsequent Seeding into Decellularized Scaffolds

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Induced pluripotent stem cells (iPSC) can be directed to become any cell in the lungs. During embryogenesis interactions between the endoderm and mesoderm are the precursor to lung bud formation. Such cells and their progeny can be subsequently used to repopulate decellularized lungs in order to generate disease models. iPSCs were separately differentiated toward an alveolar-like phenotype through endoderm and lung bud progenitor intermediaries or to a blood vasculature lateral plate mesoderm (LPM) fate. Cell phenotype was assessed throughout using flow cytometry, immunofluorescent staining and qRT-PCR for characteristic markers. Decellularized rat lung scaffolds were prepared by pressure controlled perfusion of the vasculature with 0.1% SDS following which differentiated iPSCs were implanted via the trachea and cultured in a pressure-controlled physiologic bioreactor. At each stage of differentiation marker expression was >90% including CXCR4 at definitive endoderm, FOXA2 at anterior foregut endoderm (AFE), TTF1 in lung bud progenitors and SPC at the alveolar stage. Cells taken at the AFE stage were able to repopulate the majority of a decellularized lung scaffold, integrating with and attenuating the extracellular matrix whilst also maintaining their marker expression. Cells differentiated to LPM tested >80% for GATA4, PDGFRα and VEGFR2. Our protocol can produce a high purity of cells at any stage of the embryogenesis of alveolar-like cells. Additionally these cells can be used to successfully repopulate the airways of decelluarized lung scaffolds. Currently we are assessing the co-culture of AFE and LPM into their respective compartments to reseed a decellularized lung.

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TGF-β1 Signaling Mediates Vascular Smooth Muscle Differentiation from Human Cardiac Stem Cells on Keratose Hydrogels

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Our published work demonstrated that a majority (∼72%) of human cardiac stem cells (hCSCs) cultured on keratose (KOS) hydrogels expressed the marker of vascular smooth muscle cells (VSMCs), α-smooth muscle actin (α-SMA), compared to ∼5% on standard tissue culture polystyrene plates (TCPS)[1]. The aim of the present study is to investigate the mechanisms underlying the preferred VSMC differentiation of c-kit$^+$ hCSCs on KOS hydrogels. We hypothesize that transforming growth factor-β1 (TGF-β1)[2] plays a critical role in VSMC differentiation on KOS hydrogels. Cells were cultured and differentiated both on TCPS and KOS plates and various biological assays were used to analyze gene and protein expressions. We found that treatment of 5ng/mL recombinant human TGF-β1 (rhTGF-β1) resulted in $64.5 \pm 4.2\%$ α-SMA$^+$ cells on TCPS in KnockOut serum replacement (KSR) medium. ELISA studies indicated that hCSCs produced a significant amount of TGF-β1 on KOS hydrogel (28.3 ± 0.4pg/mL) 28 days post-differentiation compared to pre-differentiation (0.3 ± 1.5pg/mL, $n = 4$, $p < 0.001$). In addition, α-SMA$^+$ cells on KOS hydrogel were significantly reduced from $71.7 \pm 3.7\%$ to $26.8 \pm 5.2\%$ ($n = 5$, $p < 0.01$) after treatment of A83-01, a TGF-β1 antagonist. These findings suggest that TGF-β1 signaling pathway likely mediates VSMC differentiation on KOS hydrogels.

References:

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Disclosures: The authors declare no conflict of interest.

Scientific Session 6: Tissue Chips and Tissue Organoid Models 2

Tuesday, December 5, 2017, 1:00 PM - 2:30 PM

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Synthetic Development of Cerebrovascular Organoids

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When clusters of pluripotent stem cells (PSCs) are differentiated into a neural lineage in 3D culture, they undergo a process of self-assembly, developing into complex cerebral organoids. These organoids can serve as powerful *in vitro* models of neurodevelopment and disease. However, existing cerebral organoid models are avascular, and, lacking adequate nutrient perfusion, develop necrotic cores once their diameter exceeds approximately 1 mm. The co-differentiation of PSCs into tissues containing both vascular and cerebral cells poses a challenge, as these tissues derive from the mesoderm and ectoderm, respectively, and thus require distinct differentiation conditions. Here, we present a synthetic approach by genomically modifying human induced-PSCs to enable simultaneous, efficient, programmable, and

rapid differentiation into vascular and cerebral lineages producing neurovascular tissues with tailorable densities of vasculature and neural cells. When these modified PSCs are cultured in three-dimensional culture, they develop into three-dimensional cerebrovascular organoids, exhibiting, ventricle-like structures, neural differentiation, and lumenized endothelium. Next, we implant these cerebrovascular tissues into 3D printed organoid perfusion-chips, wherein the organoids are fully embedded in collagen adjacent to perfusable, endothelial cell-lined channels. Via directed perfusion of nutrients through the vascular network, we aim to enhance the viability, size, and complexity of developing cerebral organoids with applications in developmental and drug screening.

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Engineering Hydrogels to Form Liver Organoids that Support Hepatocyte Phenotype and Function

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Liver function depends on healthy hepatocytes, their interactions with non-parenchymal cells (ECs, pericytes etc), and signals from the environment (ECM, vasculature, etc.) We have previously shown that endothelial cells (ECs) and pericytes co-cultured in cell-adhesive and proteolytically degradable poly(ethylene glycol) (PEG) hydrogels form stable microvascular networks.[2] Here, we co-cultured hepatocytes with ECs and pericytes in these modified PEG hydrogels to form vascularized liver organoids. Hepatocyte presence did not inhibit EC tubulogenesis and over 75% of hepatocytes localized to EC tubules, as observed *in vivo*. Hepatocytes in vascularized gels exhibited increased albumin secretion and maintained both urea synthesis and cytochrome P450 activity for at least 28 days. At day 28, hepatocytes in 3D co-cultures secreted significantly more albumin than hepatocytes seeded on collagen coated plates at day 1. The 3D co-cultures secreted significantly higher amounts of albumin than hepatocyte monocultures encapsulated in PEG hydrogels. The 3D co-cultures also secreted significantly more albumin than hepatocytes co-cultured with ECs/pericytes on 2D collagen coated plates and hepatocytes fed with conditioned media from EC/pericyte co-cultures. These results indicate that both the 3D hydrogel environment and cell-cell mediated interactions are important in maintaining hepatocyte function. Co-culture in 3D hydrogels enables robust hepatocyte-hepatocyte interactions as well as hepatocyte to non-parenchymal cell interactions that are not possible in 2D and seem to slow hepatocyte de-differentiation. Our liver tissue-like organoids create a biomimetic environment that is promising for maintaining prolonged hepatocyte function for use in models and in therapeutic devices.

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Mimicry of Neuroinflammatory Environment in Alzheimer's Disease and Discovery of Microglial Modulation in Neutrophil Recruitment to Central Nervous System

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The accumulation of immune cells in brain parenchyma is a critical step in the progression of neuroinflammatory diseases, including Alzheimer's disease (AD). The accumulation mechanisms of innate immune cells, microglia, are well studied in the context of AD pathogenesis, however, the mechanisms of peripheral immune cells are not clear, yet. Here, we reconstituted an AD microenvironment: induction of neuroinflammation by stimulating human microglia with amyloid-beta (A-beta), a signature molecule in AD, and employed this environment to investigate the recruitment mechanism of human peripheral immune cells - neutrophil - in the context of the innate-peripheral immunity crosstalk. We cultured human microglia in a central microchamber with a treatment of soluble A-beta at the concentration of 2μM for 4 days and plated freshly isolated human neutrophils to an outer chamber connected with neutrophil-exclusive migration channels. The neutrophil recruitment was assessed by measuring the speed, persistence of recruitment, and counting the number of accumulated neutrophils. We found that microglia-derived IL6, IL8, CCL2, CCL3/4, and CCL5 are effective for recruiting neutrophils in the recapitulated AD environment and observed the significantly reduced neutrophil recruitment by neutralizing antibodies for IL6, IL8, and CCL2. The increased proinflammtory factors such as IL6 are observed on recruited-neutrophil with microglia co-culture condition in central chamber. We envision that our discovery provides new insights to understand the innate-peripheral immune cell interactions in human AD brains and strategies to modulate AD neuroinflammation for the therapeutic purpose.

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Multi-tissue Interactions In An Integrated Three-tissue Organ-on-a-chip Platform

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Unfortunately, many drugs have progressed through preclinical and clinical trials, after which they existed on the market for years in some cases, before being recalled by the FDA for toxicity in humans. We have developed a multi-tissue body-on-a-chip platform that operates under a common media in a single microfluidic perfusion system for drug and toxicology screening. To date, liver, cardiac, lung, vascular, testes, gut, and brain organoids have been incorporated into the system. The organoids remain viable and functional for at least one month, with most types remaining functional for much longer. The platform has been validated in multi-organoid toxicology experiments that model integrated metabolism and downstream metabolite effects across several tissue types. As an example, recovery of cardiac sensitivity to epinephrine in the presence propranolol may be observed as the beta blocker is metabolized by the liver organoids. Another example involves bleomycin induced lung inflammation where the injured lung produces high levels of IL-1 beta that decreased cardiac organoid beat frequency. These types of responses would be impossible to detect in a single tissue platform. The multi-organoid platform has been used to predict toxicity in FDA withdrawn drugs, demonstrate toxicity of environmental pollutants and model the effects of chemical warfare agents. The future vision for the platform is a comprehensive representation of human physiology on a single microfluidic chip, with the ultimate goal of outperforming both 2D human cell culture and animal testing for predicting compound toxicity and developing countermeasures for toxic agents.

Scientific Session 6: Unlocking the Potential of Gelatin and Collagen in TERM

Tuesday, December 5, 2017, 1:00 PM - 2:30 PM

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Potential Of Gelatin In Tissue Engineering To Realize Regenerative Medicine

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Regenerative medicine is a new therapeutic trial based on the natural potential of body itself. The natural potential is physiologically regulated by the ability of cells for proliferation and differentiation. The basic idea of tissue engineering is to artificially create a local environment which enables cells to promote their ability, resulting in cell-induced tissue repairing of regenerative medicine. If a key growth factor is supplied to the right place at the right time period and concentration, the body system will initiate the natural induction

of cell-based tissue regeneration. One practically possible way to enhance the *in vivo* therapeutic efficacy of growth factors and chemokines is to make use of drug delivery system (DDS) technology. Biodegradable hydrogels of gelatin for the controlled release of biological factors have experimentally and clinically succeeded in the regeneration therapy of various tissues. This release system can be combined with cells or/and the cell scaffold to promote the therapeutic efficacy of tissue regeneration. In this paper, several concrete experimental data on promoted tissue regeneration are presented to emphasize scientific, clinical, and industrial potentials of gelatin in tissue engineering to realize regenerative medicine.

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Development of Highly Purified Medical Gelatin Called Bematrix.

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Due to its high biocompatibility and biodegradability, gelatin has been widely used in medical field applications such as DDS, medical device and regenerative medicine including cell transplantation (1, 2). When gelatin is applied in clinical applications, it is important to remove, reduce or inactivate endotoxin, viruses, bacteria and fungi. In general, those issues can be resolved by methods such as high temperature processing and/or filtration. However, these techniques cannot be effectively used for gelatin purification as heat treatment can alter the physical properties of gelatin and filtration is often not possible due to the high viscosity of gelatin solutions. A new approach using an Alkali solution treatment has allowed us to develop a highly purified gelatin we call ''beMatrix''. This purified gelatin resulted in an endotoxin level lower than 10 EU/g and viral inactivation was confirmed. Using the alkali technique, the physical properties of gelatin were also confirmed to be unchanged. In addition, safety tests including cytotoxicity, sensitization, intracutaneous reactivity and pyrogenicity were carried out. The beMatrix gelatin was found to be negative for each test suggesting it is safe for clinical applications. Having many uses in medical applications, beMatrix has the potential to rapidly expand gelatin-based regenerative therapy. (1) Young S, Wong M, Tabata Y and Mikos AG. J Control Release 109(1-3), 256, 2005(2) Kimoto M, Shima N, Yamaguchi M, Hiraoka Y, Amano S and Yamagami S. Invest Ophthalmol Vis Sci. 55(4), 2337, 2014

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Tissue Engineering Technology of bFGF Release for Various Plastic Surgical Reconstructions

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Cartilage tissue is characterized by its poor regenerative properties, and the clinical performance of cartilage grafts to replace cartilage defects has been unsatisfactory. Recently, cartilage regeneration with mature chondrocytes and stem cell have been developed and applied in clinical settings. However, there are challenges with the use of mature chondrocytes and stem cells for tissue regeneration, including high cost associated with the standard stem cell isolation methods and decreased cell viability due to cell manipulation. Previous studies demonstrated that cartilage can be regenerated from chondrocyte clusters that contain stem cells. Based upon some of the existing techniques, the goal of the present study was to develop a novel and practical method to induce cartilage regeneration. A micro-slicer device was developed to process cartilage tissues into micron-size cartilage (micro-cartilage) in a minimally-invasive manner. We evaluated the size of micro-cartilages and demonstrated that 100-400 μm micro-cartilage are optimal for generating high cell yield with collagenase digestion. In addition, autologous intra-fascial implantation of the composites of micro-cartilage and absorbable scaffold with a slow release system of basic fibroblast growth factor (bFGF) was carried out to induce cartilage regeneration. Our results demonstrated that the extent of bFGF diffusion depends on the size of micro-cartilage, and that cartilage regeneration was induced most effectively with 100 μm micro-cartilage via SOX5 up-regulation. The technique was applicable in the field of Plastic and Reconstructive Surgery and some of our clinical cases for ear and eye socket reconstructions are to be demonstrated.

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New Transportation System of 3D Tissue Constructs with Newly Developed Gelatin

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A new type of gelatin known as HTg has been recently developed. This specialized gelatin is characterized by having an increased gelling temperature and the ability to form gels more quickly than standard commercialized gelatin (1). HTg has the potential to be used extensively in fields such as DDS, cell culture, organ-on-a-chip and cell transplantation. Recently some remarkable 3D tissue constructs including cell sheet, spheroid or organoid have been developed and are expected to be widely used in regenerative medicine. Transportation of these cellular 3D constructs has proven to be very challenging. During transportation, damage to cellular 3D constructs from shear stress, vibration and environmental change can affect cellular function or break apart the complicated structures. To address this issue, we propose a new transport system of 3D constructs using HTg. This process includes the introduction of warmed HTg solution onto cells, setting of cells under 30°C to achieve HTg gel protection, transport of tissue constructs without temperature control, and finally the dissolution of HTg gel allowing for collection of cells at 37°C. In addition, HTg gel is stable through transportation even around 30°C allowing for safe transport of cells without any damage. Here, Cell-sheet of NIH3T3 cells could be collected by the cell viability greater than 90% seven days after gel protection. This result demonstrated that our proposed method using HTg is very beneficial to the safe transport of 3D tissue constructs.

(1) Ohyabu *et al*. JBB 118(1), 112. 2014.

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Hand-spun Gelatin Micro and Nanofibers for Cartilage Regeneration

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Micro/nanofibers are promising for engineering tissues including muscle, bones, and cartilages. Being an inexpensive biopolymer and a collagen derivative, gelatin is a biomaterial of choice for tissue-engineering fibers. However, existing methods to fabricate micro/nanofibers, e.g. wet-spinning and electrospinning, are difficult to use for gelatin, as these methods demand a narrow range of material properties including conductivity, viscosity and surface-tension, and often have an extremely low production rate for sub-micron size fibers.

Method: We invented a 'hand-spinning' technique to produce gelatin based micro- and nanofibers by a large, clinical-relevant quantity. Gelatin fibers were produced through repeated stretching and folding a polymer ring that encapsulated a core of gelatin. After N cycles of stretching and folding, the core of micro/nano fibers were retrieved from the polymer ring in organic solvents, methacrylated, aldehyde fixed, and freeze-dried for storage. To form a fibrous scaffold, the fibers were rehydrated, mixed with cells, and cross-linked by light (365 nm).

Chiondrogensis study: Human mesenchymal stem cells (hMSC) were encapsulated among stretched-and-folded fibers with diameters of 5 μm or 50 μm, and cultured in standard chondrogenic media four weeks. Results showed the microfiber-based scaffolds led to uniform cell distribution, cartilage-like extracellular matrix formation, and dramatically increased mechanical property. The results also suggest the existence of an optimal porosity for cartilage formation, as hMSC in the 5 μm-fiber scaffolds performed significantly higher level of chondrogenic gene expression and matrix production in comparison with hMSC in the 50 μm-fiber scaffolds.

Scientific Session 6: Bio-inspired Materials for Tissue Regeneration

Tuesday, December 5, 2017, 1:00 PM - 2:30 PM

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Cicada Wings Bioinspired Membranes for Tissue Engineering

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Biomimetic approaches are often used in tissue engineering in order to design optimized new scaffolds. For instance cicada wings have already inspired corneal transplants with antibacterial properties. The surface of wings of Mediterranean cicadas has been characterized by a multiscale approach, as used on *Dynastes hercules* [1], to elaborate biomimetic ECMs from chitosan based micro-/ nanoparticles produced with a homemade ElectroHydroDynamic Atomization (EHDA) apparatus. Thanks to an optimization of the elaboration parameters the morphology, the size and the 3D distribution of the particles have been controlled. The physical and chemical properties of these new scaffolds have been characterized by an optimized multiscale approach.

[1] Roux-Pertus C, Oliviero E, Richard V, Fernandez F, Maillot F, Ferry O, Fleutot S, Mano J.F, Cleymand F, Multiscale characterization of the hierarchical structure of *Dynastes hercules* elytra, Micron, 2017.

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Development and Characterization of Free Standing Biomembranes for Tissue Engineering Applications

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Tissue Engineering (TE) and cell therapy comprise a market for regenerative products that is expected to grow worldwide from $6.9 billion in 2009 to almost $32 billion by 2018 [1]. Advances depend on the availability of specific biomaterials that allow controlling biological systems behaviour [2]. In this context, we developed bioinspired systems from renewable resources with controlled external shape and internal architecture. Different home-made fabrication apparatus have been developed to produce controlled systems which could provide a better environment for the cells to stimulate the regenerative process. Mechanical, physico-chemical and biological characterizations assess the therapeutic potential. This presentation will report our recent advances on free-standing biomembranes such as: 1) Chitosan membranes functionalized in volume by incorporating nanoliposomes based on marin origin lecithin. [3]. 2) Electrospun micro and nanofibers of chitosan and crosslinked chitosan fibers which has been inspired by the hierarchical structure of *Dynastes hercules* elytra [4], and can be seen as new architecture to TE. [1] Tissue Engineering, Cell Therapy and Transplantation: Products, Technologies & Market Opportunities, Worldwide, 2009-2018.'' Report #S520; Med Market Diligence, LLC. [2] R. Langer, D.A. Tirrell, Nature (2004), 428, 487-492. [3] F. Cleymand, H. Zhang, G. Dostert, P. Menu, E. Arab-Tehrany, E. Velot, João F. Mano, RCS Advances, 6, 83626-83637 (2016) [4] C. Roux-Pertus, E. Oliviero, V. Richard, F. Fernandez, F. Maillot, O. Ferry, S. Fleutot, Joao F Mano, and F. Cleymand, Micron http://dx.doi.org/10.1016/j.micron.2017.05.001

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From The Hierarchical Structure Of Dynastes Hercules Elytra To Bioinspired Nanofibers Membranes

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Thanks to millions of years of natural optimization, beetle elytra (hard thickened forewings that protect the fragile hindwings) have evolved to become the model of honeycomb structures and are optimal composite materials of proteins, lipids and chitin. Their structure could provide inspiration for different applications: engineering artificial extracellular matrix with interesting mechanical properties, aeronautics, new sensors with hygrochromic properties··· In this context, the present study focuses on the elaboration of bioinspired nanofibers membranes from the hierarchical structure of beetle forewings. First, a combination of structure and microstructure characterization techniques (SEM, TEM and for the first-time X-Ray tomography) was performed on adult male *Dynastes hercules* for a fine understanding of the 3D structure of elytra[1]. Secondly, based on the determined periodic arrangement over a short distance for trabeculae, nanofibers chitosan scaffolds were produced by electrospinning. Elaboration parameters have been optimized to produce membranes with high spatial interconnectivity, high porosity, and controlled alignment. Finally, physico-chemical properties, cell-scaffold interactions and proliferation have been investigated to demonstrate the potential of home-made structured membranes for tissue engineering applications.

[1] Roux-Pertus, C., Oliviero, E., Richard, V., Fernandez, F., Maillot, F., Ferry, O., Fleutot, S., Mano, J.F., Cleymand, F., Micron, 2017, *accepted*

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Urinary Bladder Matrix Reapproximates Mechanical Strength of Native Abdominal Wall in a Long-Term Porcine Hernia Model

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Scaffolds composed of urinary bladder matrix (UBM), an ECM-based material derived from the basement membrane of the porcine bladder, have been previously shown to support constructive remodeling and site appropriate tissue deposition in many clinical applications. However in instances where continued mechanical strength is desired, the use of a degradable biomaterial may seem counterintuitive. In this study, an 8-month porcine model of a midline abdominal wall defect was utilized to evaluate the remodeling and mechanical strength of UBM scaffolds. These animals were compared to a control group that received the defect but no scaffold material, and an uninjured baseline group (n = 4/group). Histologic evaluation demonstrated that the UBM material was macroscopically degraded within 3 months and was replaced with a loose, vascularized connective tissue. There was no evidence of mineralization, encapsulation, seroma, or herniation at any time point. In contrast, the control group demonstrated a 50% herniation rate at the defect site and an increase in diastasis recti at 8 months post-operatively. Mechanical strength of the excised abdominal wall tissue increased over time for all groups, and by 8 months, there was no statistical difference in strength compared to uninjured control tissue. The loose nature of the connective tissue at the remodeling site allowed for easy dissection to re-access abdominal tissue planes. These long-term results in a large animal model demonstrate that UBM scaffolds facilitated constructive remodeling and resulted in host tissue capable of supporting the native mechanical loading of the anatomical site, despite a relatively rapid remodeling profile.

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Esophageal Extracellular-matrix Hydrogel for Treatment of Barrett's Esophagus in a Canine Model

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Background: Barrett's esophagus(BE) is the only identifiable pre-malignant condition that leads to esophageal adenocarcinoma(EAC). The high mortality of this cancer and the lack of preventative and curative treatment options requires the development of new therapies that target BE and prevent EAC. Extracellular matrix(ECM) derived from normal tissues has been shown to mitigate or reverse neoplastic cell phenotype. We evaluated a hydrogel derived from normal esophageal-ECM(eECM) for treatment of BE in a canine model.

Methods: eECM-hydrogel was produced by pepsin-digestion of decellularized porcine esophageal mucosa. Strength of adhesion of the gel to esophageal mucosa and retention over time was evaluated at different eECM concentrations(8,12,16-mg/mL). Acid reflux was induced surgically in 8 dogs by Heller myotomy, creating a diaphragmatic hernia and daily administration of pentagastrin. After 3 months, pentagastrin was replaced with omeprazole and dogs were treated with 25ml of per-os hydrogel twice daily during 1 month. Endoscopy was performed before and after treatment to evaluate disease course.

Results: Mucoadhesion of eECM-hydrogel to porcine esophageal mucosa increased with hydrogel concentration and showed continued presence after 24 hours of laminar flow *in-vitro*. Labelled hydrogel was detected by endoscopy and shown to coat the lower third of the esophagus 40minutes after oral delivery at 12mg/mL. Biopsies of dog with acid reflux showed intestinal metaplasia in the lower esophagus at 3 months and mitigation of esophagitis from >4 folds to 2 folds after 1 month of treatment.

Conclusion: eECM-hydrogel represent a viable strategy to mitigate and/or reverse esophagitis and Barrett's esophagus potentially by delivering homeostatic and anti-inflammatory microenvironmental signals.

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Matrix-bound Nanovesicles Recapitulate the Effects of ECM Bioscaffolds upon Macrophage Phenotype

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Long-term functional tissue replacement using tissue engineered technologies depends upon an appropriate host immune response. In contrast, dysregulation of the immune response is implicated in many disease processes and in poor tissue remodeling outcomes. Both the native extracellular matrix (ECM) and biomaterials composed of ECM possess the appropriate signaling milieu to facilitate immunomodulation in normal development, homeostasis, wound healing, and in the latter case, functional tissue remodeling. The specific component(s) within the ECM that activate the immune response, however, are only partially understood. Matrix-bound nanovesicles (MBV), a putative bioactive component within the ECM, represent one possible mechanism by which ECM can promote macrophage activation. In the present study, mouse bone marrow derived macrophages were exposed to MBV derived from ECM bioscaffolds or to their parent ECM. Gene and protein expression profiles and functional macrophage assays including phagocytosis and nitric oxide production show that MBV alone recapitulate the effects of ECM bioscaffolds upon macrophage phenotype. Specifically, both ECM and MBV promote a downregulation of pro-inflammatory macrophage markers and an increased expression of pro-remodeling markers. MBV derived from small-intestinal-submucosa (SIS) and urinary-bladder-matrix (UBM) were found to be enriched in miRNA 125b-5p, 143-3p, and 145-5p, inhibition of which within macrophages is associated with a gene and protein expression profile consistent with a pro-inflammatory, rather than pro-remodeling macrophages. Results implicate MBV and their miRNA cargo as a driver of the macrophage response to ECM-bioscaffolds and may have important implications in the regulation of the immune response in tissue homeostasis and normal wound healing.

Scientific Session 7: Vascular Tissue Engineering and Tissue Vascularization

Wednesday, December 6, 2017, 11:15 AM - 12:45 PM

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Application Of A Novel Small Peptide Targeting Endothelial Progenitor Cells For Improved Endothelialization And Patency In Vascular Grafts

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We screened high-throughput one-bead one-compound (OBOC) combinatorial libraries and identified LXW7, a ligand that has high-specific binding affinity for $\alpha v\beta 3$ integrin on the surface of active endothelial progenitor cells (EPCs) and endothelial cells (ECs). Culture surface coated with LXW7 strongly supported the attachment and proliferation of ECs and EPCs, and enhanced the phosphorylation of VEGF receptor 2 and the activation of mitogen-activated protein kinase ERK1/2 in ECs. LXW7 supported limited attachment of platelets and did not support attachment of THP-1 monocytes. To test the *in vivo* function of LXW7, we functionalized small-diameter vascular grafts with LXW7 via CLICK chemistry and investigated its functions on endothelialization and graft patency in a rat carotid artery bypass model. Immunohistological analyses showed that at 1 week after implantation, the LXW7 modified vascular grafts possess significantly more $CD34^+$ EPCs in the middle segment of the luminal surface than the control grafts. At 6 weeks after implantation, mature endothelialization was present throughout the whole length of the LXW7 modified grafts while only a limited number of $CD31^+$ ECs were seen in the middle of the control grafts. Patency testing results confirmed that at 6 week after implantation, 5 out of 6 LXW7-modified vascular grafts remained patent versus in the control group only 1 out of 6 grafts was patent. Collectively, our results have demonstrated that LXW7 specifically supports EPC/EC attachment and functions, and can achieved rapid endothelialization and long-term patency of vascular grafts. LXW7 and their derivatives hold great promise for endothelialization related tissue regeneration applications.

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Compliance-Induced Intimal Hyperplasia using an Ex Vivo Arterial Culture System

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A major roadblock in ''off-the-shelf'' vascular graft development is the potential for arterial re-occlusion due to intimal hyperplasia (IH). A strong empirical correlation exists between graft compliance and long term arterial patency. However, there has not been a definitive study to demonstrate that a synthetic graft with increased compliance would perform similarly to an autograft. Using an *ex vivo* arterial culture assay, the present study examines the effects of increasing graft-host compliance mismatch on the initiation of IH.

Multilayer Vascular Grafts (MLVG) of various compliances were inserted into porcine carotid artery segments via end-to-end anastomosis. The MLVGs were prepared from electrospun polyurethane sleeves with poly(ethylene-glycol) hydrogel luminal linings, the sleeve fabrication conditions varied to achieve grafts of distinct compliance

levels. Autografts and ePTFE grafts were included as high compliance and low compliance clinical controls, respectively. Grafted vessels were cultured within an *ex vivo* bioreactor for 14 days, then collected and prepared for immunostaining with IH markers.

Following 14 days of culture, even smooth muscle cell staining was present at the anastomoses of the autografts and the high compliance MLVG samples. However, as the compliance of the MLVG decreased, isolated patches of abnormally dense smooth muscle cell staining were increasingly observed in the arterial wall. The extent of these regions was similar in low compliance MLVG and ePTFE controls. Cumulative immunostaining data supports the empirical correlation between the degree of graft-host compliance mismatch and the degree of IH and serves as a foundational study for future graft development.

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Self-expandable *In-vivo* Tissue-engineered "biotube" Vascular Grafts: Proof Of Concept In A Growing Beagle Model

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Purpose: Biotube is a cell-free collagenous tube formed in a mold upon its subcutaneous embedding by in body tissue architecture technology (IBTA). In our previous study, in situ vascular reconstraction occurred in collagenous matrix of Biotube as a scaffold within several months after implantation as an arterial graft. It was our long-term dream to confirm growth potential of Biotube after its vascular reconstruction. This study aimed to evaluate the growth potential of Biotubes by angiographic examining their caliber adaptation using a juvenile beagle model for 2 years.

Methods and Results: Biotubes (ID 2, 2.5, 3 mm, length ca. 1 cm) were prepared by embedding molds into subcutaneous pouches for 8 weeks in adult beagles (age 1 year). The obtained allogenic Biotubes were implanted into common carotid arteries (ID ca.2 mm) of juvenile beagles (n = 13, age 11 weeks). In all cases, patency was maintained without aneurysm or stenosis for all observation periods. Vascular reconstruction occurred almost at 1-month and completely at 1-year of implantation. Follow-up angiogram showed that caliber sizes of almost all Biotubes were gradually expanded with growing native vessels without Biotubes with initial ID of 2 mm. In addition, elongation of Biotube length was confirmed in macroscopic observation at 1-year after implantation.

Conclusion: In this beagle model, Biotubes could be expanded and elongated according to the growing native vessels. Size mismatch between artificial vascular grafts and growing patients vessels remains a serious problem in pediatric surgery. The growth potential of Biotubes may have high potential usefulness in pediatric surgery.

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Harnessing the Vasculogenic Properties of Mural Cells to Create Perfuseable, Complex Vascular Networks *In Vitro*

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The absence of perfuseable vasculature remains a critical obstacle to the generation of engineered tissues. Current approaches rely on the use of endothelial cell (EC)-centric mediated angiogenesis. Alternatively, we have discovered that high density, 3D cultures of mesenchymal stem cells (MSCs) and vascular smooth muscle cells (SMCs) can generate complex vascular networks in fibrin hydrogels. The addition of arterial, venous or microvascular ECs contributed to further branching complexity, but was not necessary for mural cell-driven vascular network formation. Interestingly, in the presence of endothelial cells, angiogenic sprouting was led by mural cells. Whole genome profiling revealed that mural cells upregulated transcripts associated with lumen formation, pro-invasive processes, basement membrane formation and angiogenic growth factor expression when cultured under vasculogenic conditions. Utilizing this model, we have generated multi-scalar (mm-μm diameter), multi-phenotype (arterial, venous, microvascular) and dense vascular networks that can occupy 1 cm^3 of fibrin gel. Furthermore, these networks are perfuseable and can accommodate continuous and pulsatile flow for up to 7 days. Lastly, we have demonstrated that flow into the arterial channel of the engineered vascular networks passes through the microvasculature into venous channels, completing a vascular subunit necessary to support parenchymal cell viability and function. In sum, mural cell-driven vascular network formation can be used as a novel strategy to vascularize engineered tissues.

Scientific Session 7: Peripheral Nerve Regeneration-Towards Clinical Translation

Wednesday, December 6, 2017, 11:15 AM - 12:45 PM

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ESWT Accelerates Peripheral Nerve Regeneration by Reducing Schwann cell Phenotype Commitment

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The gold standard in peripheral nerve regeneration is the autologous nerve graft, where a sensory nerve is transplanted to a motor defect site. This treatment however shows inferior results for motor nerves, as the transplanted sensory Schwann cells and nerve channels differ in behaviour and structure. In this study we investigated how Extracorporeal Shockwave treatment (ESWT) improves peripheral nerve regeneration in the clinically relevant femoral nerve model (qPCR, electrophysiology,···) and how it affects on Schwann cell phenotype. *In vitro* model: Schwann cells isolated from motor, sensory and mixed nerves were treated with ESWT and evaluated concerning expression of Schwann cell specific markers (S100b, P75, MAG, P0-FACS) and proliferation in pro-proliferation (PPM)/pro-myelination (PMM) medium. *In vivo* data indicate inferior regeneration of motor axons through a sensory nerve graft. ESWT can ameliorate this effect and accelerate nerve regeneration. Cultured for 10 weeks in PPM all ESWT SCs showed no change in SC marker expression or proliferation, while marker expression and proliferation of control groups steadily decreased. Motor SC cultured in PMM were able to switch to myelination quicker and to a higher extent than sensory SC (MAG, P0), with ESWT both phenotypes showed even stronger myelin-associated protein expression. Summarizing, significant differences in all parameters were observed only between the motor/sensory control group, and ESW treated groups showed improved results *in vitro* and *in vivo*. This study indicates that ESWT is able to accelerate peripheral nerve regeneration by altering SC phenotype in a model which reflects the clinical reality after autologous nerve transplantation.

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Effects of Amniotic Fluid Derived Stem Cells (AFS) on Regeneration of Large-gap Peripheral Nerve Injuries in a rat model

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Acellular nerve allografts (ANA) have been developed to replace autografts for repairing large-gap peripheral nerve injuries. Tissue engineering strategies have attempted to mimic regenerating axons' environment by adding supportive cells other than Schwann cells. We hypothesized that ANAs can be seeded with amniotic fluid-derived stem cells (AFS) to promote and accelerate nerve regeneration. The

presence of the AFS cells provides support for the regenerating axons without the requirement of becoming Schwann cells.

Methods: ANA construct (Group 1), ANA construct with AFS cells (Group 2), or autograft (Group 3) were used to repair a 1.5 cm rat sciatic nerve injury (n = 12 per group). Walking track analysis, electromyography, nerve histology were assessed at 4 months post-injury. Fate of MPIO (micron sized iron oxide) labeled AFS cells were tracked by MRI and Prussian blue staining.

Results: Gait analysis showed group 2 had significantly better recoveries in overlap distance, paw angle degree, paw drag, stance width, axis distance and SFI compared with group1. ($P < 0.05$) Group 2 also demonstrated greater gastrocnemius CAMP ratio, sciatic axon diameter, fiber diameter, myelin thickness, G ratio and NMJ numbers compared to group 1 ($P < 0.01$). Group 2 showed no significant difference of motor recovery from group 3. MRI demonstrated that AFS cells appeared as hypointense region at 4 weeks post-surgery, which was confirmed by iron staining.

Conclusions: AFS cells may be a suitable cell source to replace Schwann cells to support and accelerate peripheral nerve regeneration following large gap nerve injury.

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Nerve Regeneration Using Lysophosphatidylcholine and Multiple Treatments of Nerve Growth Factor

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Peripheral nerve damage affects hundreds of thousands of people every year. This study tested the effectiveness of using lysophosphatidylcholine (LPC) in combination with multiple injections of nerve growth factor (NGF) to increase the healing rate of damaged left sciatic nerves in female rats using a sciatic nerve crush model. A previous study with this model found no significant difference between rats treated with a combination of LPC and NGF and rats treated with only NGF. Due to the short half-life of NGF, we concluded that a single treatment was likely insufficient to have a significant effect. Therefore, we used three separate treatments of NGF to stimulate nerve growth. The rats were randomly divided into eight groups (n > 6 per group): Crush, Crush + NGF (with 1, 2, or 3 injections), Crush + LPC, and Crush + LPC + NGF (with 1, 2, or 3 injections). The healing of the nerves was measured by monitoring electrophysiological parameters (compound muscle action potential amplitudes and nerve conductance velocities) weekly, and morphological parameters (total fascicular area, total myelinated fiber counts, fiber densities, fiber diameters, and g-ratio) after sacrifice at 6 weeks. After six weeks, we found that rats receiving 2 treatments of NGF (with and without LPC) appeared to have better recovery than rats receiving 3 treatments of NGF, but were not statistically significantly different from each other. Further research will investigate this treatment combination in transection injuries as well as other treatments for damaged peripheral nerves.

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Improved Method for Streptozotocin-induced Diabetic Peripheral Neuropathy in Murine Models

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The high prevalence of diabetes mellitus with associated comorbidities mandates the development of appropriate models for studying the etiology, symptoms, and potential treatments for diabetic peripheral neuropathy (DPN). Streptozotocin (STZ)-induced rats may be used to successfully model the progression of nerve degeneration from DPN, however high mortality can occur without providing insulin. Administration of insulin after diabetic induction more accurately models treatment of diabetes in humans and may play a role in decreased mortality due to STZ side effects. In this study, rat nerve function was assessed after providing insulin in STZ-induced diabetic rats using three sensory tests: applied force reaction time, infrared reaction time, and digit spread. The force results and time results were analyzed separately. Analysis of male vs. female, diabetic vs. non-diabetic rats was also performed. The male diabetic rats showed test results that were significantly less than the non-diabetic control rats in IR data ($p = 0.0138$), force data ($p = 0.0079$), and reaction time data ($p = 0.0115$). The female diabetic rats also had results that were significantly less than non-diabetic rats in IR data ($p < 0.001$) and reaction time data ($p = 0.0408$), but not significantly different with the force data ($p = 0.0761$). Digit spread produced insignificant results throughout testing. Mortality was decreased by 27%. Considering all diabetic rats vs. all non-diabetic rats, both tests showed a statistically significant decrease. By decreasing mortality and successfully evaluating nerve function, this study supports its use as an appropriate model for studying peripheral diabetic neuropathy.

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Biodegradable Conduits for Peripheral Nerve Injuries Modeled in Primates

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The current standard of care for peripheral nerve injuries is autografting, which unfortunately results in donor site morbidity and loss of function. In patients with multiple nerve injuries, obtaining adequate nerve tissue can be challenging. A promising alternative to autografting is an off-the-self biodegradable poly(caprolactone) nerve conduit embedded with glial cell line-derived neurotrophic factor (GDNF).

A 5-cm defect was created on the median nerve of non-human primates (NHPs). The gap was repaired with an autograft, decellularized human allograft, PCL conduit with GDNF, or an empty PCL conduit. Precision pinch was assessed using a modified Klüver board at baseline through 1-year. Percent correct pinch retrieval was recorded. Intraoperative electrophysiology was used to record compound nerve action potentials, nerve conduction velocity, and motor evoked potentials. Histological analysis was used to determine neurofilament and Schwann cell density. A biopsy of the abductor pollicis brevis (APB) muscle, which is innervated exclusively by the median nerve, was taken at 1-year post-procedure and stained to assess muscle atrophy.

The PCL/GDNF and autograft treatment groups trended towards improved functional return (60%) compared to the PCL empty group (34%) at 1 year compared to baseline. The decellularized group had higher neurofilament density than PCL/GDNF group. Nerve conduction was present in the APB of the PCL/GDNF and decellularized nerve groups suggesting the nerve was able to regenerate across the 5-cm gap and reinnervate the APB. Future work will focus on clinical translation of this research.

Scientific Session 8: Stem Cell Models for Predicting Developmental Toxicity

Wednesday, December 6, 2017, 1:30 PM - 3:00 PM

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Development of High Throughput Platforms for Generating Cardiomyocytes from Mouse Embryonic Stem Cells

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Stem cells hold great potential as an unlimited source of cardiomyocytes (CMs) for cell-based therapy of heart disease, drug screening, and toxicity testing. However, generation of CMs in large quantities and high purity remains both technologically challenging and labor-intensive. To this end, we have developed a simple, robust 96-well Eppendorf Tube Array (96w-ETA) protocol, and we compared it with an alginate-based micro-encapsulation method under the same differentiation conditions (*i.e.,* containing BMP-4, Activin-A, bFGF, and Apelin[1]). Using the 96w-ETA method, $3x10^4$ mouse embryonic stem cells (mESCs) were added to each tube, incubated for 5 days to form $\sim$640 μm embryonic bodies (EBs), and subsequently transferred to regular culture dishes with differentiation medium. Within 7-14 days after plating, we observed $\sim$99% beating EBs. Although this method is more efficient and cost-effective than the conventional Hanging-Drop method[2], it still requires manual replating of EBs and the removal of non-CMs from the resultant EBs. At present, we are examining the microencapsulation-based CM differentiation from single mESCs, which we expect will result in a higher purity of CMs and eliminate the manual step of replating. Ultimately, we anticipate that both approaches can be streamlined into high throughput platforms through the incorporation of automated robotic technologies.

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Modular Crispr-Cas9 Ribonucleoprotein Complexes For Precise Gene Editing Of Human Cells

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Writing specific DNA sequences into the human genome is challenging with nonviral gene-editing reagents, since most of the edited sequences contain various imprecise insertions or deletions. We developed a modular RNA aptamer-streptavidin strategy, termed S1mplex, to complex CRISPR-Cas9 ribonucleoproteins with a nucleic acid donor template, as well as other biotinylated molecules such as quantum dots. In human pluripotent stem cells, tailored S1mplexes increased the ratio of precisely-edited to imprecisely-edited alleles up to 18-fold higher than standard gene-editing methods and enriched cell populations containing multiplexed precise edits up to 42-fold. By exploiting the versatility of the S1mplex, assembled fluorescent labels can be used for the selection of defined sequence edits at two distinct loci, resulting in up to 10 precise edits for every imprecise edit. This work sharpens the CRISPR scalpels used in genome surgery. All components of the strategy can be chemically synthesized or recombinantly produced: thus, this approach has strong potential to generate off-the-shelf, preassembled reagents that can be customizable for any patient. 44,750 disease-associated single nucleotide or indel mutations (1-50 nucleotides in length) in the ClinVar database can be corrected, in principle, by precise editing with our S1mplex approach. These advances with versatile, preassembled reagents could greatly reduce the time and cost of *in vitro*/ex vivo gene editing applications with human cells, including pluripotent stem cells, in precision medicine and drug discovery.

Scientific Session 8: Therapeutic Applications of Biomaterials for Guided Tissue Regeneration

Wednesday, December 6, 2017, 1:30 PM - 3:00 PM

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Deterministic Single-Cell Biomaterial Encapsulation for Cell Therapy

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Intravenous infusion of stem cells, through promising preclinically in a variety of disease and injury contexts, is hampered by the short residence times of injected cells. Here, a microfluidic system was developed for encapsulation of mesenchymal stem cells (MSC) on a single-cell level in a non-Poisson determined manner. Encapsulated MSCs exhibited high viability and the capacity for trilineage differentiation. Moreover, the encapsulating alginate microgels were found to form thin, 6-um average thick layers that exhibited tunable mechanical and compositional properties. Encapsulation of MSCs into alginate significantly increased their residence time upon intravenous infusion, and manipulation of biomaterial properties was found to influence the residence time, in part by changing cell morphology. Prolonged residence time was accompanied by increases in both engineered and native secreted factors. Finally, the efficacy of encapsulated MSCs was tested in the context of bone marrow transplantation in a syngeneic murine transplant model. Infusion of encapsulated MSCs was found to accompany expedited hematopoietic reconstitution, compared to the infusion of unencapsulated MSCs and bone marrow alone. These results suggest that single-cell encapsulation of cells into biomaterial scaffolds may be an effective strategy for improving outcomes of cell infusion.

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Biomaterial Scaffolds for Recapitulating Niche Interactions for T-cell Development

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Introduction: Immune deficiency after hematopoietic stem cell transplantation (HSCT) limits its applicability as a treatment for life-threatening blood disorders. We hypothesized that the development of T-cells can be promoted by using a biomaterial-based scaffold that which specifies the T-cell lineage program in progenitor heamtopoeitic cells through the Notch-Delta pathway.

Methodology: A biomaterial comprised of alginate with tethered notch ligand Delta like ligand-4 (DLL-4) and encapsulated bone morphogenetic protein-2 (BMP-2) was polymerized into a porous hydrogel. Wild-type C57BL/6 and NSG mice with implanted human tissue (BLT mice) were transplanted with syngeneic whole bone marrow cells and human $CD34^+$ cells respectively, along with simultaneous subcutaneous hydrogel injection in the dorsal flank. Histology, flow cytometry and RNA sequencing were used to assess T-cell reconstitution.

Results: The hydrogel concentrated transplanted cells and induced a bony nodule ($\sim 90\,mm^3$). Within the hydrogel, CLPs derived from transplanted cells selectively expanded. In C57BL/6 mice, the hydrogel accelerated reconstitution of T-cells, restored thymic output, and diversified the T-cell receptor. The reconstituted T-cells conferred protection against a pathogen challenge. In BLT mice, the hydrogel accelerated human T-cell reconstitution by up to 33%, slowed onset of graft-versus-host disease and prolonged survival.

Significance: The results indicate that a programmable biomaterial scaffold with biological cues can recapitulate some niche interactions to drive immune reconstitution. By providing niche cues for T-lymphopoiesis, the reconstitution of immune competent T-cells can be accelerated after HSCT, to potentially reduce associated immunological complications.

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Fabrication of 3D-Printed, Bidirectional Growth Factor Gradient Scaffolds for Osteochondral Tissue Repair

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Cartilage damage, due to injury and osteoarthritis, is among the most common causes of disability among adults in the United States. Cartilage defects, which have limited endogenous capacity for self-repair, remain a challenge for tissue engineering, as they are highly complex despite being relatively avascular. Previous strategies have been limited

by the inability to produce mechanically sufficient articular cartilage as well as failure to mimic the complex interplay between cartilage and subchondral bone found in native tissues. Past work in our laboratory has shown that the development of heterogeneous scaffolds that address the complexity of the osteochondral unit may offer some solutions to these issues. In the present work, bilayered composite scaffolds using a porous poly(ε-caprolactone) (PCL) backbone were fabricated *via* a novel multi-material three-dimensional printing (3DP) system and used to deliver gelatin microparticle(GMP)-loaded growth factors (GFs) in a spatially controlled manner. The release of TGF-β and IGF-1, which have been shown to enhance osteochondral tissue formation *in vitro*, from GMPs is well-characterized, and these GFs were selected for use in these 3DP constructs. Uniform and bidirectional release of encapsulated growth factors was characterized using scaffolds of varying pore architectures, and the scaffold design and GF loading were optimized for use in planned future *ex vivo* and *in vivo* experiments.

Acknowledgment: This work was supported by the NIH (P41 EB023833).

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Controlled Sequential Release of Growth Factors by Cell-Free Multi-layered Scaffold for Functional Recoveries of Sciatic Nerves

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Through controlled release of growth factors by multi-layered scaffolds, regeneration of sciatic nerves has been promoted. The scaffold consists of three layers, where upper layer is fabricated by polycaprolactone (PCL) aligned electrospun nanofibers for attachment and differentiation of cells towards the direction of sciatic nerve. The middle layer is fabricated by poly (lactide-co-glycolic acid) 6535 (PLGA 6535), which consists of 65% polylactic acid (PLA) and 35% polyglycolic acid (PGA), and the last layer is fabricated by PLGA 8515 consisting of 85% PLA and 15% PGA. PLGA is known for having different degradation rates depending on the ratio of PLA and PGA. The PLGA layers act as reservoirs for release of growth factors; neurotrophin-3 (NT-3), brain-derived neurotrophic factor (BDNF), and platelet-derived growth factor (PDGF). In rat model, the injured nerve, which was rolled up by the growth factors laden and multi-layered scaffold, was recovered after 5 weeks from the time of surgery. The initially released NT-3 and BDNF from PLGA 6535 have especially promoted regeneration of the neural tissue, and the sustainably released PDGF from PLGA 8515 further enhanced the regeneration procedure to replace the function of hind limb locomotor activity. In addition, an implication of aligned PCL layer was crucial in functional recoveries of sciatic nerves. This study demonstrated that the multi-layered scaffold has performed a function that can be used to promote the locomotor activity and to enhance nerve regeneration in combination with aligned topography of fibrous scaffold and controlled release of growth factors.

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Graphene Oxide Incorporated Nanofibrous Scaffold for Solid Phase-Mediated Gene Delivery into Mesenchymal Stem Cells

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Introduction: Delivery of functional genes into stem cells via shows great application prospect in DNA-based tissue engineering. Graphene oxide (GO) with ultrahigh surface area, ultra-strong adsorption capability and good biocompatibility, has received increasing interests as a non-viral gene nanovector. To enhance the transfection efficiency of stem cells, polyethylenimine (PEI)/plasmid DNA (pDNA)- GO/poly(D, L-lactic-co-glycolic acid) (PLGA) nanofibrous scaffold was developed as a substrate for solid phase-mediated gene delivery and tissue engineering application.

Methods: Human mesenchymal stem cells (hMSCs) were obtained with IRB approval. GO was synthesized by modified Hummers method. PLGA nanofibers were prepared using a protocol developed in our laboratory. pDNA was immobilized on the scaffold via physical interactions.

Results: With the presence of GO, the nanofibers (GO/PLGA) showed an enhancement of gene immobilization, and supported long-term release of pDNA. hMSCs cultured on PEI/pDNA-GO/PLGA scaffold showed significantly higher green fluorescent protein expression than the nanofibrous scaffold without GO (PEI/pDNA-PLGA) or the scaffolds without gene immobilization (GO/PLGA and PLGA nanofibers with adding PEI/pDNA in the culture medium).

Conclusions: These findings demonstrate that solid phase-mediated gene delivery using PEI/pDNA-GO/PLGA significantly enhanced the gene transfection efficiency, and its potential utility in tissue engineering.

Acknowledgments: We acknowledge the financial supports from US Dept. of Defense, NSFJS (No. BK20150363, to He Shen) and NSFC (No. 81501074, to He Shen).

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Fibrous Scaffolds Coated with Exosomes for Immune-mediated Tissue Regeneration

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The immune system has been identified as a key mediator in tissue regeneration. Both innate and adaptive immune responses participate in tissue repair through tuning the angiogenesis, fibrosis and the local stem cell behaviors. The immune response towards *in vivo* implanted materials is inevitable and variable with the characteristics of materials, thus could be utilized to modulate the immune signaling to create a pro-regenerative environment. Mesenchymal stem cells (MSCs) provide promising repair/regenerative modalities in many diseases through their secretome. Especially, MSCs secreted exosomes exhibited the functions of immune modulation by targeting a broad spectrum of immune cells. Here, we developed cell-free scaffolds coated with exosomes as a new regenerative system with immune modulatory properties. The scaffolds with varied physical properties were designed to attract immune cells. The exosomes collected from adipose tissue derived MSCs were incorporated in the scaffolds with the purpose to educate immune cells to facilitate tissue regeneration. The result showed that electrospun fibrous scaffolds covalently modified with cationic polyetherimide could efficiently be loaded with exosomes through electrostatic interactions. The scaffolds-exosome constructs promoted the polarization of macrophage cells towards the pro-regenerative phenotype compared to the blank scaffolds *in vitro*. The exosome-loaded scaffolds will be further tested in wound healing models to provide insights into biomaterial design for immune-mediated regeneration.

Scientific Session 8: Tissue Engineering Approaches for Musculoskeletal Defects Session 2

Wednesday, December 6, 2017, 1:30 PM - 3:00 PM

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Combined Drug and Electrical Stimulation Synergistically Increase Function of Engineered Human Skeletal Muscle

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Exercise is critical to maintenance of muscle function and mass and is an effective intervention against multiple chronic diseases including obesity, type II diabetes, and sarcopenia. *In vitro* models of exercised human muscle have the potential for discovery and testing of new therapeutic targets for metabolic and muscle wasting disorders. Here we applied intermittent electrical stimulation (ES) for 7 days to 3D

tissue engineered human skeletal muscle[1] (myobundles). ES alone significantly increased (3-5 fold) contractile force due in part to myotube hypertrophy, hyperplasia, and improved sarcomeric organization. ES also increased metabolic flux as evidenced by increased glucose uptake, lactate buildup, and medium-chain acylcarnitine accumulation. To further increase force generation, we treated myobundles with creatine (CR) or the β2-agonist salmeterol (SAL) which have both been shown to improve exercise performance. ES and drug treatment induced significant and additive force increases compared to control (CTL: 1.1 ± 0.18mN, CTL+ES:3.7 ± 0.6mN, CR: 3.2 ± 0.5mN, CR+ES:7.6 ± 0.9mN, SAL: 3.5 ± 0.6mN, SAL+ES:7.9 ± 0.8mN). Myotube diameter significantly increased with both drug treatment and ES (CTL: 8.5 ± 0.5μM, CTL+ES:12.1 ± 0.7μM, CR: 11.3 ± 0.8μM, CR+ES:16.4 ± 1.0μM, SAL: 9.5 ± 0.84μM, SAL+ES: 17.3 ± 1.1μM). This system can be used to study exercise-related effects on muscle structure and function and identify new compounds able to enhance muscle performance.

Reference: 1. Madden L, *et al*. eLife 4:e04885 2015.

Acknowledgments: NIH grants UH3-TR000505 and AR065873.

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Regeneration and Osteointegration of the Anterior Cruciate Ligament with a Silk Fiber based Scaffold

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Tissue Engineering in ACL injuries has gained increasing research interest. To allow ACL regeneration, a scaffold which provides the mechanical basis, cells from different sources, and mechanical as well as biological factors are needed. The optimal scaffold for ACL regeneration is regarded to be biocompatible and biodegradable to allow tissue ingrowth, but needs to have the right mechanical properties to provide immediate mechanical stability.

Hypothesis: A degradable silk-fiber scaffold with mechanical properties similar to the ACL is able to initiate ligament regeneration and osteointegration after ACL resection under *in-vivo* conditions.

Methods: Thirty-three mountain sheep underwent ACL resection and randomization to two groups: 1) ACL reconstruction with scaffold alone (SA), 2) ACL reconstruction with cell-seeded scaffold (CS). Histological evaluation of the regenerated ligament was performed after six and twelve months. Bone histology was performed to assess osteointegration.

Results: After six months, connective tissue surrounded the silk scaffold with ingrowth in some areas. The cell seeded scaffolds had significant lower silk content and higher content of newly formed tissue. After twelve months, the density of the silk fibers decreased significantly, and the ingrowth of formed tissue increased in both groups. No differences between the two groups regarding the silk fiber degradation as well as regenerated tissue were detected anymore. Bone histology revealed good osteointegration after 12 months.

Conclusions: The silk-fiber based scaffold stimulated ACL regeneration as well as osteointegration under *in-vivo* conditions. Additional cell seeding lead to increased tissue regeneration after 6 months, whereas these differences diminished after twelve months.

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Tissue-Engineered Fascia Based on Mesh Implants for Pelvic Prolapses

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Genital prolapse is an extremely common female health condition which occurs in more than 300 million women worldwide. Severe degrees of prolapse require surgical treatment which can lead to complications. We designed tissue-engineered constructs (TECs) based on mesh implants from polypropylene, vicryl and porcine dermal collagen (Permacol) with seeded autologous rats dermal fibroblasts and bone marrow mesenchymal stem cells. Constructs and control meshes were implanted into rats and tissue reactions and collagen production were assessed. In pilot study at 2.5 and 4 months after implantation there was increasing connective tissue production forming tissue-engineered fascia and there was minimal inflammatory response in TECs groups. The most cytocompatible material was Permacol considering the number of cells and the formation of a full cell monolayer covering the entire surface of the implant. Bone marrow mesenchymal stem cells can be used to improve the biocompatibility of implanted mesh for the surgical treatment of pelvic organ prolapse.

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Osteogenic, Chondrogenic, and Angiogenic Gene Expression of Tissue-Engineered Flaps Grown in an *In Vivo* Ovine Bioreactor

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In order to generate vascularized flaps appropriate for mandibular reconstruction, we have devised a strategy in which *in vivo* bioreactors can be 3D-printed to the shape of a specific defect, filled with acellular autologous or synthetic scaffold material, and then implanted against the periosteum of the rib in order to generate custom vascularized bony tissues suitable for mandibular repair. While this strategy has previously been demonstrated to be effective in an ovine model of disease (Tatara *et al*, *Acta Biomaterialia* 2016), the cell populations that migrate within the bioreactors have not been extensively characterized. In this study, tissues grown within bioreactors filled with morcellized autologous bone or 85% beta-tricalcium phosphate/15% hydroxyapatite synthetic biphasic ceramic graft after nine weeks of implantation against periosteum in sheep (n = 9 per scaffold) were harvested from the side 1) nearest and 2) most distal to the periosteum (source of migrating cells). Genetic expression of early and late markers of osteogenesis, angiogenesis, and chondrogenesis (*Runx2, Osteocalcin, BMP2, BMP7, bFGF, VEGF, Collagen II,* and *Sox9*) were evaluated and compared as a function of scaffold and location within the bioreactor by RT-qPCR using native ovine periosteum and skin as controls. In addition, genetic expression was correlated with radiologic and histologic data from the tissue specimens. Briefly, scaffold type did not significantly change tissue gene expression (nor did it result in final tissue radiographic or histologic differences). However, preliminary data suggest that cells within the bioreactor display disparate expression profiles dependent upon their proximity to the periosteum.

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Electrospun Fibrin Nanofiber Construct for Regenerating Vascularized Skeletal Muscle in Volumetric Muscle Loss

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Tissue engineering has unprecedented potential to create a clinical treatment for volumetric muscle loss (VML) since the standard of care, an autologous muscle flap, is limited by tissue availability and donor site morbidity. Previous approaches to VML have achieved some regeneration but lack the myofiber and vascular density of native muscle.

Here we present a novel technique to substantially regenerate a murine VML defect with electrospun fibrin scaffolds that mimic the alignment and 10-15 kPa stiffness of native muscle, are suturable, and offer scale-up capabilities. C2C12 myoblasts seeded at 7.7×10^6 cells/ml and cultured for 7 days on the scaffolds formed aligned, multinucleated, and striated myotubes and demonstrate electromechanical coupling with measurable force of contraction. To test the regenerative capacity of engineered grafts murine VML defects were created by resecting ~30% of the tibialis anterior (TA) and 4 grafts were bundled and sutured into each defect site. C2C12-seeded scaffolds enabled remarkable regeneration with negligible fibrosis after 2 and 4 weeks. The defect sites were densely populated with myofibers positive for myosin heavy chain, embryonic myosin, and laminin as well as dense, highly organized vascular networks as in native muscle. Acellular scaffolds did not result in similar regeneration. Preliminary data also demonstrates that engineered grafts provide functional improvements over untreated VML defects and approach the performance of native TA muscle. In summary, the electrospun fibrin microfibers support the formation of functional muscle grafts and robust regeneration of VML defects, and have the potential to advance the development of improved treatments for VML.

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Repair Large Articular Cartilage Defect Using 3D Bioprinted Micro-Cartilages through a Minimally Invasive Approach

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Articular cartilage has very limited capability of self-healing and osteoarthritis is the leading cause of disability. 3D live cell bioprinting is an emerging technology which has strong potential to regenerate damaged tissues. Previously, we have successfully bioprinted micro cartilages using our novel 3D bioprinting system and accomplished the implantation to the cartilage defect through a minimally invasive approach. In the current study, we further validated the clinical feasibility of the bioprinted micro-cartilage using a large articular cartilage defect model, which approximates the defect size that is normally observed clinically. The bioprinted micro cartilages show standard morphology and biochemical and molecular profiles comparable to the native cartilage. The micro-cartilages were successfully implanted arthroscopically and completely covered the cartilage defect. An orthopaedic surgeon can accomplish the implantation of the bioprinted micro-cartilages using the standard arthroscopic surgical techniques. Only a few days post implantation, the implanted micro-cartilages show effective adhesion to the surrounding cartilage and subchondral bone remaining in place even in the present of shear force. The self-assembly of the micro cartilages started hours after implantation and assembled cartilage shows continuous growth and mature. Histology and immunohistology results show good integration of the bioprinted cartilages to the surrounding cartilage and subchondral bone. The results from this study demonstrate that our bioprinted micro-cartilage is promising for the clinical application.

Scientific Session 8: Skin Regeneration and Immunomodulation

Wednesday, December 6, 2017, 1:30 PM - 3:00 PM

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Glycosaminoglycan-based Hydrogels To Control Pro-inflammatory Chemokines And Rescue Wound Healing Deficiency

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Excessive production of inflammatory chemokines can cause chronic inflammation and thus impair the cutaneous wound healing process. Accordingly, capturing such chemokine signals may stop chronic inflammatory processes and thus become a powerful treatment option for chronic wounds. Here, a modular hydrogel based on derivatives of the glycosaminoglycan (GAG) heparin and star-shaped poly(ethylene glycol) (starPEG) was customized for maximal chemokine sequestration based on the GAG's natural ability to bind and sequester a wide range of cytokines. The resulting scavenging characteristics of the materials were compared in binding assays using recombinant chemokines MCP-1 and IL-8, inflammatory conditioned media and wound exudates of human patients suffering from chronic venous leg ulcers, respectively. The effects of hydrogel-based chemokine sequestration were investigate in a murine model of delayed cutaneous wound healing (db/db mice) to evaluate the overall pro-regenerative effect of the starPEG-GAG wound dressings. The developed material effectively scavenged the inflammatory chemokines MCP-1, IL-8, MIP-1a and MIP-1ß from conditioned medium and wound fluids from patients with chronic venous leg ulcers and to reduce the migratory activity of human monocytes and neutrophils. In an *in vivo* model of delayed wound healing (db/db mice) starPEG-GAG hydrogels were able to reduce inflammation, as well as promote the formation of granulation tissue, angiogenesis and wound closure. Wound dressings formed from GAG-based gels were demonstrated to act as an efficient 'molecular sink', neutralizing high amounts of chemokines thereby preventing further recruitment of immune cells to ultimately resolve the inflammatory process.

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Macrophage Infiltration into Macro-porous Electrospun Scaffolds Initiates a Reparative Inflammatory Response

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A recurring problem encountered with electrospun matrices is the restricted penetration of cells into the interior of such fibrous scaffolds. This limitation has been attributed to the density of the ensuing electrospun mesh and lack of pores large enough to allow cell infiltration. Upon *in vivo* implantation, scaffolds with large pores will be populated by infiltrating cells, including macrophages, whereas they accumulate adjacent to the surface facing scaffolds with small pores. The inability of macrophages to infiltrate the scaffold leads to their fusion and eventually fibrotic encapsulation of implanted scaffolds. In this study, we developed a unique macro-porous, cell permeable, soy-protein-based electrospun scaffold as an immunomodulative structure and compared cell penetration *in vitro* into the macro-porous scaffolds to that into conventional (microporous) scaffold electrospun from the same material. We then implanted both types of scaffolds subcutaneously in a rat model and evaluated differential influx and perseverance of macrophages as indicators of the severity of *in vivo* inflammation. Our *in vitro* results demonstrated enhanced penetration of macrophages into the macro-porous scaffold compared to zero penetration into control traditionally spun scaffolds. Our *in vivo* results suggest that infiltration of macrophages into macro-porous implants plays a pivotal role in modulating the inflammatory response of the host. In extending our prior work on the use on electrospun soy scaffolds as bioactive wound dressings, we are currently carrying out experiments in a rat model of delayed wound healing comparing re-epithelialization and dermal tissue regeneration in full thickness excisional wounds treated with macro-porous and control scaffolds.

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Allogeneic Graft Tolerance without Immunosuppression via Engineered Immunomodulatory Signal Presentation

D. Headen[1], K. Woodward[2], J. Weaver[1], **M. M. Coronel**[1], p. shrestha[2], W. Bowen[2], C. Johnson[1], L. Shea[3], E. Yolcu[4], A. Garcia[1], H. Shirwan[2];

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Type 1 Diabetes (T1D) is an autoimmune disease in which autoreactive T cells target and destroy the insulin-producing beta cells in the pancreas. Clinical islet transplantation (CIT), a cell-based therapy has been an attractive approach to establish physiological insulin production in T1D patients. Whereas initial success in restoring metabolic control has been attained, long-term insulin independence (>3 years) has only been achieved in a small subset of patients in part due to the progressive graft loss associated with the allo and auto immune responses to the transplanted graft. We have developed synthetic surface functionalized microparticles for the localized presentation of SA-FasL, an immunomodulatory signal, to abrogate T-cell responses. Herein, we sought to evaluate the effect of these microparticles on islet function *in vitro* as well as graft acceptance and tolerance in an animal model of diabetes. For this purpose, SA-FasL functionalized microparticles (SA-FasL-PEG) were co-culture with pancreatic islets for 24 hrs. Metabolic activity, live/dead and functional assessments demonstrated no difference between islets co-culture with or without SA-FasL-PEG particles, validating the cytocompatibility of our platform. Significantly, allo-transplantation of SA-FasL-PEG particles with islets in the kidney capsule lead to sustained diabetes reversal (> 6 weeks) without the need for long-term immunosuppression. Contrariwise, control islets were promptly rejected within two weeks post-transplantation. In conclusion, we have engineered a platform for the controlled loading, presentation, and retention of SA-FasL protein within the graft microenvironment for immunomodulation. Thus, this technology has the potential to heighten the performance of allografts without the need of systemic immunosuppression.

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In Vitro Platform for Characterizing Immunological Responses to Encapsulated Cells

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Alginate encapsulation has been extensively studied as an approach to protect transplanted cells from host immune recognition and destruction. Despite widespread use, the mechanisms by which alginate encapsulation affects host immune responses to transplanted cells remain unknown. While it is suspected that alginate encapsulation blocks contact-dependent direct antigen recognition, it remains unclear if indirect recognition occurs, whereby shed donor antigen is presented and recognized by the host. To explore these mechanisms, we generated an *in vitro* platform capable of delineating direct and indirect recognition processes using ovalbumin (OVA) as a model antigen. Cells (splenocytes) isolated from membrane-bound OVA (mOVA) mice were used as stimulators and co-cultured with antigen-specific responders (OTI). Proliferation and activation of the clonally-specific OTI $CD8^+$ T cells were quantified via flow cytometry. Co-culture of OTI splenocytes with encapsulated mOVA cells resulted in robust $CD8^+$ T cell proliferation and activation, indicating the presence of strong indirect recognition. To parse out the roles of responder cell populations, OTI $CD8^+$ T cells were purified and co-cultured with unencapsulated or encapsulated mOVA cells either alone or with supplemental antigen presenting cells (APCs). Purified $CD8^+$ T cells responded to unencapsulated mOVA cells but were not activated in the presence of encapsulated mOVA cells, unless supplemented with APCs, lending further credence to the indirect recognition hypothesis. This work demonstrates the impacts of alginate encapsulation on host immune responses, and the ability for adapting this model to an efficient immunological screening platform for additional encapsulation approaches.

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Functionalization of Immunomodulatory IL-10 to PDMS Porous Surfaces Attenuates Inflammatory Macrophage Responses

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Inflammatory responses can impart detrimental effects to tissue engineered grafts. In the context of islet transplantation, significant islet loss is attributed to early inflammatory events and islet apoptotic stress. Macrophage infiltration and their subsequent phenotype plays a dominant role in early inflammatory progression. The biomaterial properties of the implant, such as surface chemistry, porosity, and topography, can influence macrophage polarization towards an M1 (inflammatory) or M2 (anti-inflammatory) phenotype at the transplant site. Herein, we sought to 1) understand the impact of surface topography and porosity of a PDMS (polydimethylsiloxane) scaffold on macrophage phenotype; and 2) examine the capacity to direct the resulting macrophage phenotypic response towards M2 via the surface functionalization of IL-10, a potent anti-inflammatory cytokine. Bone marrow-derived macrophages were seeded and cultured on the 2-D PDMS porous surfaces and characterized *in vitro*. Cytokine and gene analysis of macrophages after culture exhibited a unique pro-inflammatory M1-inclined phenotype, with elevated production of pro-inflammatory cytokines (IL-6, TNFα, MCP-1, and MIP-1α) and M1-associated genes (*Il-1b*, *Il-6*, *Cox2* and *Nos2*). PDMS porous surfaces where then functionalized with IL-10 via thiol-maleimide chemistry, as verified via XPS. IL-10-functionalized PDMS porous surfaces significantly reduced macrophage production of pro-inflammatory cytokines, such as IL-6 and TNFα, despite no changes in overall macrophage presence or viability. This study demonstrates that the surface functionalization of porous surfaces with IL-10 provides a promising approach to modulate macrophage responses *in situ*.

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Fabrication of Vascularized Skin Tissue Grafts using 3D Bioprinting Technology

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Skin microvasculature not only promotes graft survival by supplying skin cells with oxygen and nutrients but is also known to modulate inflammation and immune cell migration to the wound site. Vascularization of skin grafts is crucial for successful skin engraftment. Here, using a 3D bioprinting platform, we show successful integration of a vascularized bed in a 3D printed human skin model. In addition, we show the influence of different cell sources (fibroblasts, endothelial cells and pericytes) in controlling the formation of vasculature. We have further investigated the inclusion of CRISPR/Cas9-edited endothelial progenitor cells in 3D bioprinted vascularized skin grafts to evade immune response. We anticipate that this approach will reduce the capacity of human endothelial cells to initiate rejection events in human organ transplants. Moreover, we hypothesize that the pre-vascularized skin construct will enable faster integration with the host skin, and accelerated recovery of blood perfusion to the wound site to promote healing. These advances will allow the fabrication of human skin tissues suitable for clinical translation.

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Volume 23, Supplement 1, 2017

Poster Abstracts

Poster Session 1

Sunday, December 3, 2017, 6:00 PM - 8:00 PM

Biobanking & Cryopreservation

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Mechanisms of Xenon Effect on Skin and Red Blood Cells

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The usage of Xenon (Xe) is known in anesthesia and biobanking areas. It is considered preservation effect of Xe is associated either with clathrate formation - solid gaseous structures or dissolution of Xe molecules in liquid phase without physical state modification (so-called hyperbarium) [1]. This study is addressed to establish differences between hyberbarium or clathrate Xe actions as well as its applications on various bioobjects with anaerobic - red blood cells (RBCs) and aerobic (skin fragments) metabolism. Xe clathrates and hyperbarium storage were simulated under 277 K and 620-725 kPa and 300-350 kPa, respectively. RBCs were divided at three groups: clathrates, hyperbarium and CPDA-1 as control and stored for 30 days. It was demonstrated that the hemolysis index in clathrate group (29.65 ± 3.71, CI95%) was significantly higher than in hyberbarium (17.81 ± 2.75, CI95%) as compared to control (15.68 ± 1.11, CI95%). Skin fragments were harvested from rat tails and divided on hyberbarium, clathrate and dimetylsulfoxide cryopreserved as control group and stored for 7 days. Assessment was performed by point-score method including epidermal-dermal integrity various assays and engraftment index. It was shown that scores in clathrate group (7.7 ± 0.7, CI95%) was significantly higher than in hyperbarium (5.3 ± 2.2, CI95%) and control (6.0 ± 1.7, CI95%). The results highlighted the differences between anaerobic and aerobic metabolism. The advantages of either gaseous (hyperbarium) or solid Xe state (clathrates) for tissue and RBCs have been clarified.

Reference: 1. Rodin V, Ponomarev A, *et al*. Xenon-water interaction in bacterial suspensions as studied by NMR. International journal of biochemistry and biophysics 5(1), 26-36, 2017.

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Deciduous Dental Pulp Stem Cells Cryopreservation

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Current strategies for tissue engineering depend on autologous cells and in this context Deciduous Dental Pulp Stem Cells (DDPSC) are great promise as an alternative source of cells for treating damaged tissue. All children will lose their deciduous teeth and this make them a non-invasive source of stem cells. The multipotent capacity of DDPSC open new avenues for researchers to start a cryopreservation of DDPSC start-up company named R-Crio and located in Campinas, São Paulo, Brazil. Our Aim is to describe how DDPSC can be collect and cryopreserved by a start-up company respecting the International Society for Cell Therapy recommendations.

Methods: The R-Crio company has registered dentists and pediatric dentists who receive their specific training to perform the tooth extraction. When the tooth arrives at the company after checking the patient 's registry data record and their pre-collection blood tests the tooth will be send to the Good Practice of Manufacturing (GMP) laboratory which has the required operating Brazilian licenses at ANVISA, as well as, it has the Federal Council of Dentistry registry. Using specific process developed by R-Crio Company to isolate and to characterize DDPSC this cells are cryopreserved (4 cryotubes with 10^6 DDPSC) in liquid nitrogen.

Conclusion: to deliver for society DDPSC cryopreserved, in GMP conditions, open new avenues for the use of these cells in tissue engineering and cell therapies.

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Cryopreserving Tissue Engineered Constructs With Dmso-free Cryoprotectants: Toward Clinical Solutions For Regenerative Medicine

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Cryopreservation of composite tissues and organs is a critical need in regenerative medicine. While bioengineered tissues and organs may help alleviate issues arising due to organ and tissue shortage, they cannot support more than minimal cell losses due to cryopreservation in order to maintain full function. As the search for clinically safer cryoprotectants (CPAs) that are devoid of DMSO progresses, so does our understanding of the biophysical cues that direct the cells to respond differently to the presence of different stabilizers and cryoprotectants. This makes developing cryopreservation media reliant on understanding the cryoprotectant candidates' effects on cells' and tissues' biophysical and mechanical properties. Work in our laboratories has focused on developing novel DMSO-free formulations based on combinations of natural biomolecules, amino acids, carbohydrates and antioxidants, tailored for a range of cell types in order to cryopreserve regenerative medicine products: from fragile immune cells to more complex tissue constructs and composites. Here we present recent work from our lab on developing solutions that specifically focus on structures that are used in regenerative medicine from cell therapy to tissue transplantation. These solutions address the shortcomings associated with traditional, largely DMSO-based cryoprotectants, and highlight factors such as freezing procedure, cryoprotectant loading and type on optimal recovery of cells post-thaw. Viability is measured alongside functionality to determine cryoprotectant performance and eliminate temporary, acute effects that mask longer term CPA performance. The impetus behind this line of work is to generate clinically-relevant tissue preservation solutions to advance the safety and efficacy of tissue engineered therapy.

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Generation Of Fertility In Klinefelter Patients: Describing Model Of A Multidisciplinary Program

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Introduction: Klinefelter Syndrome (KS) is the most common genetic disorder affecting 1/660 newborn males with dramatic loss of spermatogonial stem cells (SSC) following the onset of puberty.

Material and Methods: To establish a multidisciplinary referral program to offer clinical-experimental fertility preservation options

to KS patients. Newly diagnosed KS patients at any age are referred by either pediatrician or medical geneticist to urology clinic. Each patient is enrolled in a long term follow-up to monitor his endocrine profile, pubertal development and testicular structure to detect early fibrosis with Elastography-Ultrasound. At Tanner stage III or higher, one step fertility preservation is offered, including semen collection, microsurgical testicular sperm extraction (micro-TESE) and SSC cryopreservation. The extracted sperm is stored in a clinical setting for future IVF/ICSI and testicular tissue containing SSCs is stored in experimental testicular tissue bank for future use for *in vitro* or *in vivo* spermatogenesis (auto-transplantation).

Result: December-2014 to May-2017, 17 patients have been enrolled. Three patients (11, 13 and 17 years-old; none-mosaic XXYY, XXY and XXY respectively) went through electroejaculation or self-collection with successful semen collection. No sperm found. Micro-TESE was performed in both testes and no testicular sperm were found by an embryologist presented in the operating room. A biopsy from each testis was stored to preserve SSCs. Diagnostic pathology confirmed the absence of testicular sperms and presence of spermatogonia in <10% of tubules in all patients**.**

Conclusion: We have established an effective, comprehensive and safe multidisciplinary team approach for potential early fertility preservation in Klinefelter patients.

Biofabrication and Bioreactors

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Fabrication Of Tissue-Engineered Constructs For Bile Duct Reconstruction

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Iatrogenic bile duct injury is still a challenge for surgeons. Up to 2.7-0.05% of patients are require to reconstructive bile duct surgery after cholecystectomy. The standard surgery includes suturing of the duct with small intestine, but this reconstruction can lead to various complications. No one method was proposed as the best option for the repair and native reconstruction of common bile duct, though the use of bioengineered materials and methods is keeping an experimental surgical research. We have constructed a multilayered tube consisting of three synthetic polymers layers and two types of cells: bone marrow mesenchymal stem cells (MSCs) and bile duct epitheliocytes. All polymers were tested for biocompatibility with the use of two cell lines: fibroblasts 3T3 and epitheliocytes MCF7 cells. The inner layer was formed with bile duct epitheliocytes and PCL modified by EGF during a electrospinning fabrication, which allows to stimulate a proliferations of epitheliocytes. The medium layer was formed by PCL thin film. The outer layer of the tube included a copolymer PCL/PLGA (70:30) with seeded MSCs. The copolymer was modified with VEGF165 plasmid (Neovasculgen^{TM}), which increase of angiogenesis on 70% after subcutaneous implantation in rats. The construct is biodegradable in various model mediums: deionized water, phosphate buffer, bile, full culture medium. The next step is pre-clinical trials on rabbits and minipigs for assessment of implantation safety and efficacy. We suppose that this tubular multilayered tissue-engineered construct will be capable of integration after implantation and may be used to reconstruct damaged bile ducts.

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Drug Release Evaluation from Dexamethasone-Loaded PCLA Scaffold fabricated by 3D Printer

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For effective drug delivery, well-controlled and desired release period are very important in medicine treatment to cure disease because it could induce appropriate duration of remedial effect and reduction of side effects. In this study, we examined effect for melting points on drug release of drug-loaded scaffold. We synthesized poly(ε-caprolactone-ran-lactide) (PCLA) copolymers with varying compositions of various monomer rations. The melting points were measure by differential scanning calorimetry (DSC) and PCLA copolymers melting points decreased as the LA content increased. Dexamethasone (Dex)-loaded PCLA scaffold were prepared by solid freeform fabrication and the drug loading efficiency of scaffolds were above 90%. The *in vitro* and *in vivo* drug release behavior of Dexa-loaded PCLA scaffold showed similar sustained release profile to 30 days after initial burst of Dexa. The *in vitro* and *in vivo* order of the Dex release was good agreement with the melting point order of the drug carrier. Scanning electron microscopy (SEM) and optical image were used to examine the morphology of the *in vivo* Dexa-loaded PCLA scaffolds. We confirmed that the possibility of the control of Dexa release using Dexa-loaded PCLA scaffold with different melting points.

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The Effects of Cell Density on the Printability of Collagen Bioinks for Cartilage Bioprinting

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A major advantage of bioprinting is the ability to incorporate cells into the hydrogel bioink being printed allowing for precise control over cell placement within a printed construct. We have previously found that the shape fidelity of constructs printed with collagen bioinks can be predicted by the storage modulus of the collagen before gelation. The effect of cell density on collagen rheological properties and shape fidelity has not been assessed. Therefore, the objective of this study was to determine the effects of incorporating cells on the rheology and printability of collagen bioinks. We incorporated primary chondrocytes (0-100x10^6 cells/mL corresponding to 0-20% volume fraction) into 8 mg/mL collagen bioinks and measured the storage modulus before, during, and after gelation and measured the footprint area of dots printed using a commercial 3D printer, a surrogate measure of bioink shape fidelity. We found that the addition of cells had no effect on the storage modulus of the collagen before gelation. However, the storage modulus after gelation and the rate of gelation decreased with increasing cell density. The addition of cells was found to have no effect on the shape fidelity of collagen bioinks with no differences found in the footprint areas of printed dots. These findings show that the addition of cells decreases gel mechanics and gelation kinetics but does not impair the printability of collagen bioinks suggesting that cartilage constructs can be printed using chondrocyte-laden collagen bioinks with the same degree of geometric accuracy as those printed using acellular collagen bioinks.

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Large-scale Preparation Of Hair Follicle Germs Through Self-organization Of Epidermal And Mesenchymal Cells

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Regenerative medicine has been increasingly expected as a new approach for the treatment of hair loss. Recent studies have shown that transplantation of a compartmentalized hair follicle germ (HFG), which was fabricated by integrating two respective aggregates of mesenchymal and epithelial cells, led regeneration of hair follicles. This approach is excellent, but one drawback is to require labor steps for the preparation of the germs particularly considering that hundreds of thousands of hair follicles are necessary for a single patient. In this study, we propose a robust approach for a large-scale preparation of HFGs through self-organization of cells. We revealed that

when the two types of hair cells, mouse epidermal and mouse/human mesenchymal cells, were mixed in suspension and seeded in a microwell they initially formed a randomly-distributed single cell aggregate but then spatially separated each other and exhibited typical morphological features of HFG in three days of culture. The HFGs efficiently generated hair follicles and hair shafts after intracutaneously transplanted in the back of nude mice. This finding facilitated a large-scale preparation of compartmentalized HFGs (~5,000 HFGs). We fabricated a microwell array culture device with oxygen-permeable silicone and demonstrated that oxygen supply through the bottom of the silicone device was crucial for the spontaneous formation of HFGs and subsequent hair shaft generation. This simple HFG preparation may provide a practical and robust approach for hair regenerative medicine.

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Evaluation of Bi-layer 3D Printed-Scaffold in Osteochondral Regeneration

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Osteochondral defects usually caused by trauma or disease, with damage of both articular cartilage and underlying subchondral bone. In order to repair osteochondral defects, the needs of the bone and cartilage must be approached together. The combination of biodegradable materials and ceramic in a bi-layered composite scaffold is promising in this area. Three-dimensional (3D) bioprinting technology is considered have great potential and broad applications in tissue engineering, it is enabled printing of a multitude of biocompatible materials and geometric structures. In this study, we used water-based light-cured polyurethane (PU) and calcium silicate (CS) to fabricate a bi-layered 3D printed-scaffold and evaluated the ability of osteochondral repair. To investigate the potential of the PU or PU/CS scaffold for regeneration of osteochondral tissue, the 3D printing cartilage (PU) and bone (PU/CS) mono-scaffolds were analysis with cell viability, degradation rate, mechanical properties, Alcian blue staining, Alizarin red staining, and ALP analysis. The 3D printing PU or PU/CS scaffold seeded with human Wharton's jelly mesenchymal stem cells (hWJMSCs), the SEM and fluorescent images results showed an excellent cytocompatibility. Regarding *in vitro* bioactivity, the different content PU/CS scaffolds were soaked in simulated body fluid, the scaffold surface was cover abundant apatite spherulites after immersion for 28 days. In summary, the PU/CS integrated bi-layered 3D printed-scaffolds might have potential in osteochondral tissue engineering.

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A 3D-Printed Multi-Channel Conduit for Peripheral Nerve Repair

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Although the gold standard for the surgical treatment of peripheral nerve injury, the autograft is associated with many drawbacks, including a second surgical procedure, donor site morbidity, mismatch of donor nerve size, and limited donor nerve length. In this study, we developed a liquid resin preparation process of water-based polyurethane (PU) based photosensitive materials applied with polydopamine (PDA) combined extracellular matrix (ECM) with application of the materials for 3D printed nerve conduits as an alternative to the autograft. Nerve guidance conduits may be used to promote neuronal growth and guide axonal extension after nerve injury. The 3D printing conduit cultured with human Wharton's jelly mesenchymal stem cells (hWJMSCs), the SEM and fluorescent images results indicated an excellent cytocompatibility. The 3d-printed conduits not only supported the attachment, proliferation and survival of the seeded hWJMSCs but also up-regulated the expression of neurogenesis-related proteins *in vitro*. In addition, 10 mm sciatic nerve transection rat model was used to evaluate the efficacy of the conduit up to 8 weeks after nerve transection and conduit implantation. Finally, the regeneration assessment was examined electrophysiological activity, functional and histological assessments to compared with commercialize silicon conduit in this study. Consequently, the resulting PDA/ECM promotes macrophage migration at injury sites to re-construct microenvironments and thus facilitates nerve regeneration. This study proves the 3D-printed PU nerve conduit that has potentially clinical application in promoting the neurorrhaphy.

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Voxelated Tissue Engineering with 3D Bioprinted Auxetic Metamaterial for Controlled Patterning of Multiple Cell Types

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Bioprinting is one of the most promising solutions to global shortages of tissues and organs for transplant. The integration of additive manufacturing technologies remarkably expanded the design space for the scaffold. However, not many application cases have exploited and demonstrated the full potential of additive manufacturing. In this study, we demonstrate a novel scaffold design which employs the latest advances in 3D printed auxetic metamaterials. The concept of this innovation is that it places the target tissue into interconnected, submillimeter-scale voxels that consists of cell-laden thermosensitive hydrogel with any desired cell types. Pluronic F-127 is used as the support material during printing and is removed by soaking the final build in serum-free medium at 4 °C after the build is made. The cleaned 3D printed object has carefully designed voids inside it, which serve as channels for circulation of cell cultural medium. The dimensions of the channels are controllable by adjusting the overall volume of the auxetic construct, which can be utilized to facilitate a microfluidic circulation throughout the construct during cell growth. The auxetic metamaterial design provides a new way to pattern different cell types and regulate nutrient/waste exchange while the cells populate, both of which are critical challenges in engineering large solid tissues. Furthermore, this voxelated concept for tissue engineering and its associated setup can also be a platform for sophisticated control strategies that aim to 1) accelerate tissue regeneration, 2) minimize variation in cells in different locations, 3) achieve cell self-alignment, and 4) enhance tissue's mechanical properties.

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Manufacturing of Artificial Tissues using Hybrid Biomaterials and a Low-Cost Three Dimensional Printer

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Every 10 mins, someone is waiting for a tissue or organ transplant as there is no abundance of tissues Bioprinters are a great solution, but on average cost $95,000 with some successful prints as most use one biomaterial. Herewith, we present a novel bioprinter system by taking a $600 industrial printer (RepRap Prusa i3) and modifying it to include microvalves, a pressure-regulated system for dispensing, and a water-

jacket bound incubation chamber. All of these components maintain the integrity and homeostasis of the cells and their environment and are controlled by the printer's central processing unit via Arduino. Though the printer sets a high advantage, the biomatrix developed, the cell-cell interactions, and the composition of the material are of equal importance. Therefore, a method to develop a hybrid biomatrix that serves to cultivate an interior tissue complex that builds structures and an exterior tissue that maintains its integrity. What makes up this matrix are two biomaterials (Type I Collagen and Alginate), which are cross-linked with Ethlyenediaminetetraacetic acid (EDTA) to keep cells stable during the printing process. Experimentation proved successful, where all cells encapsulated remained unchanged and had a viability of 90%. With an inexpensive printer and the proper biomatrix, it is possible to print artificial tissue structures that are capable of holding any cell line and be able to structure several tissue types.

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Characterization Of Decellularized Extracellular Matrix On 3d-printed Ceramic Scaffolds For Promoted Osteogenesis Differentiation

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Currently available orthopedic implants are extremely biocompatible but lack certain extents of biological characteristics that allow further interaction between the biomaterial and the surrounding tissues. Nature extracellular matrix (ECM)-coated 3D bioscaffolds was received considerable interest for hard tissue regeneration due to their ability to promote cell behaviors and decreased inflammatory responses. This research was focused on the design and manufacturing of 3D-printed ceramic that is made out of calcium silicate (CS), and extracellular matrix (ECM) from MG63 cell, generating a enhancing bone tissue engineering strategy that revolves around the concept of promoting osteogenesis by creating an osteoinductive microenvironment through the incorporation of bone-growth-promoting MG63's ECM. Therefore, we performed decellularization on MG63 cell cultured on ceramic scaffolds to obtain the ECM-coated 3D scaffold and further analyzed the biological behaviors of the ECM-coated scaffolds. The data indicated that the ECM-coated ceramic scaffolds showed excellent bioactivity and effectively promoted cellular adhesion, proliferation, and differentiation of human Wharton's Jelly mesenchymal stem cells by increasing the expression of osteogenic-related genes, such as ALP, BSP and OC, and presented anti-inflammatory characteristics by showing a decrease in expression of TNF-α and IL-1. Overall, our study presented a valuable technique of producing promising 3D ceramic bioscaffold that augment bone tissue regeneration effects in numerous aspects.

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Autologous Blood Vessel from Human ADMSCs

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Differentiation potential of adipose derived mesenchymal stem cells (ADMSCs) has been widely studied. Ability to become endothelial cells (EC) and smooth muscle cells (SMCs) is an important advantage for constructing non-immunogenic tissue engineered small diameter vascular grafts (TEVG). This study intends to demonstrate construction of functional TEVG by seeding pre differentiated SMC and EC. Major EC function demonstrated is nitric oxide (NO) release into culture medium. Ability of ADMSC-derived SMC to deposit collagen and elastin to maintain compliance of the constructed vessel is another aspect. The pre-differentiation protocol for obtaining EC comprised exposure to hypoxia and shear stress. To obtain SMC, platelet growth factor and BMP4 were the induction factors used. Human adipose tissue obtained (IEC and IC-SCR approved) from same donor was employed for obtaining well-characterized ADMSC and after expansion separate induction protocol was used to generate both ECs & SMCs. Replicate donor tissue was used to demonstrate repeatability. Lineage commitment of hADMSCs to ECs was evidenced by gene expression of endothelial markers (eNOS and CD31) and differentiation was confirmed by Ac-LDL uptake assay, CD31 immunofluorescence and NO production. Similarly, lineage commitment of hADMSCs to SMCs upon induction was detected by gene expression of specific markers calponin and smooth muscle actin (SMA) and differentiation was confirmed by α-SMA and calponin immunofluorescence. Passaging of differentiated SMC and long-term culture resulted in ECM deposition. Decellularized culture substrates were employed to demonstrate collagen and elastin deposition by qualitative and quantitative methods. The study established that functional TEVG may be constructed using autologous hADMSC.

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Growth Factor Loaded Bioinks for Enhanced Extracellular Matrix Deposition

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Introduction: Cartilage is a tissue with complex geometry and unique shape for every individual, therefore, replacement of this tissue can be elegantly addressed with 3D bioprinting. Recent studies have shown strongly increased deposition of extracellular matrix (ECM) in scaffolds of sulfated polymers, which have high affinity to certain growth factors (1). In this study, we created a bioink based on alginate-sulfate (AlgS) and gellan gum and investigated the chondrogenic potential of bioprinted scaffolds with Transforming Growth Factor beta (TGFβ) either supplemented in the media or preloaded in the ink.

Methodology: Bovine chondrocytes were isolated, expanded to passage 3, mixed with bioink and printed into cylindrical scaffolds. Scaffolds were cultured either in TGFβ1 containing media or loaded with TGFβ1 and subsequently cultured in TGFβ1-free medium for 21 days.

Results: Scaffolds were successfully bioprinted with post-printing viability of over 90%. Cells proliferated and exhibited spread morphology. Although atypical for chondrocytes, spread morphology was also observed in casted AlgS scaffolds that induced strong chondrogenic phenotype (1). Deposition of collagen II and collagen I was observed in TGFβ1 supplemented scaffolds. Scaffolds loaded with TGFβ1 showed increased ECM deposition compared to unloaded, however, less then constant supplementation of TGFβ1.

Conclusions: In this study we could demonstrate the feasibility of loading bioinks with growth factors for enhanced deposition of ECM. Patient-specific grafts without the need for *in vitro* pre-culture can be created and could be used for clinical applications in the maxillofacial and orthopedic fields.

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3D Printed Biphasic Elastomeric Scaffolds with Cell-Laden Hydrogels for Blood Vessel Tissue Engineering

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The total mortality rate of all cardiovascular disease-related diseases is higher than other diseases, causing about 17.1 million deaths worldwide each year. The World Health Organization (WHO) predicts

that about 23.3 million people will die from cardiovascular diseases by 2030. Although artificial blood vessels have been widely used for many years, current grafts applied in clinical suffer from the problems of infection, progressive obstruction, calcification, poor long-term durability, and a lack of growth potential. In 70-100% of cases, graft failure occurred about 10 to 15 years after surgery. With the development of 3D printing technology, 3D printed and biomimetic blood vessels may provide a great potential in blood vessel tissue engineering. Here, biphasic elastomeric scaffolds for blood vessel tissue engineering were manufactured by digital light processing (DLP) 3D printing technology. The design of the scaffold structure uses connected porous and nonporous phases as the basis for mimicking the media and intima layers of the blood vessel, respectively. The biodegradable and photosensitive polyester elastomers were applied to provide similar mechanical properties of blood vessels. The scaffolds can recover from deformation, and have good cytocompatibility and flexibility. To establish the media layer of the blood vessel, the porous structure was filled with cell-laden hydrogels. Furthermore, the customized and Y-shaped biphasic elastomeric blood vessel scaffolds can be fabricated by DLP 3D printing technology. The biphasic elastomeric scaffolds with cell-laden hydrogels may improve the development of blood vessel tissue engineering.

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Tissue-engineered Bioequivalent Based on Hybrid Matrix and Spheroids from Buccal Epithelium for Urethral Reconstructive Surgery

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To date, the common way to treat urethral stricture is substitute urethral reconstruction using a buccal mucosa graft or an acellular matrix. However, many studies showed that the use of these materials can lead to the development of fibrosis, recurrent stricture, necrosis, and graft rejection (Atala *et al*., 2017). This occurs because of insufficient cell number, possible changes in cell phenotype (epithelial-mesenchymal transition), and absence of blood supply in a graft. We therefore sought to develop a tissue-engineered urethral wall bioequivalent, which can be used to treat urethral strictures longer than 2 cm. We developed a three-component system: hybrid matrix, modified fibrin, and cell spheroids. Matrices consisted of reconstituted type I and III collagen reinforced with glycolide and L-lactide fibers and provided mechanical support. Spheroids were obtained from human buccal mucosa epithelial cells and ensured epithelial phenotype preservation. Fibrin hydrogel, which immobilized cells on a matrix surface, was prepared in accordance with our previous data (Koroleva *et al*., 2016; Shpichka *et al*., 2017) and induced vasulogenesis. Thus, the developed bioequivalent is promising; and after required trials its use can be easily translated into clinical practice.

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A Conditioning Platform for Kidney Tissue Engineering using a 3D Printed Bioreactor

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T P Burton and A Callanan Institute for Bioengineering, University of Edinburgh. Kidney tissue engineering is an accelerating field, with recent advances using induced human pluripotent stem cells to create kidney organoids[1]. For advancement in kidney tissue engineering a greater control of the growth environment is required, in terms of both 3D structure where cells grow and the bioreactor stimulus they receive. Decellularized tissue is the main focus of this research at present[1]; however, the decellularized tissue is often poorly characterised with recellularization proving an daunting challenge.

We showed how a 3D printed bioreactor can be used alongside polymer scaffolds as a conditioning tool for tissue engineered kidney constructs, providing a highly controlled and customizable environment within which to influence cell differentiation, survival and function. The device is capable of delivering a range of shear stresses representative of *in vivo* conditions, and houses any electrospun polymer scaffold. The dual purpose device can be used as a preconditioning tool for kidney cells, or as a testing bed; providing a controlled environment in which to investigate disease states or treatments. This system provides a much needed tool within the field of kidney tissue engineering and bioengineering at large.

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Development of Protein-based Hydrogels for 3D Printing of Tissue Constructs

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Substrate topography, along with mechanical properties and presence of cell-relevant ligands and cues, has been established as a crucial design parameter that affects cellular behavior in *in vitro* tissue models. Understanding cellular behavior in engineered human tissue is expected to lead to greater physiological relevance of these models, which may ultimately facilitate application of the model for *in vitro* drug screening or disease modeling. In the small and large intestine, villi and crypts, respectively, are the major morphological features that contribute to tissue function. Recent examples of *in vitro* tissue models have sought to incorporate crypt topographies more representative of large intestine morphology into culture substrates through different means, including the use of decellularized extracellular matrix, formation of hydrogels around sacrificial components, or casting scaffolds against patterned substrates. While previous work with these models have shown that topography affects cellular response, there are two main limitations with these models: 1) the constructs degrade quickly in many cases, and 2) the topography cannot be easily varied. This research focuses on preparing intestine-like structures by 3D printing silk protein hydrogels. Silk proteins can be enzymatically crosslinked into biocompatible hydrogels with long-term stability and tunable mechanical properties. This work develops a new synthetic approach that enables 3D printing of the enzymatically-crosslinked hydrogels. Rheological experiments were used to determine flow behavior and gelation kinetics, and constructs were then 3D printed using robotic dispensing. Intestinal cell lines were cultured on and within the gels to quantify cell attachment, proliferation, and morphology on the printed constructs.

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Electrospinning for Resorbable Vasrcular Scaffolds with Complex Shape and Bifurcation

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0 0 1 194 1112 Tecnologico de Monterrey 9 2 1304 14.0 Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:''Table Normal''; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:''''; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;}
While autologous tissue is the graft of choice in most surgical bypass procedures, the next ideal option is the use of synthetic vascular grafts, particularly in the case of pediatric applications. While significant advances have been reported in the use of electrospinning for vascular grafts both at *in vitro* and *in vivo* level, most of the work is limited to straight, tubular shapes with uniform diameters. In order to generate complexly-shaped resorbable scaffolds with curving and bifurcated tubular shapes with non-uniform diameters, this study proposes combination of directed electrical field and dynamic positioning of electrospun fibers aimed at a custom, 3D printed mandrel. The proposed approach produced a woven membrane of electrospun fibers. In this study, the fibers used were polycaprolactone (PCL). They were spun onto a 3D printed (in ABS plastic) bifurcated tubular mandrel. Conventional tubular scaffolds base on PCL have tensile strength in the range of 3 and 4 MPa in order to be considered adequate for *in vivo* testing as vascular grafts inside models. In tension tests, the scaffolds produced with the proposed novel method can reach up to 1.90 MPa. The results show a promising new process in order to achieve resorbable vascular scaffolds with complex shape and bifurcation.

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Three-dimensional Tissues Using Human Pluripotent Stem Cell Spheroids as Biofabrication Building Blocks

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A recently emerged approach for tissue engineering is to biofabricate tissues using cellular spheroids as building blocks. Human pluripotent stem cells (hPSCs), including human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), can be cultured *in vitro* over a long term to generate large numbers of cells. And they can presumably be differentiated into all the cell types of human body. Additionally, patient-specific iPSCs and their derivatives induce minimal or no immune rejection in the patient. These characteristics make hPSCs ideal cell source for biofabrication. We previously developed a thermoreversible hydrogel-based cell culture system that can economically produce large numbers of hPSC spheroids. With hPSCs and this culture system, there are two potential methods to biofabricate the desired tissue. In Method 1, hPSC spheroids are first utilized to biofabricate a hPSC tissue that is subsequently differentiated into the desired tissue. The key for Method 1 to succeed includes: (1) hPSC spheroids can quickly fuse to form the hPSC tissue and (2) hPSCs in the hPSC tissue can be differentiated into the desired tissue cells. In Method 2, hPSC spheroids are first converted into tissue spheroids in the thermoreversible hydrogel-based cell culture system and the tissue spheroids are utilized to biofabricate the desired tissue. The key for the success of this method is tissue spheroids can quickly fuse to form a cohesive tissue. In this paper, we explore these questions and study which method is more appropriate for tissue biofabrication with hPSCs.

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A Novel Integrated Bioprinter, Biopaper, Bioreactor System for the Fabrication and Culture of iPSC Derived Brain Tissue Constructs

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With the growth of the biofabrication and bioengineering fields, the ''tools of the trade'' have improved in number and ease of use, with better output quality and reduced cost. Contemporary tissue engineering must employ more than a single, novel technology by integrating multiple tools/processes and transfering tissue constructs between biofabrication/analysis platforms. This non-trivial challenge must be addressed in multi-tool, multi-process laboratories. We have developed a tissue engineering system combining a laser-induced forward transfer based cell printer, a framed biopaper substrate/scaffold, and a perfusion bioreactor system that supports bilayer and stacked tissue constructs that integrates with the bioprinter. The bioprinter was built as previously reported with modifications to ensure pattern registration to the dimensions of the bioreactor aseptic environment maintenance. Human iPSCs were differentiated microvascular endothelial cells and astrocytes. The framed biopaper, an electrospun nanofiber mat attached to a laser-cut cyclic olefin copolymer (COC) frame, was loaded into the bioreactor. The bioreactor was 3D printed. The astrocytes were seeded on the biopaper and microvascular endothelial cells were printed onto this biopaper. The heterotypic tissue construct was cultured under perfusion and remained viable in the bioreactor for 14 days. This work was supported by the Chemical Biological Technologies Directorate under project number from the Department of Defense Chemical and Biological Defense program through the Defense Threat Reduction Agency and the Defense Health Agency. This information is to be released under Distribution A.

Biomaterial Scaffolds

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The Influence of Strontium Doped Biphasic Ceramic on the Osteoporotic Rabbit Bone

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Introduction: The still unclear Strontium (Sr) influence on the different bone molecular events in case of combination of biomaterial and Sr ions requests the evaluation of effect of Sr doped biphasic material on the distribution of osteocalcin (OC), osteoprotegerin (OPG), nuclear factor kappa beta 105 (NFkB105), bone morphogenic protein 2/4 (BMP2/4) in 12 weeks long implantation in osteoporotic rabbit bone.

Methods: Osteoporosis was induced in 37 matured female rabbits. A 4 mm bone defect of right trochanter was filled with granules composed of 70% hydroxyapatite (HA), 30% tricalcium phosphate (TCP) and 5% of Sr in 7 rabbits; HA(70)/TCP(30) in 7 rabbits. Following groups (7 rabbits each) contained the above mentioned material without Sr. Sham surgery was done in 8 rabbits. Left legs was set as a relative control, but the last group consisted of intact bone (5 animals). Bone samples were analysed by immunohistochemistry and morphometrically by Image Pro Plus 7 program. Semi-quantitative method was used for interpretation of results.

Results: Only the volume of intact bone statistically significantly differed from indicators of all other groups. The relative number of OC, OPG, NFkB105 and BMP2/4-containing cells was higher in the femur with biomaterial. Only OPG elevated significantly, but number of BMP2/4 positive cells was slightly increased after biomaterial and Sr implantation.

Conclusions: Different biomaterial and Sr implantation of 12 weeks doesn't change the osteoporotic bone volume in rabbits. The Sr doped biphasic ceramic seems to increase the suppression of osteoclastogenesis and shows a slight tendency for stimulation of bone regeneration.

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Early Response in Peripheral Nerve Injury Repaired With Decellularized Nerve Allograft

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Peripheral nerve injuries can result in a defect, which requires the use of autologous nerves or acellular nerve allografts (ANAs) to bridge the defect to facilitate regeneration. While alternatives to autologous nerve grafts, such as ANAs, are desired, axonal regeneration is significantly limited across long ANAs (>3cm). To determine how longer ANAs limit regeneration, we examined the cellular repopulation of short (2 cm) compared to long (4 cm) ANAs for up to 4 weeks post-grafting in a rat sciatic nerve defect. In this model, axons regenerate across 2 cm ANAs robustly, while axon regeneration across 4 cm ANAs is severely limited. We observed that both long and short ANAs were repopulated with cells at similar rates and densities for the first 2 weeks, where macrophages (CD68+) were prominent in either ANA (42% *vs* 38%). Interestingly though, by 2 weeks differences in angiogenetic processes were emerged. The total length of blood vessels (RECA-1+) in the proximal and distal regions of long and short ANAs were similar, but in the mid-region, total blood vessel length was significantly higher in the short ANAs than the long ANAs (10280 units vs 3020 units). Concomitant to these differences in the mid-region of long compared to short ANAs, regions of hypoxia in the mid-graft of long ANAs were present, as visualized via hypoxyprobe and nuclear localization of HIF-1α staining. Taken together, these data suggest that longer ANAs are deficient or delayed in angiogenesis, which may play a role in limiting axonal regeneration across long ANAs.

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A Nano-scaled And Multi-layered Recombinant Fibronectin/cadherin Chimera Composite Selectively Concentrates Osteogenesis-related Cells And Factors To Aid Bone Repair

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Easily accessible and effective bone grafts are in urgent need in clinic. The selective cell retention (SCR) strategy, by which osteogenesis-related cells and factors are enriched from bone marrow into bioscaffolds, holds great promise. However, the retention efficacy is limited by the relatively low densities of osteogenesis-related cells and factors in marrow; in addition, a lack of satisfactory surface modifiers for scaffolds further exacerbates the dilemma. To address this issue, a multi-layered construct consisting of a recombinant fibronectin/cadherin chimera was established via a layer-by-layer self-assembly technique (LBL-rFN/CDH) and used to modify demineralised bone matrix (DBM) scaffolds. The modification was proven stable and effective. By the mechanisms of physical interception and more importantly, chemical recognition (fibronectin/integrins), the LBL-rFN/CDH modification significantly improved the retention efficacy and selectivity for osteogenesis-related cells, e.g., monocytes, mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), and bioactive factors, e.g., bFGF, BMP-2 and SDF-1α. Moreover, the resulting composite (designated as DBM-LBL-rFN/CDH) not only exhibited a strong MSCs-recruiting capacity after SCR, but also provided favourable microenvironments for the proliferation and osteogenic differentiation of MSCs. Eventually, bone repair was evidently improved. Collectively, DBM-LBL-rFN/CDH presented a suitable biomaterial for SCR and a promising solution for tremendous need for bone grafts.

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Thiolated Poly(Lactic-co-Glycolic Acid) Macromers for Biomolecule Conjugation by Alkyne-Azide Click Chemistry

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Synthetic hydrogels offer high tunability of their structural characteristics and physico-chemical properties for tissue engineering applications. However, hydrogels usually require the conjugation of biomolecules to confer biological activity and induce tissue formation and regeneration *in vivo*. To meet this need, our objective is thus to develop thiol-terminated poly(lactic-*co*-glycolic acid) (PLGA) macromers with alkyne moieties for the conjugation of biomolecules via *alkyne-azide ''click'' chemistry*. By exploiting the high selectivity and yield of the alkyne-azide cycloaddition reaction,[1] we aim to achieve ''click'' binding of tissue-relevant biomolecules to this polymer. Our methodology to generate this polymer involves the functionalization of PLGA termini with 1,4-butynedithiol, producing alkyne and thiol groups at the chain termini. Following this, biomolecule conjugation can be achieved by reaction of alkyne groups with azide-linked biomolecules. Preliminary results have demonstrated the successful synthesis of this macromer with expected structure and controlled molecular weight. Ultimately, this novel, tunable polymer demonstrates significance in its presentation of nucleophilic thiol termini for crosslinking with other polymer systems, in addition to highly orthogonal alkyne-azide ''click'' chemistry for biomolecule binding.

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The Enhancement Of Bone Formation With The Micro And Nano-scaled Surface Coatings On Biodegradable Mg-based Bone Scaffolds

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Magnesium (Mg) alloys are biodegradable and can enhance bone formation while maintaining desired mechanical strength in the wound healing process. However, Mg-based implants with desired degradation rate remain a challenge. Previously, we conducted a pilot study by comparing electrochemical and long-term biocompatible properties of

hydroxyapatite (HA) and fluorohydroxyapatite (FHA) coated Mg within similar body fluid. We observed that the FHA coating displayed preferable corrosion resistance and had a suitable topography for culturing cells. In this study, human mesenchymal stem cells (hMSCs) were used to examine cell viability, cell proliferation, and osteogenic differentiation on the uncoated biodegradable Mg, $Mg(OH)_2$-Mg, HA-Mg, and FHA-Mg scaffolds. Our results confirmed that the degradation rates were regulated by different kinds of surface modification. MTT assays demonstrated that the initial high concentration (1300 ppm) of Mg^{2+} released from uncoated Mg scaffold resulted in low hMSC viability, whereas the lower concentration (400 ~ 500 ppm) of Mg^{2+} released from $Mg(OH)_2$-Mg and HA-Mg caused no harm to cells. Both SEM images and Alamarblue assays showed that the FHA-Mg scaffold, which had the least released Mg^{2+} concentration (150 ppm), significantly enhanced the proliferation of hMSCs. Compared with micro-scaled surface of $Mg(OH)_2$-Mg and HA-Mg scaffolds, the nano-scaled needle-like structures on FHA-Mg scaffolds were observed. Furthermore, osteogenic differentiation of hMSCs on FHA-Mg scaffold was noticeably observed two weeks after cell seeding in the presence of osteogenic inducing medium. The results indicate that the nano-scaled needle-like FHA-Mg scaffold may provide an efficient approach to improve early biocompatibility of the Mg-based implantation for bone tissue engineering.

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In Vivo Vascularization of a Modular Bone Regeneration Platform

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Introduction: Inadequate vascularization limits attempts to regenerate functional bone. Modular tissue engineering promises the ability to generate well vascularized tissue constructs from endothelialized, cell-laden modules. We examined the *in vivo* vascularization of implants composed of fused glycosaminoglycan (GAG)/chitosan/hydroxyapatite (HAP) microcapsules containing osteoprogenitors (OP), and evaluated how accessory cells could enhance vascularization.

Methodology: OPs and HAP microgranules were co-encapsulated in GAG/chitosan microcapsules and the capsules were coated with endothelial progenitors (EP) or undifferentiated mesenchymal stem cells (MSC). Cell-coated capsules were fused into disc constructs (6×5 mm) and implanted subcutaneously in rats. Doppler Ultrasound imaging of constructs was conducted at 1, 2 and 4 weeks post-implantation to characterize blood flow within the constructs. The vascular area fraction (VAF) was calculated from the US images. Results were compared to acellular fused constructs, and OP constructs without accessory cells. Implants were harvested for histology at 1, 2 and 4 weeks.

Results: At 1 week post-surgery, all implant conditions had similar average VAF, between 3.4% and 4.4% of total implant area. After 4 weeks, implants with accessory cells (OP+EP and OP+MSC) had higher average VAFs (8.7±5.1% and 6.3±2.6% respectively) than either acellular (2.0±1.9%) or OP-only (1.9±1.4%) implants. Given the Doppler resolution limit, microvessel ($D < 30$ μm) density comparisons requires analysis by quantitative histology (ongoing). Microvessel densities will be reported: preliminary observations suggest that microvessel densities were higher in conditions containing EP and MSC. Results suggest that the microcapsules with external accessory cells are an effective new design for achieving well-vascularized bone grafts.

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Living Nanofiber Yarn-Based Woven Biotextiles for Tendon Engineering Using Cell Tri-Culture and Mechanical Stimulation

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Tendon grafts are essential for the treatment of various tendon-related conditions due to the inherently poor healing capacity of native tendons. In this work, we combined electrospun nanofiber yarns with textile manufacturing strategies to fabricate nanofibrous woven biotextiles with hierarchical features, aligned fibrous topography, and sufficient mechanical properties as tendon tissue engineered scaffolds. Comparing to traditional electrospun random or aligned meshes, our novel nanofibrous woven fabrics possess strong tensile and suture-retention strengths and larger pore size. Cell proliferation and infiltration, and the expression of tendon-specific genes by human adipose derived mesenchymal stem cells (HADMSC) and human tenocytes (HT), were significantly enhanced on the woven fabrics compared to those on their electrospun nonwoven counterparts. Co-cultures of HADMSC with HT or human umbilical vein endothelial cells (HUVEC) on woven fabrics significantly upregulated the functional expression of most tenogenic markers. HADMSC/HT/HUVEC tri-culture on woven fabrics showed the highest upregulation of most tendon-associated markers. Furthermore, we conditioned the tri-cultured constructs in the dynamic conditioning and demonstrated that dynamic stretch promoted total collagen secretion and tenogenic differentiation. Our nanofiber yarn-based biotextiles have significant potential as engineered scaffolds to synergize the multiple cell interaction and mechanical stimulation for promoting tendon regeneration.

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Characteristics of Nanofibrous Scaffolds Made By Animal Fiber Protein

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Keratin is a fibrous protein that constitutes the intermediate filaments in epithelial cells of vertebrate animals and is a chief constituent of hair, nail, horn, and beak. A key characteristic of keratin is its amino acid composition and high cysteine content that permits the formation of many disulfide bonds. These bonds maintain a firm steric structure and make the protein water-insoluble. Keratin also contains amino acid sequences such as RGD and LDV that are involved in cell adhesion and have similarities to fibronectin, which is a known cell-adhesive protein. Thus, much like fibronectin, high cell affinity is expected for keratin if it is used as a biomaterial. In this study, keratin protein extracted from wool fibers and human hair was processed into nanofiber sheets which have different diameters. In order to improve the mechanical property and functionality of the scaffold, multilayer nanofiber sheets were also prepared by using several kinds of bioabsorbable synthetic polymers as auxiliaries. The physical properties of each of the nanofiber sheets were evaluated for hydrophilicity, mechanical strength, and surface structure. Biocompatibility of keratin nanofiber sheets were also evaluated by culturing fibroblast on them. From an evaluation performed for mechanical properties of keratin nanofiber sheets, it had been proved to hold strength durable enough to ordinary use. Fibroblast culture on the keratin nanofiber sheets resulted in proliferation in cells with the lapse of days. Moreover, recipient cells were well attached on the keratin nanofiber sheets without remarkable inflammation when they were implanted in rats.

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Surface Modification Of Poly(lactic Acid) Nerve Regeneration Tube Using Oligo(d-lactic Acid) Bioactive Peptide Conjugates

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Nerve injuries, typically result in significant nerve gaps leading to the loss of motor and sensory functions. When the gap is too large, an autologous nerve graft from the patient's own body is used to bridge the injury site. However, autologous nerve grafts have several disadvantages including permanent loss of donor function and size mismatch between the injured nerve and the graft nerves. Therefore, artificial nerve conduit is of great interest for bridging the gap between severed nerve stumps. Poly(lactic acid) (PLA) and its

copolymers are preferred because they are not enzymatically hydrolyzed to low toxic lactic acid and are metabolized via the tricarboxylic acid cycle *in vivo*. In addition, its mechanical properties, shaping and molding properties are excellent, and it is easy to be molded. However, since PLA lacks congenital biological activity, it is usually modified with biologically active molecules. We then focused on the bioactive peptide sequence, isoleucine-lysine-valine-alanine-valine (IKVAV), which was discovered from laminin by Tashiro *et al*. in 1989, to modified PLA. IKVAV known to promote neural cell adhesion, proliferation and neurite outgrowth. In this study, we developed a PLLA nanofibrous nerve conduit, modified with a conjugate of oligo(D-lactic acid) (ODLA) and the neurite outgrowth, thereby promoting peptide IKVAV to improve the nerve regeneration. PLLA/ODLA-IKVAV nanofiber was fabricated by electrospinning, then, the neurite outgrowth of PC-12 on PLLA/ODLA-IKVAV nanofiber and on control PLLA nanofiber. We also modified the ODLA-IKVAV chemical structure improve the hydrophilicity and exposing efficiency of IKVAV sequence to the outmost surface of nanofibers.

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Gellan Gum Spongy-like Hydrogels Reinforced with Hydroxyapatite for Bone Tissue Engineering

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Bone tissue engineering with cell laden hydrogels has been attracting a lot of attention. In fact, they can resemble in some aspects the extracellular matrix, as the high water content. In a forward thinking manner, da Silva engineered hydrogels where cells adhered and proliferated without the need of any backbone modification, which they called gellan gum (GG) spongy-like hydrogels [1]. In this work, we took advantage of these characteristics and combined it with hydroxyapatite to reinforce the gellan gum (GG-HAp) spongy-like hydrogels. Since, there is limited knowledge about the interaction between osteoclasts and biomaterials, we assessed the ability of developed hydrogels to support osteoclastogenesis. First, mechanical properties were evaluated. Then, isolated bone marrow cells were cultured in the spongy-like hydrogels and treated with 1,25-dihydroxyvitamin D3 (Vitamin D3) to promote osteoclastogenesis. Cultures without Vitamin D3 and standard 2D cultures were used as control. After 7 days, cell viability and differentiation was assessed. It was shown that the addition of HAp to GG spongy-like hydrogels enable the formation of lager pores and thicker walls, allowing to withstand higher loads and resist fracture to a higher extent. Additionally, cells were viable and metabolically active. Finally, it was observed that the GG-HAp spongy-like hydrogels supported cell differentiation into pre-osteoclasts, as suggested by the presence of aggregates of TRAP-stained cells, and the expression of DC-Stamp and Cathepsin K, necessary for cell fusion and resorption capacity, respectively. Overall, GG-HAp spongy-like hydrogels showed to support osteoclastogenesis, an important feature when establishing new biomaterials for bone tissue applications.

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Rapid Photocrosslinking of Core/shell Bioink for in situ Intra-surgical application

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Abstract: Three-dimensional (3D) bioprinting is driving major innovations in cartilage tissue engineering. Extrusion-based 3D bioprinting requires a phase change from a liquid hydrogel to a semi-solid crosslinked network. The hardening of the hydrogel can be achieved by a photoinitiated free radical polymerization that is highly efficient but generates cytotoxicity. Thus, we aimed to define the conditions to match cell viability with stiffness and rapid photocrosslinking that can be exploited for intra-surgical application. We have previously developed a handheld bioprinter for in situ anatomic application, ''Biopen'' (1, 2), that is customized in the present work for the co-axial extrusion of a stem cell niche in a non- crosslinked Core, while the photocrosslinking procedure is segregated in the stiff outer Shell. Confocal imaging was used to characterize the Core/Shell organization of a gelatin-methacrylamide/hyaluronic acid-methacrylate (GelMA/HAMa) bioscaffold and a stiffness of 200KPa was reached in only 10 seconds of UV photocuring with a selected photoinitiator. The bioprinted Adipose Derived Stem Cells maintained 90% viability after bioprinting exerting a 30% increase in cell number in only 10 days. Our Core/Shell 3D bioprinting couples the rapid generation of high modulus bioscaffold with cell survival, resulting ideal for cartilage engineering surgical techniques.

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Novel Biomanufactured Seamless Collagen Scaffolds As A Substitute For Biologically-Sourced Materials In Reconstructive Urological Applications

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Objectives: Biological-sourced materials such as decellularized human epicardium (DHE) and decellularized porcine small intestinal submucosa (SIS) are used for complex reconstructive urological operations such as tunica reconstruction for severe Peyronie's disease. Although effective, they are expensive and have theoretical risks of infection and inconsistency given their allogenic and xenogenic sources. We have engineered a novel molded collagen type I scaffold that can be used as a reconstructive material in complex urological repairs. We sought to compare the mechanical and biological properties of our molded collagen scaffolds with conventional biological-sourced materials.

Materials and Methods: Stress and strain were measured for DHE, SIS, porous molded collagen (PMC) and cross-linked molded collagen (CMC). All four materials were evaluated by scanning electron microscopy (SEM) and implanted subcutaneously in rats and harvested 3d, 7d, 14d, and 28d and evaluated for persistence, gross appearance in situ, and cellular infiltration assessed by immunohistochemistry (IHC) and quantitative polymerase chain reaction (qPCR).

Results: PMC and DHE had SEM architecture and stress and strain properties similar to SIS and DHE, respectively. All materials persisted to 28 days. None of the materials appeared grossly infected or inflamed. All materials had evidence of cellular infiltration by IHC by 28d. qPCR demonstrated expression of genes associated with neovascularization and inflammation in all materials.

Conclusions: Engineered molded collagen scaffold may provide an inexpensive, consistent, and safe source of materials that can be customized and used for complex reconstructive urological applications.

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Biogelx: Designer Gels for Cell Culture

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Biogelx is a biomaterials company that designs tuneable peptide hydrogels, offering artificial tissue environments to cell biologists for a range of cell culture applications.

The hydrogels are highly tunable, cell-matched biomaterials, capable of revolutionizing the way cell biologists control and manipulate cell behavior in the laboratory. This is of direct relevance to fundamental cell research, including the study of stem cell biology and disease models within academic and medical labs. Biogelx's hydrogels also have a potentially dramatic impact on harnessing the capabilities of 3D bio-printing; where they are being used as the 'bio-ink' in the printer.

Biogelx offers a range of hydrogel platforms that are three dimensional (3D), 99% water and have the same nanoscale matrix structure as human tissue. This gives control back to the cell biologist, as the gels can be tuned to meet the needs of any given cell type.

This presentation showcases the underlying chemistry of Biogelx's peptide hydrogels, highlighting the range of chemical and mechanical modifications that can be implemented within the gels, in order to address a wide range of cell based applications. Some examples of academic and industrial collaborative work shall also be presented, including how the gel tunable properties, can be used to influence the differentiation pathway of stem cells.

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Biodegradability And Thrombosis Assessment Of Magnesium-based Alloys Using A Microfluidic System

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Magnesium (Mg)-based stents are extensively explored to alleviate atherosclerosis due to their biodegradability and hemocompatibility. To ensure the quality, safety and cost-efficacy of bioresorbable scaffolds and full utilization of the material tunability afforded by alloying, it is critical to access degradability and thrombosis potential of Mg-based alloys using improved *in vitro* models that mimic as closely as possible the *in vivo* microenvironment. In this study, we investigated biodegradation and initial thrombogenic behavior of Mg-based alloys at the interface between Mg alloys' surface and simulated physiological environment using a microfluidic system. The degradation properties of Mg-based alloys WE43, AZ31, ZWEK-L, and ZWEK-C were evaluated in complete culture medium and their thrombosis potentials in platelet rich plasma, respectively. The results show that 1) physiological shear stress increased the corrosion rate and decreased platelets adhesion rate as compared to static immersion; 2) secondary phases and impurities in material composition induced galvanic corrosion, resulting in higher corrosion resistance and platelet adhesion rate; 3) Mg-based alloys with higher corrosion rate showed higher platelets adhesion rate. We conclude that a microfluidic-based *in vitro* system allows evaluation of biodegradation behaviors and platelets responses of Mg-based alloys under specific shear stress, and degradability is related to platelet adhesion positively.

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WITHDRAWN

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Digital Additive Biomanufacturing of 3D Cell-Synell Co-Cultures

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Synells are cell-sized bioreactors containing one or more genes and the enzymes, cofactors, and substrates required for their transcription (TX) and translation (TL) into proteins [1].

In this work, lipid vesicle synells are fabricated by flow-focusing microfluidic devices that allow control over the relative concentrations of TX-TL components necessary for the expression of functional proteins. Multiphase flow simulations of synell generation allow the optimization of synell size and polydispersity. Various configurations of three-dimensional (3D) synell - human mesenchymal stem cell (hMSC) co-cultures are demonstrated using a repertoire of direct writing additive biomanufacturing (ABM) processes [2].

In one example, 3D microscale fibrous poly-ε(caprolactone) (PCL) structures are fabricated using a melt electrospinning writing (MEW) process. The produced structured material substrates entrap synells within their lattice pore microarchitecture with subsequent hMSC seeding. In another exemplar case, synell- and hMSC-laden 3D fibrous self-assembled peptide hydrogel structures are fabricated using a direct-write ABM process. Confocal microscopy reveals divergent synell-hMSCs spatial configurations in 3D between the two cases.

This work can inform the reliable fabrication of complex 3D biologically-relevant systems with superior spatial patterning and functional control over different scales.

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Chitosan-halloysite Nanotubes Scaffolds Containing In Situ-Reduced Silver Nanoparticles As Antimicrobial Dressings For Tissue Engineering Applications

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Incorporation of nano-structures into chitosan (CTS) usually improves its properties and microstructure. Naturally occurring halloysite nanotubes (HNT) can be used as reinforcement nano-fillers while silver nanoparticles (AgNP) can be used to increase the antibacterial action of CTS. Therefore, in this work we developed novel CTS-HNT-AgNP nanocomposite scaffolds by combining a solution-mixing, an *in situ* reduction process and a freeze-gelation technique, aiming to obtain antimicrobial and mammalian cells cytocompatible scaffolds for their potential application in tissue engineering. The microstructure of the scaffolds was improved with the incorporation of HNT, the compression stress of the CTS-HNT scaffolds was 5 times higher compared with bare CTS scaffolds due to the strong interactions between HNT and CTS functional groups, which was confirmed by infrared spectroscopy. AgNP were successfully obtained *in situ* by using only CTS as reducing and stabilizing agent. Transmission electron microscopy revealed that AgNP were spherical in shape with an average size of 17 nm. The obtained CTS-HNT-AgNP scaffolds inhibited the proliferation of Gram-negative and positive bacteria depending on AgNP concentration. *In vitro* human fibroblasts viability assays showed that HNT addition improved the cytocompatibility of the scaffolds even with AgNP incorporation. Our results demonstrated that the CTS-HNT-AgNP scaffolds with improved mechanical, antibacterial and cytocompatibility properties could be a promising material as cellular dressings for wound healing.

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Osteogenic Differentiation of Mesenchymal Stem Cells Encapsulated in Feather Keratin Hydrogel

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Keratin is a family of fibrous proteins found in nature as the major component of wool, hair, and horn of mammals and birds feather. Keratin has a relatively high fraction of cysteine residues compared to other proteins for the formation of inter- and intramolecular crosslinks. The disulfide crosslinks in combination with other structural features impart high strength to keratinized tissues. The objective of this work was to synthesize a photo-polymerizable hydrogel based on keratin extracted from poultry feather and evaluate the hydrogel as a matrix for encapsulation and delivery of mesenchymal stem cells (MSCs) in regenerative medicine. Keratin was extracted from feather barbs by reducing the disulfide bonds in cysteine residues to sulfhydryl groups (-SH). Next, the free thiol groups were converted to dehydroalanine (Dha) by oxidative elimination using O-(2,4,6-Trimethylbenzenesulfonyl) hydroxylamine. Then, the Dha moieties were converted to S-allyl cysteine by reaction with allyl mercaptanol to produce keratin allyl thioether (KeratATE). KeratATE hydrogel was dissolved in aqueous solution, MSCs were added, the cell-suspended hydrogel precursor solution was crosslinked by ultraviolet radiation, and cultured in osteogenic medium. The KeratATE hydrogels had a porous, interconnected, honeycomb microstructure and the gels degraded in trypsin solution. MSCs seeded in KeratATE hydrogel had elongated spindle-shape morphology and the cells produced a mineralized matrix upon incubation in osteogenic medium. KeratATE Hydrogel derived from poultry feather is a viable alternative to collagen as an in injectable, photo-polymerizable hydrogel with tunable degradation for encapsulation and delivery of stem cells in tissue regeneration.

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WITHDRAWN

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Fabrication of Biodegradable, 3D-Printed, Oxygen Releasing Scaffolds

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Oxygen is a crucial metabolic substrate and regulator of the survival and regenerative capacity of cells transplanted to regenerate bone defects.[1] To improve cell viability under hypoxia, we reported the use of polyacrylonitrile microspheres, or *microtanks,* that provided controlled oxygen release to their microenvironments.[2] However, polyacrylonitrile is non-biodegradable and its high oxygen permeability led to a relatively short oxygen release half-life. To address these limitations, we have fabricated microtanks composed of an inner polyvinyl alcohol (PVA) layer surrounded by an outer poly(lactic-co-glycolic acid) (PLGA) shell using a water/oil/water double emulsion. Both PVA and PLGA are biodegradable and PVA has lower oxygen permeability than polyacrylonitrile, allowing for longer oxygen release. Following fabrication, microtanks were centrifuged to remove compromised tanks and filtered to obtain an average diameter of 42 μm. We incorporated microtanks into scaffold struts using a 3D printing process. By changing the weight/weight ratios, we could vary the microtank concentration in porous polycaprolactone scaffolds. We loaded scaffolds with oxygen using a hyperbaric oxygen chamber and utilized resazurin dye to visualize controlled release of oxygen into the pore spaces. We are currently investigating the potential of these oxygen-releasing scaffolds to extend the viability and osteogenic differentiation of cells maintained in hypoxic microenvironments.

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Marrow-Isolated Adult Multilineage Inducible (MIAMI) and Annulus Fibrosus Cell Co-Cultures on Electrospun Scaffolds for Intervertebral Disc Tissue Engineering

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Intervertebral disc (IVD) degeneration accounts for most cases of low back pain, affecting 80% of the adult population[1]. Although tissue-engineering shows potential for treatment, IVD cells are sparse and not readily available, rendering their use for implantation difficult. In this study, we propose co-cultures of marrow-isolated adult multilineage inducible (MIAMI) with native annulus fibrosus (AF) cells as an alternative source of cells. MIAMI cells are an immature sub-population of bone marrow MSCs[2]. We hypothesize that such co-cultures will more efficiently guide MIAMI differentiation towards IVD lineages, thereby enhancing functional outcomes, compared to AF/MSC co-cultures previously proposed[3]. In our study, we fabricated nano-fibrous, randomly-oriented, polycaprolactone scaffolds using a custom electrospinning apparatus. AF/MIAMI co-cultures are seeded on the scaffolds and the optimal cell ratio is determined based on the strongest expression of markers characteristic for the AF portion of the disc (collagen I and V, Sox9 and HtrA1 genes). Under SEM, scaffolds appeared to possess uniform diameter fibers smaller than a micron. GFP-MIAMI cells adhered to the scaffold upon culturing for 72hr, and GFP expression demonstrated that MIAMI cells were present both on surface and within the scaffold. Our polycaprolactone scaffolds are therefore suitable biocompatible cell carriers. Further studies will involve RT-PCR, SEM imaging, DNA and sGAG content on AF/MIAMI, to prove the benefits of MIAMI cells over MSCs in AF co-cultures.

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Role of Nanofibrillar Scaffolds on Endothelial Function and Survival

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Research Background & Objectives: Endothelial cells (ECs) are a promising cell type for the treatment of peripheral arterial disease (PAD). We previously showed that anisotropic nanofibrillar scaffolds augment the angiogenic function of human ECs. In order to optimize the biomechanical properties of nanofibrillar scaffolds, we examined the effect of nanofibril size and crosslinking on endothelial survival and function.

Methodology: Aligned nanofibrillar collagen scaffolds were fabricated using a shear-based method. Scaffolds formulations of varying fibril diameters were prepared by altering the ionic strength of monomeric collagen. Scaffolds were further crosslinked at varying levels. Scaffolds were characterized for surface topography and degradation rate. Human ECs were seeded onto the scaffolds for assessment of migration and survival under hypoxia (1% O_2).

Results: Scaffold formulation with low ionic strength had low diameter (LD) fibrils ($\sim$270 nm), compared to the high ionic strength formulation group ($\sim$630 nm). HD scaffold showed higher

elastic modulus than LD group (20.74 ± 11.73 mPa vs. 13.23 ± 6.17 mPa). The mean direction of migration with respect to the fibril axis was 37.06°, compared to 48.45° in the LD group within 24 hours, regardless of the degree of crosslinking. Cell survival was 9% higher on HD scaffolds than that on LD group under hypoxia after 3 days. These data demonstrate that aligned scaffolds of HD scaffolds promoted greater cell survival and alignment along the fibril direction.

Significance: Aligned nanofibrillar scaffolds that improve survival, migration and angiogenesis of ECs provides a viable clinical strategy for the therapeutic neovascularization of PAD patients.

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Surface Modification of TiO_2 Nanotubes with Bioactive Molecules to Improve the Osteocompatibility of Ti6Al4V based Bone Implants

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Titanium alloys are among the most used metallic biomaterials, particularly for orthopedic applications, as they have low specific weight, excellent mechanical properties, immense resistance to corrosion in biological fluids, good wear resistance and very low toxicity towards the host. Further, to improve bone fixation and enhance the biocompatibility of the Titanium based implants, bone cements are used in conjugation with the implant materials during the joint replacement surgeries. However, such implants fail due to de-bonding of cementing material followed by accumulation of the particulate causing toxicity and cell death. Also, bone loosening might occur at the cement-prosthesis interface and/or cement - bone interface, thereby increasing the chances of implant failure by two-folds. Therefore, in the present study the surface of Ti6Al4V was modified through the synthesis of TiO_2 nanotubes using anodic oxidation. Furthermore, the modified surface was coated with a naturally derived, biodegradable, non-toxic polymer (silk fibroin protein). The as-modified surface was characterized for its morphology, surface roughness, wettability and osteocompatibility using MG-63 cell line. It was observed from the study that the suggested modification significantly enhanced the biocompatibility and osteo-integrative property of the Ti6Al4V implant surface.

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In vitro and *In vivo* Study of Controllable Biodegradability of Magnesium Implant for the Repair of Bone Defects

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Magnesium is a lightweight metal with many advantages such as accelerating bone formation and being biodegradable. These make magnesium be proposed as a revolutionary biodegradable material for orthopedic applications. However, magnesium corrodes too quickly in electrolytic aqueous environments. These drawbacks make magnesium be abandoned as a kind of orthopedic application. In this study, we applied a new approach in the design of an Mg-based implant material to control the biodegradation rate. By mixing magnesium particles into PLGA matrix to fabricate an Mg/PLGA composite, like a drug release system, the corrosion resistance decreased and the biodegradation rate became controllable. This study was designed to test the biological performance of Mg/PLGA composite both *in vitro* and *in vivo*. *In vitro* results showed that the extract from Mg/PLGA composites exerted favorable effect on osteoblast behavior including cell proliferation and osteogenic differentiation. *In vivo* study revealed that a significantly large amount of new bone formation was observed on the Mg/PLGA composites compared to the pure PLGA evidenced by Micro-CT and histology analysis. These results demonstrate that Mg/PLGA composite has excellent osteogenesis properties and suggest the potential clinical applications of the Mg/PLGA composites as a kind of sustained-release material in bone defect treatment. Furthermore, the mechanism underlying this process was explored. We found that Mg^{2+} could promote the biological behaviour of rat calvarial osteoblasts by activating the PI3K/Akt signalling pathway. These findings advance the understanding of cellular responses to biodegradable metallic materials and may attract greater clinical interest in magnesium.

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Biological Scaffold of Gelatin-Endogenous Cells Composite Prepared by Bio-3D Printing

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Tissue and organ weakness that can be used for organ transplantation is one of the major challenges facing the medical profession today. Due to traditional manufacturing technology is difficult to complete the precise construction of complex organizations, and also can't avoid the rejection of the material in the human body, so many patients suffering from pain, and no satisfactory treatment results are available. Emerging bio-3D printing technology has become a key research method in the field of material preparation with its unique ability to form complex structure and precise similarity. On this basis, the addition of endogenous cell complexes makes the original chemical material become a bioactive tissue functional material. This provides the possibility of implantable human endogenous functional materials.

In this paper, we will add endogenous cells in the biological 3D printing process added to the cross-linked structure of the gelatin stent gap, and then have excellent biocompatibility of tissue cells containing gelatin tissue-like scaffold, The tissue-like scaffold obtained by printing is immersed in a fluid capable of providing cell growth. When the cells have a high survival rate, the cells are more likely to be self-propagating through cell differentiation and chemotaxis Organization, so that can quickly repair the organization and organs at the same time reduce the occurrence of rejection response.

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A New Technology For Making Multi-branched Vascular Scaffold With Controlled Release Of Growth Factors

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The small diameter vascular system that with well functioning is the essential condition for the metabolism of cells in the tissues, a creative way that combines 3D printing and electrostatic self-assembly technology is proposed to create a small diameter vascular scaffold with multiple layers and multi-branched structures. Using a 3D printed mould, the mould has a primary vessel with diameter of 2 mm, and several secondary vessels with diameter of 1 mm. To create the hollow vessels, hydrogel solution mixed with growth factors was covered onto the mould. After cross-linked, the mould was removed. Using self-assembly technique, a dense layer that is 10μm thick composed of chitosan and heparin was added to the outer surface of the hollow structure. This multi-layer surface with heparin contributes to anticoagulation, and also used as outer shell for achieving the controlled release of the internal growth factors. This multi-layer, multi-branched structure has been tested, and results demonstrated that the small diameter blood vascular structure had good anticoagulation properties and greatly reduced the probability of thrombus generation. Furthermore, because of the controlled release of the growth factors, there is a wide application of this structure, such as stem cell induction, targeted drug delivery and so on.

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Amnion MSCs Remodel Biomimetic Nucleus Pulposus Replacement for Intervertebral Disc Regeneration

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Intervertebral disc (IVD) degeneration (IDD) is an aberrant cell-mediated process in which proteases degrade the nucleus pulposus (NP) disrupting the mechanical function of the IVD eventuating in low back pain. Biomaterials used for NP regeneration must have similar mechanical characteristics to the native NP and support cell-mediated remodeling. We recently demonstrated the formation of a mimetic, extracellular matrix-based biomaterial hydrogel from decellularized bovine tail IVD NP tissue which support human amniotic mesenchymal stromal cell (hAMSC) viability. To further investigate the ability of the biomaterial to support regeneration, hAMSC seeded hydrogels were cultured for 14 days prior to evaluation for matrix remodeling using unconfined compressive dynamic mechanical analysis at physiologically relevant testing frequencies. We hypothesized that hAMSCs would remodel the biomaterial yielding increased viscoelastic properties over time in culture. Results demonstrated that the complex, storage, and loss moduli of the hAMSC seeded hydrogels at day 14 were significantly ($p < 0.05$) greater than their respective values at day 3 and to non-seeded hydrogel controls. Furthermore, these properties were shown to be test frequency-dependent. In general, the viscoelastic properties of the hAMSC seeded hydrogels were found to be within the range of values reported for human NP tissue. Together, the observed increase in mechanical properties of the hAMSC-seeded biomaterial with time in culture demonstrate that hAMSCs remodeled the biomaterial and create a tissue that resembles human NP. Funding: NIH(NIGMS:5P20GM103444-07)

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Freestanding Multilayered Membranes patterned with Micro-Reservoirs as potential Regenerative Paths

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Polymeric membranes have been investigated as biomaterials for tissue regeneration, most of them presenting a flat geometry or a patterned texture at the nano/micrometer scale. Bottom-up strategies have been selected to produce such systems and layer-by-layer technique has been one of the most interesting. Taking advantage of this technology, we propose a new concept of a flexible freestanding membrane featuring well arrays forming pore-like environments to accommodate cells. Briefly, chitosan and alginate are alternately deposited over a patterned PDMS substrate, repeating this procedure 100 times using a robotic system. After drying, the multilayer system can be easily detached from the PDMS substrate. The photolithography technology used to produce the molds allows obtaining an array of wells on the final membranes with a tuned shape and micro-scale precision [1]. The influence of crosslinking, fibronectin immobilization and patterning on the behavior of SaOs-2 cells is investigated. The results suggest that the presence of patterned wells influences positively cell adhesion, morphology and proliferation and that cells colonize preferentially the well regions. Such positive outcomes should result of the more 3D environment felt by the cells on the surface of the membrane. These patterned membranes with arrays of one-side closed pores may adequate groups of cells that could be faced to the tissue to regenerate while blocked to migrate from the location.

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Heart dECM Hydrogel by Supercritical Carbon Dioxide for Enhancing the Effect of Angiogenesis

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Decellualrization by various detergents such as sodium dodecyl sulfate (SDS) and triton X-100 can remove the cell nuclei in tissue organs. However, this leads to ECM structure denaturation, less presence of various ECM proteins and cytokines and loss of mechanical properties. To overcome these limitations, in this study, we developed a super critical carbon dioxide ($scCO_2$) decellularization method with rat heart tissues, which is a detergent-free system that prevents ECM structure disruption and retains various angiogenic proteins in the heart dECM. The heart was placed into the $scCO_2$ reactor and decellularized at 37°C and 350 bar. After $scCO_2$ decellularization, the native $scCO_2$-treated group and detergent-treated group were evaluated by DNA, collagen, glycosaminoglycans (GAGs) quantification and H&E and immunofluorescence staining. As a result, similar to the native group, the $scCO_2$-treated group contained more ECM components such as collagen, GAGs, laminin, fibronectin and *myosin heavy chain* (MHC) as well as angiogenic factors including vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) and others when compared to the detergent-treated group. In addition, to estimate the effect of angiogenesis, collagen and the dECM gel solution were injected in a rat subcutaneous layer ($n = 6$ in each group: collagen, $scCO_2$-treated and detergent-treated group). After 3 days, the gels were harvested and estimated by immunofluorescence staining and the ImageJ program for angiogenesis analysis. Here, we suggest that heart-derived decellularized extracellular matrix (HdECM) with $scCO^2$ decellularization is a promising highly angiogenic material for ischemic tissue regeneration and wound repair.

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An Efficient Protocol For Porcine Liver Decellularization In Three Days Supports Subsequent Recellularization

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Decellularized porcine livers have a wide range of applications in regenerative medicine, however previously existing protocols for organ decellularization still need to be adjusted to a full size porcine liver.

Objective: to establish a protocol that enables effective decellularization of the entire porcine liver in a short period.

Method: Livers ($n = 3$) were perfused with decellularizing solutions through the portal vein during 3 days at room temperature. To analyze the extracellular matrix (ECM), after decellularization, histology and electron microscopy were performed. The vascular tree integrity was evaluated, by magnetic resonance and computed tomography, injecting gadolinium and iodine, respectively. Toluidine blue dye was also used. The presence of residual cells was analyzed by DAPI and quantification of DNA by spectrophotometry. Collagen

IV presence was detected by immunohistochemistry. To recelularize 10^6/ml of HEPG2 cells were cultured over the matrix. Cell presence in the recellularized matrix was visualized by DAPI and secretion of albumin was detected by ELISA.

Results: After 3 days, this new protocol, based on the use of sodium-deoxycholate as detergent, was effective in preserving all ECM structures and the vascular system. DNA quantification indicated that >97% of cells were removed. Electron microscopy showed the presence of collagen in ECM and cells could not be detected. Cell seeding showed that ECM could be recellularized after 15 days.

Conclusion: By enabling the achievement of liver scaffolds with preserved tissue integrity in 3 days at room temperature, this novel protocol brings new perspectives to the development of bioartificial livers.

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In Vitro and *In Vivo* Vascular Cell Response and Release Profile for Carbon Monoxide Releasing Molecules Incorporated within Electrospun Scaffolds

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Carbon monoxide (CO) has the potential to improve endothelial function in small-diameter vascular grafts. CO is a cell-signaling molecule produced naturally in the body with anti-inflammatory properties at appropriate concentrations. We previously reported controlled CO release from scaffolds with visible-light activated carbon monoxide releasing molecules (CORMs). However, incubation time limitations for the anthracene-based diketone CORM (DK1) were found. In this study, we aim to extend the culture time that allows CO release by using a more hydrophobic CORM and determining the endothelial cell (EC) response to this material. *In vivo* response was investigated in a pilot study. Both DK1 and the aliphatic chain modified diketone (DK3) were synthesized. Poly(ε-caparolactone) with 0-2% w/w CORMs were electrospun. Scaffolds were activated with 470nm light and CO release was tracked through fluorescence signal. We determined activation profile comparison of wet versus dry for all scaffold conditions. Maximum relative fluorescence was at 180s for DK3 ($n=7$). The ability to extended incubation time prior to activation and CO release was demonstrated (71.4 and 79.1% for 1 and 24 hours of incubation versus the 3 min reading, respectively; $n=3$). Further, cell studies with DK3 are ongoing. Preliminary DNA analysis shows a significant increase in EC density from day 3-14 when seeded on DK3. In addition, *in vivo* pilot study results demonstrate patency, viability and cell infiltration through the graft wall when conduits are implanted into the aorta of rats. Von Willebrand Factor of non-activated samples illustrates endothelial cell layer was formed in the lumen.

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Mg-based Helical Stent Scaffold Biodegradation Analysis : Static, Dynamic, and Porcine Ex Vivo Models

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A bioresorbable metallic helical stent was explored as a new device opportunity (magnesium scaffold), which can be absorbed by the body without leaving a trace and simultaneously allowing restoration of vasoreactivity with the potential for vessel remodeling. In this study, we evaluated Mg-based helical stent scaffold deployment, expandability and subsequent degradation using static, dynamic, and porcine *ex vivo* models. Our presentation showed that a Mg-based helical stent was uniformly expanded up to 100% compared to its predeployment diameter. By assessing stent degradation in three different environments, we observed: 1) stress- and flow-induced degradation; 2) a high degradation rate in the dynamic reactor; 3) production of intermediate products ($MgO/Mg(OH)_2$ and Ca/P) during degradation; and 4) intermediate micro-gas pocket formation in the neighboring tissue *ex vivo* model. Overall, the expandable Mg-based helical stent employed as a scaffold performed well, with high expansion rate (>100%) in porcine *ex vivo* model.

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Bioprinting Of Prevascularized β-cell Sheet To Improve Islet Transplantation

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Bioprinting of Prevascularized β-cell Sheet to Improve Islet Transplantation Seunggyu Jeon, Hyun-Wook Kang

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Type I diabetes is an incurable autoimmune disease, which is failure in producing sufficient insulin because of loss of β cells in pancreas. Islet transplantation has been considered as a viable option for healing the disease. However, absence of vascular network and low mechanical stability of islet construct are still big obstacles in its clinic applications. Therefore, the way to fabricate prevascularized β-cell construct having reliable stability was studied to overcome the difficulties in this research. Mouse insulinoma cell line 6 (Min6), human umbilical vein endothelial cells (HUVEC) and polycaprolactone (PCL) were applied to fabricate a prevascularized β-cell laden sheet by co-bioprinting of the multiple materials. Min6 and HUVEC laden hydrogel were printed individually within PCL lattice structure having 300μm channel. Time-series micrographs of vessel formation within the sheet was observed. And viability, proliferation and functionality test of β cells were also conducted to show its usefulness. These results successfully demonstrated that the bioprinted β cell sheet having prevascular structures has great potential to treat type I diabetes in clinics.

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Sterilization of Chemically Cross-linked Polyvinyl Alcohol Hydrogel

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Polyvinyl alcohol (PVA) tubular scaffold, crosslinked with trisodium trimetaphosphate, was used as a vascular graft and being tested in a rabbit *in vivo* model. Sterilization of the PVA graft prior to implantation is necessary to eliminate disease-transmitting agents such as bacteria, viruses, spores and fungi. There are a few established terminal sterilization method of a medical device that are approved by regulatory agencies such as FDA, among which γ-radiation (25kGy) and ethylene oxide (EtO) were chosen as potential sterilization methods. Studies have shown the effects of these sterilization techniques to the physical properties of PLA and PLGA[1,2]. We hypothesize that γ-radiation and EtO also affect physical properties of PVA. Grafts with 2 mm ID were fabricated with dip casting method[3]. We fabricated grafts with 2 different radial compliance ranges (0.1-0.4% and 1.0-1.4%). The γ-radiation softened PVA, increased the compliance by 0.6-1.3% and reduced Young's modulus by 0.2-0.4 MPa, while EtO decreased PVA compliance by 0.5-0.8% and increased Young's modulus by 0.1-0.2 MPa. In addition, sterilizations of micro-patterned PVA film with gratings, convex and concave lenses topography further show the effect of both

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Collagen Bioinks for 3D Bioprinting

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This poster discusses native extracellular matrix bioinks. Presented are a number of unique collagen-based bioinks that have been shown to support the growth and proliferation of various cell types and can be printed into a variety of structures. A detailed comparison of completely native collagen bioinks with other bioink formulations that consist of partially native and synthetic materials. Bioinks being compared include: methacrylated collagen, high concentration collagen, mixture of methacrylated/high concentration collagen, methacrylated gelatin, mixture of gelatin/alginate and collagen/hyaluronic acid. Presented will be bioprintability, mechanical and structural properties, printing resolutions, shear thinning, gel stiffness, extrudability and cell viabilities.

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Biomimetic Self-Mineralizing Polysaccharide Material for Bone and Cartilage Tissue Engineering

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Creation of engineered tissue constructs requires a biomaterial scaffold that not only provides structural support but also mimics the extracellular matrix (ECM) for cellular processes until native tissue forms *in vivo*. Specifically, cell-instructive scaffolds for tissue engineering applications mimic important characteristics of ECM-based microenvironment. In this study, bacterial cellulose (BC), a biomimetic self-mineralizing polysaccharide material, was used to evaluate its biocompatibility for application in bone and cartilage tissue engineering. The fabricated BC and BC-modified hydroxyapatite (BC-HA) scaffolds were characterized for its morphology, topography, elemental information and thermal properties using Scanning Electron Microscope (SEM), Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy, X-Ray diffraction, Thermogravimetric Analysis (TGA), and Differential Scanning Calorimetry (DSC). This study also examined the biological properties and osteoconductivity of native BC and BC-HA scaffolds using human-derived placental stem cells (PSCs). BC is highly crystalline material having a cellulose-I structure with entangled fibrils of 40-60 nm in diameter. The crystallinity is severely damaged between 250°C-350°C and decreases for modified BC. The results showed that BC scaffold supports cell adhesion and proliferation of PSCs while supporting osteogenic differentiation of PSCs on BC-HA as compared to native BC for bone tissue engineering.

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Surface Topography of Silk Films Influences the Functional Behavior of Vascular Cells

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Fabrication of vascular grafts by considering the native like micro architectural features may ultimately determine the graft success. Herein, we investigated the role of surface topography on functional behavior of endothelial (ECs) and smooth muscle cells (SMCs). Both patterned and flat silk films from mulberry (*Bombyx mori* - BM) and Indian endemic non-mulberry silk (*Antheraea assama* - AA) were implemented. Patterned silk films were able to guide unidirectional cellular alignment whereas cells cultured on flat films exhibited random distribution. Post 7 days culture, SMCs cultured on flat BM films initiated self-assembly and resulted in formation of cell aggregates whereas flat AA films supported uniform monolayer culture. Patterning of SMCs on BM and AA films induced their functional contractile phenotype as evinced by upregulation of SM-MHC, Calponin and α-SMA genes. AA films also favored higher expression of extracellular matrix (ECM) proteins - Elastin and Collagen, a prerequisite of mechanical resilience. On the other hand, ECs cultured on patterned silk films showed upregulation of eNOS and secreted higher amount of Nitric Oxide (NO) that helps maintaining the blood vessel tone. This study delineates the impact of alignment on functionality of vascular cells.

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Comprehensive Examination Of Mechanical And Diffusional Effects On Cell Behavior Using A Decoupled 3d Hydrogel System

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Hydrogels possess several physical and chemical properties suitable for engineering cellular environments for tissue engineering applications. However, it is still a significant challenge to **independently control the mechanical and diffusional properties** of hydrogels, both of which are well known to influence various cell behaviors in 3D cell culture systems. Here, we have employed **a polymeric crosslinker that allows for the adjustment of the number of crosslinkable functional groups to control the mechanical properties of the hydrogel**. By using the polymeric crosslinker with varying degrees of substitution (DS), **the moduli of the resulting hydrogels could be controlled in a wide range without changing their concentration.** Furthermore, **their diffusional properties were not significantly affected by the changes in DS** as characterized by their swelling ratios, pore diameters and drug release rates. The viability of cells encapsulated in this hydrogel system successfully demonstrated the varying effects of mechanical properties on different cell types, whereas the cells in a conventional hydrogel system were not significantly influenced by changes in diffusion.

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Spontaneous Differentiation of mESC Cultivated on 3D Poly(Lactide-Co-Glycolide) Scaffolds Coated with Fibronectin

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The use of adhesion molecules play an important role in mouse embryonic stem cells (mESCs) balance between pluripotency and differentiation. The aim was to evaluate the spontaneous differentiation of mESCs cultivated on 3D PLGA scaffolds coated with fibronectin. For the electrospinning the polymer poly(D,L-lactide-

co-glycolide) was dissolved in dichloromethane:ethanol (8:2). The scaffolds were hydrolyzed by NaOH and arranged in control, gelatin or fibronectin groups. Gelatin-coated plates were used as control. The scaffolds were characterized by SEM for fiber diameter and contact angle. A total of 1.5x104 cells/cm2 was seeded and analyzed after 5 days without LIF. The morphology (Confocal microscopy), viability (MTT test) and differentiation (Nestin, alpha-SMA and Sox-17 immunofluorescence) of the mESCs/scaffolds were evaluated. The fibers were well-shaped, with a diameter around 4.1 μm; the contact angle of the gelatin was the lowest ($P < 0.05$). All the groups presented cells with morphological changes and viability tests with similar results, but the cells on the plate presented a significantly higher viability ($P < 0.05$). The results show few Nestin+ and Alpha-SMA+ cells on the scaffolds and no positivity for Sox-17 in all the groups. Therefore, the scaffold structure allows for the mESCs to maintain pluripotency for longer than the plate. The study shows that the 3D scaffold structure allows the mESCs to change the balance between maintaining pluripotency and differentiation, favouring maintenance of pluripotency and assisting in inhibiting differentiation. Therefore, the 3D system for a mESC culture can be considered an important tool for the study of cell fate determination. Financial support: CNPq/CAPES/FAPERGS/IPCT.

Cancer

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Electrospun Three Dimensional Models of Osteosarcoma Tumor Microenvironments

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Osteosarcoma (OS) is the most common primary tumor of the bone, comprising 56% of all bone cancers, and is the third most common tumor of adolescence. Though modern treatments have increased the 5-year survival to ~70% when disease is localized, the survival for patients who present with metastatic disease—roughly 30%—hasn't changed in more than four decades. The inability to cure metastatic OS patients is due in large part to phenotypic heterogeneity that exist both within the same tumor and among different tumors in the same patient. This problem is compounded by the lack of accurate pre-clinical models of OS that rely heavily on standard 2D monolayer culture on hard plastic surfaces which has been shown to alter differentiation and phenotype. Recent attempts at the identification of aggressive tumor cell phenotypes in cancer have involved hydrogels, organoids, microfluidic devices, and suspension culture. Three-dimensional environments have been shown to generate more aggressive cells characterized by increases in drug resistance and tumorigenic potential. This works aim to develop three-dimensional microenvironments in electrospun poly-caprolactone (PCL) suitable for assessing cancer biology, including critical pathological pathways and drug resistance mechanisms. Osteosarcoma cells in our systems have been shown to have differential activation of the IGF-1 and Wnt pathways which are critical to OS progression, differential growth patterns and phenotypes, and reduced responsiveness to chemotherapy that may more accurately represent the behavior of tumors *in vivo*. Our model system provides a new platform for the study of divergent cancer phenotypes, cancer biology, and mechanisms of resistance.

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Involvement of Extracellular Matrix in the Metastasis of Breast Cancer Cells

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Breast cancer, as the most common cancer among women, affects approximately one out of every eight women over their lifetime. The malignancy of breast cancer is mainly attributed to its invasiveness and its metastasis to remote sites (e.g., lung, bone) for secondary tumor. Despite the tremendous progress in therapeutics and diagnosis, it remains a big challenge to treat those metastatic ones. Thus, mechanistic understanding of the regulation of metastasis would be of great benefits. While identifying the essence of extracellular matrix (ECM) in mediating chemotaxis to breast cancer cells, development of an *in vitro* system that can recapitulate the key physicochemical attributes of ECM becomes highly desirable. In this regard, efforts were specifically made to create tissue-specific ECM-like matrices by electrospinning the solutions containing tissue-ECM-specific macromolecules into fibers (average fiber diameter = 500-600nm). These fibrous matrices were morphologically, compositionally and dimensionally similar to the tissue ECM. Breast cancer cells (MDA-MB-231 and MCF-7) cultured on these matrices were analyzed for various metastatic markers like vimentin, vinculin, focal adhesion kinase, and e-cadherin. Interestingly, cancer cells exhibited varying morphology, protein expression and migration profiles on different tissue-mimetic matrices. which might undergo non-canonical pathways. Taken together, the prepared biomimetic matrices are effective to unveil the effect of tissue-specific composition on phenotypic expression of breast cancer cells.

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Enhanced Cytotoxic Effect of Two Novel Curcumin Derivatives on Chemoresistant Cell Lines

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Cancer remains the second leading cause of death in the United States despite the numerous drugs and therapies developed for its treatment, largely due to the occurrence of multiple drug resistance, distant metastases, and tumor recurrence. As a result, there remains a demand for new anti-neoplastic compounds. Natural products, such as those isolated from medicinal plants, have been a historically rich source pharmaceutically active compounds, especially in the context of cancer. One such natural product which has garnered much attention and study as an antioxidant, anti-inflammatory, and anti-cancer agent among other properties is curcumin, an active constituent of turmeric. However, the clinical effect of curcumin in humans has been hindered by its poor bioavailability, likely a result of its rapid metabolism or excretion and its poor absorption. Two novel, semi-synthetic derivatives of curcumin were synthesized with the aim of increasing the bioavailability and efficacy of the compounds against cancer. These derivatives were exposed *in vitro* to a doxorubicin resistant ovarian carcinoma cell line and a 5-fluoro-2'-deoxyuridine (FdUrd) resistant colorectal carcinoma cell line. The curcumin derivatives were able to induce cell death in both cell lines and exhibited higher toxicity than curcumin in the resistant colorectal carcinoma model. Further, the derivatives showed an enhanced ability over curcumin to act synergistically with FdUrd to inhibit cell proliferation. The strong cytotoxic effect demonstrated by these derivatives may indicate a better potential clinical efficacy for their use in the treatment of cancer.

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WITHDRAWN

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3D bioprinted Environments For Toxicological Studies On Multi-cellular Tumor Spheroids

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Using 3D-bioprinting, we've developed a method to create 3D constructs capable of maintaining cell culture for extended periods of time (> 30 days) by embedding cancer cells into a hydrogel composed of alginate/gelatin. Within that time the embedded cancer cells reorganize into multi-cellular tumor spheroids (MCTS). Breast cancer cells (MDA-MB-231 and MCF-7) were independently loaded ($1x10^6$ cells/ml) into the hydrogels, sequentially printed and the cell-laden construct was crosslinked with $CaCl_2$ (10 mM) before culturing. Cell reorganization into MTCS was monitored using fluorescence microscope. Drug-response tests were performed at different periods of time considering different conditions (single cell and different MCTS sizes between 100-500 μm). Cell live/dead counts after exposure to either doxorubicin or 5-fluorouracil were studied using Live/Dead assays (Thermofisher) and the viability was evaluated by MTS assays after digesting the gels and by the signal ratios from controls and treated samples. Drug uptake is confirmed by fluorescent microscopy after 24 h of exposure. The signal ratio after 250 h of exposure of green (live) to red (dead) and fluorescent images indicate that the MTCS maintain their shape and viability. Overall, toxicological responses against anti-cancer drugs suggest that cancer cells in 3D bioprinted environments respond significantly different compared to conventional cell culture toxicology screens.

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An *In Vitro* Model of Glioblastoma

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The aim of this project was to create a bioengineered 3D *in vitro* model of glioblastoma (a brain cancer arising from astrocytes) which faithfully recapitulates the tumor architecture along with its intratumor heterogeneity and microenvironment seen *in vivo* in humans. The cell lines U138, U373, U87, U87EGFRviii, A172, and human astrocytes were labeled with individual fluorescent dyes and employed to create multicellular spheroids, thus encompassing all the major mutations observed in glioblastoma. Spheroids were encapsulated in a hyaluronic acid/gelatin hydrogel that mimics the brain extracellular matrix. We studied tumor growth, migration distances, proliferation and viability of the different cell lines at different time points and in different stiffness environments. Metrics were analyzed using microscopy and macro-confocal imaging. We observed that cell lines with EGFR mutation or upregulation like U87EGFRviii, A172 and U373 proliferated at a higher rate than other cell lines in the spheroid in a stiffer environment. We believe that the observed phenomena - different cell lines dominating the spheroids at different time points - replicated tumor evolution of changing heterogeneity which is observed in glioblastoma clinically. Our results demonstrate that this platform is a good model to study disease progression kinetics and cancer evolutionary dynamics under different conditions and it is amenable to drug testing as well. We are currently adapting this system for use in personalized precision medicine by employing patient tumor biospecimen-derived cells within the platform to track clonal evolution and assess individual patient response to therapies.

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Rheology vs Alignment: Two Operators of the Mechanical Tumor Microenvironment

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The tumor microenvironment is a complex space consisting of a number of stimuli such as: stromal cells, signaling and paracrine factors, as well as extracellular matrix (ECM) components. Each of these factors push and pull cancer cells in different directions and the sum of the interactions defines a cancer cell's final disposition. Much has been made of the microenvironment in recent years; one hotly researched aspect is ECM mechanics. In terms of the mechanical interaction of the ECM and the cancer cell, a number of observations have been made, such as: stiffer environments produce more aggressive cancer cells and facilitate migration and invasion, and aligned matrices are associated with healthy tissues while unaligned matrices are associated with poor prognosis. Although there is a wealth of literature describing the interaction of both stiffness and alignment, there are few studies that determine the relative input of either stimuli. In this study, we produce submucosal constructs with varying ECM alignments and stiffnesses to generate a toolbox of ECM compositions. Using HCT-116 cell spheroids, we then integrate a tumor compartment into the submucosal construct. Finally, we use WNT activity and epithelial-to-mesenchymal transition (EMT) markers to assess the relative effects of stiffness and alignment on cancer cell progression. In all, we observe matrix alignment produces a dominating effect on cancer cell phenotype across the stiffnesses assayed. Higher stiffness is associated with increased WNT activity, but this activity is significantly decreased when spheroids are cultured in highly aligned matrices.

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High-throughput Screening of Brain Cancer-ECM Interactions in 3D using Gradient Hydrogels

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Brain cancer is a devastating disease given its extreme invasiveness and delicate location, yet effective therapies remain elusive. Glioblastoma multiforme (GBM) is one of the most fatal forms of brain cancer. One key hallmark of brain tumors is the significantly increased tissue stiffness as the cancer progresses, however, how varying matrix cues modulate GBM progression in 3D remains largely unknown. The objectives of this study is to develop a facile method that allows fabrication of 3D gradient hydrogels with brain-mimicking biochemical cues and tunable stiffness as *in vitro* brain tumor models, and apply such biomaterials platform for evaluating GBM-niche interactions in a high-throughput manner. Gradient hydrogels were fabricated using our recently published method (2017). The resulting gradient hydrogels exhibit brain-mimicking stiffness ranging from ~40 Pa to 1000 Pa. While all zones support high GBM viability and proliferation, increasing hydrogel stiffness led

significant decreases in GBM spreading in 3D, as shown by actin staining. This is coupled by stiffness-dependent up-regulation of genes responsible for the ECM degradation, which is essential for cancer metastasis. When encapsulated in gradient hydrogels, GBM 270 exhibited differential resistance to Temozolomide (TMZ), a drug clinically used for treating GBM, highlighting the important role of matrix cues on drug responses of GBM. Together, our results validate such 3D gradient hydrogels as a biomimetic cancer cell niche, which enables high-throughput screening studies to accelerate the discovery of the role of niche cues in cancer progression using reduced materials, cells and time.

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Microscale Methods For Investigating Car T-cell Killing Efficacy

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Chimeric Antigen Receptor (CAR) T-cell therapy utilizes T-cells engineered with a CAR to target cancer cells. While CAR T-cell therapy using an anti-CD19 CAR has shown promise in addressing B-cell lymphomas and leukemia, it has not shown equivalent promise in treating solid tumors. We demonstrate a novel microwell based imaging platform for assaying the effectiveness of anti-GD2 CAR T-cell therapy (GD2 is a biomarker for certain neuroblastomas and melanomas) *in vitro*. We developed a mathematical model to fit imaging data to analyze the growth of engineered CAR T-cells and their cytotoxicity. We integrate these platforms to further our investigations. We demonstrate that microwell based imaging can be used to estimate time course of cell death. Parameter estimation can be used to compare CAR T-cells performance within the assay. Refinement and further data collection is required to advance both platforms.

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Modular Tissue Engineering of Bone Microenvironment for Propagating Circulating Tumor Cells

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Circulating tumor cells (CTC) are precursors of all distant metastases, and an important clinical biomarker for cancer diagnosis. Further, understanding their progression to metastasis, particularly in key sites such as bone, is crucial for developing new therapeutic strategies. However, widespread utility of CTC as a clinical and research tool remains exceptionally limited by the inability to reliably propagate and expand their population. We seek to reengineer the close interaction of invading CTC with stromal cells of mesenchymal identity and perivascular location (mesenchymal stem cells - MSC as pericytes), as well as secretory osteoblasts. An *in vitro* recapitulation of the metastatic dissemination to bone could provide 'natural' habitats in which CTC thrive. A two-compartment fluidic device was fabricated in-house using rapid prototyping techniques such as laser engraving, 3D printing, and micromilling. The top compartment, containing osteoblasts derived from human MSC (hMSC) seeded on a semi-permeable membrane, was connected to osteoblast media flow. The bottom compartment, containing hMSC embedded in Matrigel™, was connected to independent connectors that flowed endothelial growth medium, to maintain/drive them towards a pericyte phenotype. Each compartment was reversibly, and individually sealed. By modifying the fluid pressures, each compartment was perfused with their own media. Ongoing studies include phenotypic analysis on the cancer cells to determine their response to the bone microenvironment, as well as seeding CTC cells, isolated from breast cancer patients, in the bottom compartment for analysis of their proliferation profile.

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Biomimetic Surface Modification for the Development of *in vitro* Tumor Models

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Traditional chemotherapy regimens put a high degree of emphasis on the use of historical data to predict a cancer patient's response to a proposed therapy. Unfortunately for the patients, this often leads to continuous rounds of trial-and-error in the search for a compatible treatment, decreasing their chances for survival. Tumor engineering seeks to alleviate this issue by growing patient tumors outside of the body, providing a high throughput avenue for treatment discovery. Utilizing a variety of techniques for 3D culture, researchers have created models that more closely resemble and predict *in vivo* tumor drug responses; however, there is still more room for improvement. In particular, these *in vitro* models consistently exhibit poor cell proliferation and distribution, which severely limits their predictive capabilities. To combat this major issue, we have leveraged our patented biomimetic surface modification platform for tumor engineering applications. In particular, we have identified various moieties specific to certain tumors that are integral to cellular adhesion, and have used these to modify our scaffolds and trick the cancer cells into exhibiting higher rates of adhesion. For instance, in terms of prostate cancer, poly(L-lactic acid) (PLLA) scaffolds were modified to express n-cadherin, which is a highly upregulated protein used for cellular adhesion. After cell seeding, we were able to significantly increase PC3 seeding efficiency and potentially improve cell physiology without compromising the mechanical and degradation properties of the underlying PLLA.

Cardiovascular

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Piezoelectric Vascular Prostheses

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This work focuses on a novel vascular prosthesis, based on an electrosprayed elastomeric porous materials doped with piezoelectric nanoparticles. The nanocomposite prosthesis was seeded with human endothelial precursor cells (EPCs) and stimulated with ultrasonic waves, thus to exploit the indirect piezoelectric effect and to generate electrical charges on the prosthesis surface. Results highlighted an improved differentiation of EPCs on the piezoelectric prosthesis when stimulated by ultrasound, in comparison with non-stimulated counterparts.

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inCITE Optical Clearing affords Unprecedented Interrogation of Macroscale Architecture and Cell/Matrix Content within Bioengineered Heart Valves

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Objective: Long-term function of bioengineered heart valves relies on *in vivo* restoration of native tissue-like cell/matrix content and 3-D architecture. This study explores the utility of novel quantifiable multiplexed multispectral immunohistochemistry (MMI) (up to 8 markers on one slide) and 3-D analysis of cleared tissues using **inCITE -** (**in**dex-matched **C**lear **I**maging for **T**issue **E**valuation) to evaluate these parameters in self-seeding bioengineered heart valves.

Methods: Decellularized pig valves were conjugated with CD133 (a marker for endothelial progenitor cells) (n = 6) and implanted in the pulmonary position of sheep for 3 months. MMI and inCITE

analysis were used to evaluate the expression and spatial relationships of smooth muscle actin, vimentin, collagen, elastin as compared to age-matched native control heart valves.

Results: Cell (endothelial and interstitial) and matrix content (elastin, collagen), as well as biomechanical properties, in engineered valves leaflets were restored to near native values by 3 months. However, both MMI and inCITE analysis revealed that many of the interstitial cells were still myocyte positive, and myofibroblast negative, suggestive of continued maturation. Architectural analysis revealed numerous regions of disorganized cell/matrix and anisotropic matrix/matrix distribution. Specifically cells and matrix had not aligned with flow, as seen in the native leaflets.

Conclusion: While CD133 conjugated implants had native-leaflet like cell and matrix content, they had not established native leaflet-like cell/matrix architecture 3 month post implantation. We propose that inCITE and MMI technology may be more useful than standard histological techniques in predicting successful regeneration of tissues.

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Antithrombogenecity of a PMPC-grafted PEEK Mechanical Heart Valve in Porcine Model

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Lifelong dosing of anticoagulants such as warfarin is inevitable after the implantation of mechanical heart valves. This fact accompanies decreased quality of life. Here, we have been developing and evaluating a polymer-based blood compatible mechanical valve, which requires less dose of anticoagulants. A bileaflet mechanical valve was made of poly(ether-ether-ketone) (PEEK) because it shows good workability and endurance. PEEK has a benzophenone-like structure in its unit, where radicals are generated by photo-irradiations[1)]. Thus, antithrombogenic poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) was directly graft-polymerized on the surface of the PEEK valve by a 27-mW/cm^2 UV irradiation at 60°C for 30 min. The existence of PMPC on the valve surface was confirmed by strong nitrogen and phosphorus XPS peaks. Short-term (<26 h) evaluation of the PMPC-grafted PEEK (PEEK-g-PMPC) valve was conducted by an aortic valve replacement in porcine model, where no anticoagulants were used after the operation. Six miniature pigs were divided into two groups: PEEK-g-PMPC valve transplantation (n=4) and PEEK valve transplantation (n=2; as the control). SEM observation showed that blood clots formed by hematocytes and fibrous materials attached to the PEEK valve surface within 2 h post-implantation. These clots were likely to grow to cover a part of the surface by 10 h. In contrast, such growing clots were not observed on the PEEK-g-PMPC valve even at 26 h post-implantation. Long-term antithrombogenicity of the PEEK-g-PMPC valve is now being investigated. The authors thank Profs. Hirano and Nagasaki for their support of the operation. 1) ACS Appl Mater Interfaces 1, 537, 2009.

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Neointima Formation Process On Peptide-modified Acellular Graft

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Introduction: Our previous report demonstrates an excellent patency of the peptide-modified acellular grafts measuring 20-30 cm in length and having 2 mm inner diameter as femora-femoral crossover bypass in minipig transplantation model[1]. Neointima was observed on the graft surface after transplantation for one week. However, it was not able to identify how rapid endothelialization was occurred with long-bypass graft. In this presentation, we discuss the rapid endothelialization process on the graft in minipig transplantation model.

Materials and Methods: After transplantation into minipig for 1, 3, and 7 days, the luminal surface of the peptide-modified grafts was stained with antiCD31, CD105, CD34, and Flk-1 antibody to identify the cell type on luminal layers.

Results: After one day transplantation, the cell indicating CD34 and Flk-1 positive were observed on center part of the graft where was 10 cm far from anastomotic site. The cells formed layer on the surface, and expressed CD31, 34, 105, and Flk-1 after 3 day.

Conclusion: The neointima was formed by the capturing of blood circulating endothelial progenitor cells during 1 to 3 day after transplantation.

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Conflict of interest: The authors declare that they have no conflict of interest.

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Beating Clusters Created with Cardiac Extracellular Matrix from Decellularized Porcine Hearts and Repopulated with Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes

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Beating human heart tissue was created *in vitro* using non-immunogenic scaffolds generated from decellularized porcine hearts combined with cardiomyocytes (CMs) derived from human induced pluripotent stem cells (hiPSCs). Whole porcine hearts were decellularized to create 3D scaffolds capable of supporting the human cells mechanically and biochemically. From the left ventricle of the decellularized hearts, 300 μm thick, 10 mm round slices were prepared and mounted on glass coverslips. Human iPSCs were differentiated into cardiac progenitors and 4 days after differentiation, these cells were seeded onto the decellularized tissue samples. Ten days after recellularization, clusters of differentiated CMs started to beat spontaneously. Immunofluorescence images showed confluent coverage of CMs on the decellularized slices and the effect of the scaffold was evident in the alignment of the CMs in the direction of the collagen fibers. A resazurin-based viability assay showed attachment and survival of 75% of the seeded cells up to 14 days after recellularization. The clusters continued to beat for 60 days. To improve the beating function of the CMs implanted in the tissue scaffold, we are using mechanical, electrical and pharmacological stimulation. By combining mechanical stimulation through a mechanical stretching device, electrical stimulation through transmitting electrical frequencies ranging from 1 to 1.6 Hz, and pharmacological stimulation through drugs, such as epinephrine, we hypothesize that we will increase the beating function of the cardiac tissue from 10 beats per minute (current rate) to 60 beats per minute.

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In Vitro Fabrication of Functional Anisotropic 3D Constructs using Silk-Cardiomyocyte Monolayers

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Cardiac tissue engineering seeks to develop a construct with ability to restore the structural and functional aspects of infarcted myocardium[1]. In this context, our study uses mulberry (*Bombyx mori*) and non-mulberry (*Antheraea assama*) silk fibroin to fabricate 3-D construct of cardiomyocyte monolayers using patterned silk films. These fabricated films exhibited effective swelling, biodegradability and enhanced mechanical properties. They provided contact guidance to the growing cardiomyocytes allowing them to form unidirectionally aligned cell monolayers

and exhibited low immunogenicity both *in vitro* and *in vivo*. Enhanced proliferation and maturation of primary rat cardiomyocytes (PCMs) and H9c2 cells was observed on non-mulberry films, owing to the presence of intrinsic cell binding motifs (RGD)[2]. This was further attested by the upregulation of cardiac specific genes on non-mulberry silk films. The patterned silk-cardiomyocyte monolayers were then stacked onto each other to form 3-D construct. Histological assessment exhibited uniform cardiomyocyte distribution and maintenance of an intact 3-D structure. Moreover, increased DNA content of the construct indicated the absence of cell necrosis. Collectively, this study highlights the suitability of non-mulberry *A. assama* silk fibroin as a potential biomaterial and the prospects of using silk-cardiomyocytes monolayers for cardiac tissue engineering applications.

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Pulmonary Artery Reconstruction using In Body Tissue Engineered Bovine Biosheet: Xenotransplantation in a Beagle Model

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Purpose: Upon implantation as a scaffold of autologous or allogenic collagenous tissues prepared subcutaneously in molds using our developed in-body tissue architecture technology (IBTA), which can form membranous (Biosheet) or tubular (Biotube) tissues, in situ reconstruction to target cardiovascular tissues, such as arteries or heart valves, could be successfully occurred within several months after implantation. However, this approach was encumbered by long production times (1-2 months). The solution would be transition to a xeno graft that is available off-the-shelf. This study presents the first use of bovine Biosheet to demonstrate the feasibility and safety for transitioning to a xenoimplantation in a beagle model.

Methods and Results: Collagenous tubular tissues were formed in stainless steel molds by their embedding into bovine subcutaneous pouches for 2 months, harvesting, and removing. Upon cutting open the prepared tubes, Biosheets (thickness 0.5mm) were obtained as membranous tissues and stored in alcohol solution before use in implantation. Under anesthesia, pulmonary arterial defects (size; 10×10 mm) in beagles were made, and patched repair was performed with the bovine Biosheets. Follow-up ultrasound examination indicated no thrombosis or stenosis. After 1-month implantation, histological observation showed vascular remodeling on the implanted Biosheets with no signs of degradation or dilation. There was no evidence of immune response.

Conclusion: In this study, xenogenic bovine Biosheet could be successfully engrafted in short-term implantation into beagles. It may have potential to use of bovine Biosheets for implantation during any time of need as ready-made xenogenic tissues.

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Multi-factor Analysis of Synthetic and Natural/Protein Content in Engineered Vascular Tissues using Raman Spectroscopy

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Tissue-engineered vascular grafts (TEVGs) are a promising alternative for small-diameter arteries. It is important that TEVG composition prevents compliance mismatch and improves viability. Raman spectroscopy has been used to assess components of tissue-engineered scaffolds. However, Raman has not been applied to semi-quantitatively assess 3D engineered-tissues and cellular remodeling of natural/synthetic-blend materials. Electrospun meshes were prepared with 100% poly(ε-caprolactone)(PCL), 90% PCL/collagen, and 90% PCL/fibrinogen. Meshes were characterized using SEM, XPS, and Raman. Meshes had similar fiber diameters (0.63 ± 0.20—0.92 ± 0.39μm), and were characterized without incubation, with incubation in media, and with culture of smooth muscle cells for 7-21 days. Differences in cell proliferation and contractile markers (RT-PCR) were observed, especially with collagen incorporation. Raman peak integration showed increases in 740cm^{-1} ($p < 0.0001$) and 1050cm^{-1} ($p < 0.0001$) peaks for protein-blended samples. However, the strong PCL signal prevented resolution of the 1550cm^{-1} peak found in pure fibrinogen but not collagen. Subtracting the PCL spectra showed characteristic fibrinogen peaks in blended materials, but these results were not quantitative. Thus, multivariate-factor analysis (MFA) was used to evaluate changes in mesh composition after culturing, with factor scores plotted against wavenumbers. Factor 1 spectrum is from PCL, whereas Factors 2-3 are from a linear combination of incorporated proteins and other components post-culture. Overall, this work demonstrates that MFA analysis of Rama spectra can assess natural material incorporation into TEVGs. We are currently performing non-negative least squares (NNLS) analysis to determine if additional resolution can be obtained to detect small changes with remodeling that cannot be reliably assessed with traditional techniques.

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Designing A Novel Pouch To Minimize Peritoneal Adhesions With *In Vivo* Bioreactor- Based Vascular Graft Strategies

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Tissue engineered vascular grafts require strategies to allow graft remodeling but avoid stenosis and loss of graft mechanics. In our approach, we use the peritoneal cavity as an ''*in vivo* bioreactor'' to recruit autologous cells and improve graft patency. In a previous study, we generated vascular grafts by implanting poly(ε–caprolactone)-based electrospun conduits enclosed within an ePTFE pouch into the rat peritoneal cavity for 4 weeks. The results demonstrated that the constructs remained patent after being grafted autologously for 6 weeks into the abdominal aorta, and peritoneal pre-implantation reduced overall lipid oxidation, intimal layer thickness, and expression of macrophage markers. In this study we plan to overcome the potential side effect of peritoneal adhesion formation by designing a new pouch composed of poly(ethylene glycol) diacrylate (PEGDA) to replace ePTFE pouches. While PEG is used to provide resistance to protein adhesion and cell attachment, our strategy is unique in simultaneously creating pores to enable tissue generation in the enclosed conduits. A pilot study was performed to determine the response in a rat model, and the results demonstrated a notable decrease in formation of peritoneal adhesions. Immunofluorescence images showed similar cell infiltration and expression of endothelial cell and contractile markers as the samples from an ePTFE pouch. Compressive properties of these pouches are being studied at different PEGDA concentrations and pore diameters to find the pouch parameters that give desired mechanical properties. Overall, the results showed apparent benefits of peritoneal pre-implantation and effective reduction in peritoneal adhesions when a PEG pouch was utilized.

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Evaluation of Novel Therapeutics To Treat Neonatal Coagulopathy

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Post-operative bleeding is a serious complication for neonates undergoing corrective cardiac surgery requiring cardiopulmonary bypass (CPB). Mitigating post-CPB bleeding is currently addressed through transfusion of adult blood products, including platelets and cryoprecipitate (fibrinogen component). However, the effectiveness of such transfusions is inconsistent in neonates; additionally, multiple infectious and noninfectious risks are associated with blood product transfusion. Moreover, neonatal clotting components display qualitative and quantitative deficiencies, including in the primary coagulation protein fibrinogen[1]. The goal of this study is to 1) evaluate new hemostatic therapies and 2) establish *in vivo* models of neonatal coagulopathy. We aim to determine how different pro-coagulant agents alter neonatal fibrin matrix properties. Plasma was obtained from neonates scheduled for cardiac surgery and CPB. Samples were collected before and after CPB and following the transfusion of cryoprecipitate. Pro-coagulant therapeutics were added to post-CPB samples. Confocal microscopy and Atomic Force Microscopy (AFM) were utilized to examine clot structure and mechanics. Our long-term goal is to decrease transfusion requirements by determining more efficacious and safe methods of enhancing clot formation in neonates following complex cardiac surgery. In order to facilitate future preclinical studies for neonatal specific therapies, we additionally aim to validate a porcine model of neonatal coagulopathy.

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Cellular Cross talk And Paracrine Interactions Between Epicardial Adipose Tissue And Myocardium

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Epicardial adipose tissue (EAT) plays an important role in normal and pathological cardiac function. There is interest in investigating the cross-talk between EAT and the myocardium. What is needed is a preclinical translatable model system in which these interactions can be studied and physiologically relevant treatments performed. We aim to understand the molecular regulatory mechanisms of EAT in order to provide insights into the treatment of cardiovascular diseases and regenerative medicine. We set up a co-culture system of human cardiac and EAT tissues (from the same patient) using two-organ-chips. The circulating conditioned medium and the tissue pieces were collected for proteome profiler, metabolomics and histological analysis. Our preliminary proteome analysis indicates a higher expression of proteases involved in extracellular matrix degradation and remodeling under dynamic co-culture, which may in turn result in vascular formation in both cardiac tissues and EAT. It has been reported that cardioprotective factors reverse some harmful effects of EAT-derived factors in heart failure patient. In our experiment, the expression of a cardioprotective factor was higher in the co-culture under dynamic condition. A higher level of matrix proteases as well as a higher expression of these protective factors stimulate endothelial cells and vascular formation under dynamic condition as compared to static culture. Our ongoing metabolomics analysis indicates the difference between the dynamic and static condition. We believe that our final results will identify clinically relevant processes/pathways or secreted factors, as potential new targets, which might allow pharmacological interventions in the field of cardiovascular and metabolic diseases.

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Optimization of MSC/iPSC-EC Vascular Network Formation in Fibrin Scaffolds Using Statistical Design of Experiments

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Engineered tissues with pre-formed vascularization may enhance regeneration. Induced pluripotent stem cell-derived endothelial cells (iPSC-EC) are a promising EC source for clinical application, but their investigation for tissue engineering applications has been limited. Design of experiments (DOE) methodology can help optimize multivariable systems and determine effects and interactions that may not be elucidated in traditional experimental design. This study aims to utilize DOE to optimize the *in vitro* formation of vascular networks of iPSC-EC and mesenchymal stem cell (MSC) co-culture spheroids in fibrin scaffolds. 5000-cell spheroids of iPSC-ECs and MSCs (0, 25, 50, 75, or 100% MSC) were encapsulated in fibrin hydrogels (1, 3, or 5 mg/ml) and cultured for 1, 2, or 3 weeks. Immunostaining for CD31 was used to assess EC outgrowth and quantify EC network area, mean vessel length, and mean vessel diameter. iPSC-EC/MSC spheroids formed extensive networks throughout the scaffold that varied with ratio, fibrin concentration, and culture time. The highest EC network area ($2.53 \pm 0.48\,mm^2$) and mean vessel length ($969.6 \pm 79.3\,\mu m$) was observed with 75% MSC spheroids in 3 mg/ml fibrin after 3 weeks. The *in vitro* results were used to create a DOE predictive response model (MODDE Pro Software) and generate contour plots for each response. Based on the model, a spheroid composition of approximately 60% MSC will result in the highest EC area, and approximately 67% MSC will result in the highest vessel length. iPSC-ECs show promise as a potential autologous EC source for vascularization of tissue engineering strategies.

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Fabrication of Modular Tissue Engineered Blood Vessels

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Tissue engineered blood vessels (TEBVs) enable *in vitro* disease modeling and drug testing. However, existing TEBV fabrication approaches create homogenous tubes, which lack the capacity to model focal diseases such as intimal hyperplasia or aneurysm. Alternatively,

we developed a modular system to fabricate TEBVs from tissue ring building units. By combining individual ring units, spatially distinct tissue regions can be created along the tube length. To create rings, human smooth muscle cells are seeded into custom agarose molds, where they self-assemble into 3D tissue rings. In previous work, we showed that gelatin microspheres can be incorporated within rings during self-assembly to allow local growth factor delivery and creation of tissue ring units with distinct structure and function. Here, we use ring building units to create and dynamically culture tissue tubes on a custom designed bioreactor. Custom electrospun polymer cuffs were fused onto tube ends to create reinforced material for improved handling and cannulation. After 3 days of ring culture and 7 days of ring fusion, tubes were cannulated. Cuffs fit snugly over cannulas, without the need for sutures, enabling dynamic culture (2 dyne/cm^2 flow) for 7 days before fixation. To create tubes with focal heterogeneities, rings with incorporated microspheres were fused in a region between rings without microspheres. Smooth muscle cells within rings with microspheres maintained their spatial position, indicating the potential of this system for creating distinct tissue regions characteristic of focal vascular diseases. In ongoing work, we are evaluating localized microsphere-mediated growth factor release to simulate intimal hyperplasia.

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TG2 Expression in Arterial TEVGs

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Objectives: Tissue-engineered vascular grafts (TEVGs) require adequate extracellular matrix (ECM) to withstand arterial pressure. Tissue transglutaminase (TG2) is an enzyme that cross-links ECM proteins and plays central role in the development of vascular stiffness associated with aging. The purpose of this study is to investigate ECM-TG2 expression and vascular stiffness in TEVG remodeling.

Methods: Fast- and slow-degrading electrospun scaffolds were fabricated from polydioxone (PDO) and poly(L-lactide-co-caprolactone) (PLCL) copolymer, with a PDO/PLCL ratio of 9:1 being fast-degrading and 1:1 being slow-degrading. The two types of small-diameter arterial TEVGs were implanted in rats ($n=5$/group) as abdominal aorta interposition conduits. The grafts were harvested at 1 month for evaluation of patency, vascular neotissue formation, TG2 expression, and mechanical properties.

Results: All TEVGs were patent and did not display any dilation at 1 month. ECM area in fast-degrading TEVGs was significantly larger than that in slow-degrading TEVGs ($42.91\pm4.21\%$ vs. $14.5\pm2.33\%$, $p<0.001$), with significantly larger TG2 area as well ($26.26\pm8.55\%$ vs. $7.55\pm1.83\%$, $p=0.0014$). The mechanical properties of fast-degrading TEVGs were akin to that of native aorta, as demonstrated by strain-stress curve. The area of remaining scaffold was significantly reduced at 1 month in fast-degrading TEVGs compared to the slow-degrading TEVGs ($2.84\pm0.86\%$ vs. $18.53\pm6.65\%$, $p<0.001$).

Conclusions: At one month, rapid and well-organized ECM in fast-degrading TEVGs led to increased TG2 expression and native-like mechanical properties, compared to slow-degrading TEVGs.

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3D Tissue-engineered Model of Pressure-overload Induced Cardiac Fibrosis

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Heart failure is characterized by extensive fibrosis contributed by activated and proliferative cardiac fibroblasts (CFs). CFs interact with cardiac myocytes and dynamically modulate heart function, representing a potential target for therapeutic interventions [1]. Here, we developed an *in vitro* model of cardiac fibrosis by 3D co-culture of CFs from failing adult mouse hearts and healthy neonatal rat cardiomyocytes. Transverse aortic constriction was applied for 12 weeks to induce pressure overload and heart failure in adult mice. Distinct fractions of CFs from failed and age-matched sham-operated (control) hearts were isolated using FACS based on the expression of surface markers and were immediately encapsulated within fibrin-based 3D engineered cardiac tissues without 2D subculture known to profoundly alter cell phenotype [2]. Compared to use of control CFs, engineered cardiac tissues with CFs from failing hearts exhibited significantly elevated Collagen I and III, prolonged action potential duration, and decreased contractile force, closely resembling the phenotype of pressure-overload fibrosis and heart failure *in vivo*. Interestingly, these structural and functional effects were CF-fraction dependent. This 3D model of cardiac fibrosis represents a well-controlled platform for systematic studies of the fibroblast-induced changes in cardiomyocyte phenotype aimed at discovery of novel fibroblast-targeted therapies for heart failure.

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Commercialization of Regenerative Medicine Products

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Automated Closed-System Bone Marrow-derived hMSC Isolation for Regenerative Medicine Biomanufacturing

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With over 500 clinical trials investigating the use of mesenchymal stem/stromal cells (hMSCs) for Regenerative Medicine, there is a critical need for economical biomanufacturing processes capable of generating billions to trillions of cells for commercial applications. An efficient hMSC isolation process is the foundation for realizing economically viable hMSC-based therapeutics. The traditional method for hMSC isolation from buffy coat, using manual density gradient separation, is an open process susceptible to contamination and substantial operator dependent variability. We hypothesize that an automated closed-system process will reduce variability and maintain cell quality while also streamlining the regulatory process. The Sepax processing system (GE/BioSafe) was evaluated for isolating hMSCs from bone marrow (BM). Fresh BM aspirates (AllCells), from multiple distinct donors, were processed in parallel using a manual or Sepax method. The resulting mononuclear cell fractions were cultured in RoosterNourish^{TM}-XF media (RoosterBio) for 9-10 days, and cell expansion, morphology, identity (surface marker expression, tri-lineage differentiation) and potency (cytokine secretion, immunomodulatory potential) quality parameters were assessed. The use of the Sepax system yielded a 3x fold increase in hMSCs over the manual method while maintaining cell quality parameters and reducing the time and labor needed for cell processing by ≥50%. Thus, closed automated processing via Sepax is a robust, consistent and reproducible alternative for hMSC isolation. The improved yield along with reduced time, labor, and risk of this method will contribute to the decrease in cost of goods (COGs) of hBM-MSCs for regenerative medicine applications.

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A Post-manufacturing, Therapy Distribution Model

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We present a graph-based supply chain model of (autologous) CAR-T cell therapy that serves as the basis for the development of strategies to: 1) deliver cell therapy products that are safe and have a high level of efficacy, 2) minimize fulfillment time and variability, and 3) reduce total manufacturing and logistics costs while reducing

the risk of patient morbidity and mortality. We then provide a more detailed optimization model of a daily post-manufacturing, therapy distribution problem considering two objectives: minimize total distribution cost and maximize total service level, where service level is directly related to the health of the patient and the health of the therapy and is enhanced with fast fulfillment. A solution to this model would address such questions as: given a set of therapies for distribution and the time when they would be released for distribution, how many delivery vehicles to use, what therapies should be assigned to which vehicles for delivery, what should be the sequence of deliveries for each vehicle, and what should be the departure time for each vehicle from the production facility? The problem objective is to generate a set of non-dominated solutions from which the decision maker (DM) would choose a most preferred non-dominated solution, based on the DM's preferred trade-off between cost and service level. This model could be used to address questions such as: what is the value of real-time decision making based on real-time patient and therapy health information? What is the value of transporting live cells versus cryopreservation?

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A Novel System For The Rapid Isolation Of Human Adipose-derived Stem Cells Using A Gmp Collagenase

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Introduction: Translating basic science discoveries to approved products in the marketplace remains one of the greatest challenges in healthcare. Autologous adipose-derived stem cells (ASCs), when used in clinical practice, will need to meet critical quality attributes (CQAs) to include identity, potency, purity, and safety.

Purpose: This project tests the feasibility of a kit (''Stem cell Isolation Kit'' designed by Reviticell Holdings, Inc., Jacksonville, FL) to isolate ASCs from lipoaspirate to meet the CQA of identity within regulatory, practitioner, and engineering criteria.

Methods: Fresh human lipoaspirate was processed with a GMP collagenase (Corase™, Reviticell Holdings, Inc., Jacksonville, FL), in a closed system. Both short term incubation and centrifugation were included in the processing. ASCs were then cultured. The CQA of identity of the ASCs was matched against ISCT/IFATS criteria.

Results: Each isolation from lipoaspirate took 30 minutes. Cellular viability of the initial stromal vascular fraction (SVF) was >90% with a minimum viable cell count of 160,000 nucleated cells per gram of tissue processed. Cellular phenotypic ontology revealed plastic adherent cells with confirmatory corresponding flow cytometry markers (+CD105, CD73, and CD90, - CD34, CD45, CD11b, CD19, and HLA-DR). Differentiation to adipocytes, osteoblasts, and chondroblasts was confirmed.

Conclusions: The kit-based system, from Reviticell Holdings, Inc., isolates ASCs as identified by the ISCT/IFATS criteria. The kit, with its modular system, can be easily configured to accommodate to new isolation protocols for ASCs of different CQAs when needed. Assays respective to ASC potency, purity, and safety will need to be completed.

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Automated Quantitative Image Analysis and Precision Selection of Desired Sub-Population of iPS Cells Using Cell X™ and Colonyze™ Platform

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Induced pluripotent stem (iPS) cells are being developed for a broad range of research and therapeutic applications. However, reprogrammed iPS clones are inevitably heterogeneous. Value judgements must be made regarding which clones to use or exclude. iPS labs currently make decisions by visual inspection and manual selection, which are prone to large variation and lack the standardization needed for clinical translation. Automated methods are needed that enable rapid, quantitative, repeatable and reproducible: i) imaging and quantitative analysis of iPS clones, ii) objective determination of critical quality attributes (CQAs) (e.g. morphology or markers) that predict long term biological performance and iii) precise automated selection of desired cells or removal non-desired cells. This paper describes the development and validation of Cell XTM, a robust robotic system that enables each of these critical aspects of iPS cell processing and analysis. Large field of view images are acquired from standard tissue culture plates (6 to 96 wells) with 1μ/pixel resolution in 1-8 minutes, using phase-contrast or fluorescence. Integration with ColonyzeTM software enables Automated Multimodal Large field of view Image Analysis (AM-LIA) based on metrics of proliferation, migration, surface markers, or cell and colony morphology, using principles outlined in ASTM Standard F2944-12. This integration enables automated image capture, analysis and ''picking'' or ''removal'' of desired or undesired colonies based on defined and reproducible criteria. Cell XTM and ColonyzeTM together eliminated labor-intensive manual processing steps, and provide unprecedented rigor in the analysis and management of heterogeneous iPS cell populations.

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Application Case Study All-in-one Bio 3D Printer, *IN VIVO* from ROKIT Inc

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For about recent a year, approximately 60 customers purchased our all-in-one 3D printers for mostly their research purposes. For the last a year, we can feel sudden increasing movement of tissue engineering and biofabrication research using bio 3D printers and we are introducing its applications case studies through the consulting with our customers. First, cell bio printings with bio-inks is a one big category. Vascularized kidney, cardiac myocyte regeneration, Neuron regeneration for dementia patients, liver function regeneration, Oncology using target therapy, and pancreatic cell regeneration are proposed for tissue engineering researches using our product of 3D bio printers. All the cases include live cell as a printing bio-materials and consist with scaffold design. Second application category is human skin equivalent. Skin is relatively easier than other organs for fabrication sue to is structure. Dermis and epi-dermis are not containing large vascular, so many researchers are working on this topic. One third of our customers were also purchased our 3D bio printers for human skin equivalent. The final applications for HSE are cosmetics test for substitution of animal tests and artificial skin for wound healing for burnt patients.

Third application cases are bone and teeth, hard tissue application. Not just simple or uniform shape of bone-replace scaffold, non-uniform, which is gradually increasing pore size toward outside. This structure may prevent clogging or fouling on the surface and cannot fill the designed structures. In fact, more than half of the studies related to bio-implant is recently focused on scaffolds' internal structure design.

Imaging

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Nano Onion Carbon Dots - a tool for *in vitro* imagining and accelerated wound healing

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Nitrogen, Sulphur and Phosphorous co-doped water-soluble carbon nanodots are synthesized from culinary waste onion peel powder (OPP) by short microwave treatment. Onion Derived Carbon Nano Dots (OCND) comprises of hydrophilic groups decorated amorphous nanodots and exhibited bright stable fluorescence at excitation energy of 450 nm and emission wavelength at 520 nm along with free radical

scavenging property. The OCND exhibited excellent stability at different pH and UV exposure. Although extracted polyphenols degraded in the extract, but interestingly it was evidenced to be cytocompatible and blood compatible as observed during cytotoxicity, fluorescence imagining of cell and haemolysis study. The present work not only focuses on synthesis of OCND from OPP extract, but also provides an interesting fact that even after degradation of polyphenols in the extract; they are non-toxic to human cells (HFF & MG63) and RBCs. Moreover, OCND had no adverse effect on migration rate of *Human Foreskin derived Fibroblasts (HFFs)* as observed from scratch assay. In addition to accelerating migration rate of fibroblast, the OCND altered intra- and extracellular reactive oxygen species (ROS) by enhancing antioxidant mechanism of fibroblast under oxidative stress. Further, OCND was observed to accelerate wound healing in full thickness (FT) wound in rat model on topical application, which can be attributed to its radical scavenging potential. In summary, study leads to a new source of OCND synthesis route, which is inherently co-doped with phosphorous, sulfur and nitrogen and holding a great promise for myriad biological applications including bio-imagining, free radical scavenging and wound healing.

Matrix & Extracellular Matrix Biology in Regenerative Medicine

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Regenerative Nanotherapeutics to Modulate the Tumor Microenvironment in Non-Small-Cell Lung Cancers

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Response rates to emerging immunotherapies for Non-Small Cell Lung Cancers (NSCLCs) are <20%, largely owing to the tumor microenvironment (TME) that favors immune-evasion and pro-tumorigenic pathways such as macrophage polarization from a pro-inflammatory (M1) to an immunosuppressive and pro-tumorigenic/angiogenic (M2) phenotype. The TME is also compromised by chronic enzymatic breakdown and lack of natural regenerative repair of the alveolar elastic matrix with generated elastin peptides also stimulating M1 to M2 phenotypic switch. In prior work, we developed doxycycline (DOX)-releasing polymer nanoparticles that both augment elastogenesis and inhibit elastolysis, effects attributed to both the DOX and cationic amphiphiles functionalized on the NP surface. Since DOX is known to inhibit M1-M2 phenotypic switch[1] in this work we explored the utility of our NPs to accomplish this. We formulated cationic DOX-loaded poly(ethylene glycol)-poly(lactic glycolic-acid; PEG-PLGA) NPs covalently conjugated with an antibody against interleukin 4 receptor (IL4R) on the M1 macrophages, since IL4 activation of IL4R triggers macrophage polarization. The structure-property relationship of the synthesized diblock copolymer and fabricated NPs were characterized as also targeted binding of the NPs to M1 macrophages. We showed that steady and sustained (>30 days) release of DOX from these NPs is possible in the 1-10 μg/ml dose range and at these doses, the released DOX inhibited M1 macrophage polarization as assessed via immunofluorescence, cytokine arrays, and functional assays for pro-angiogenic effects.

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JNK-inhibitory Nanotherapeutics to Augment Vascular Elastic Matrix Regenerative Repair

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Abdominal aortic aneurysms (AAAs) are localized aortal wall expansions resulting from matrix-metalloproteinase (MMP)-mediated disruption and loss of elastic fibers in the AAA wall. Elastic fibers do not naturally regenerate/repair which prevents AAA arrest/regression. We have shown that low μg/ml doses of doxycycline (DOX) stimulate elastic matrix neoassembly and crosslinking by cultured aneurysmal smooth muscle cells (SMCs) by upregulating TGF-β1 upon inhibition of the regulatory protein c-Jun-N-terminal kinase 2 (JNK 2). Using JNK 2 inhibition as a metric for pro-regenerative matrix effects of DOX, we presently demonstrate that sustained, steady-state release of DOX at the useful dose, from poly(ethylene glycol)-poly(lactic glycolic acid) nanoparticles (NPs), provides pro-elastogenic and anti-proteolytic effects more efficacious than exogenous DOX alone, an outcome attributed to previously determined functional properties of cationic amphiphile-functionalized polymer nanocarriers. Released DOX inhibited expression and phosphorylation of JNK which increased expression of TGF-β1 and increased elastogenesis and lysyl oxidase-mediated crosslinking of the elastic matrix. Independent of DOX-release, the surface-functionalized cationic amphiphile reduced expression and activity of MMP-2. Our results suggest that JNK inhibition is a useful metric to assess pro-elastic matrix regenerative effects and point to the combinatorial regenerative benefits provided by our multi-functional DOX-encapsulated NPs.

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C-Jun N-terminal Kinase Gene Silencing as an Effective Modality to Augment Elastic Matrix Regenerative Repair in a Proteolytic Milieu

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Inherently poor regenerative repair of elastic matrix disrupted by chronically overexpressed matrix metalloproteases (MMPs) prevents restoration of tissue homeostasis, as in abdominal aortic aneurysms (AAAs). We previously showed that at doses <10 μg/ml, doxycycline has both anti-MMP and pro-elastogenic effects on aneurysmal smooth muscle cells (SMCs), and that these occur downstream of JNK2 inhibition and then upregulation of TGF-β1. These pro-regenerative effects positively correlated to severity of JNK inhibition. Accordingly, we investigated *JNK2* gene silencing as a modality to more efficiently inhibit JNK2 to augment elastic fiber neoassembly and anti-MMP effects. Cultured AAA SMCs from a rat model were stimulated with cytokines (30 min) and then transfected with a pool of 3 anti-JNK2 siRNA probes, with scrambled siRNA (negative control) or cultured with no siRNA (treatment control). Healthy rat aortic SMCs served as cell controls. We showed efficient transfection of siRNA probes, their inhibition of *JNK2* with no non-specific effects, and subsequent decrease in JNK2 protein synthesis. *JNK2* inhibition positively correlated to TGF-β1 increases, elastic matrix amounts, lysyl oxidase production and enzyme activity and desmosine crosslinking of elastin and elastic fiber formation and negatively to synthesis and activity of elastolytic MMPs 2 and 9. Importantly, our results showed these effects to be more pronounced than that previously achieved by DOX treatment. Our study indicates siRNA-mediated JNK2 knockdown as an efficient mechanism to augment elastogenesis in a proteolytic tissue milieu. This work was supported by funding from the NIH (HL132856), AHA (16IRG27250113), and NSF (1508642) awarded to A.R

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Characterization Of Tissue- And Age- Specific ECM-based Gels For 3D Cell Microenvironment

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The extracellular matrix (ECM) consists of structural and functional molecules secreted by the resident cells. The 3-dimensional organization and composition of an ECM is distinctive for each tissue type. Solubilized gel-forming ECM could potentially be used to

develop products such as 3D culture substrates, growth factor delivery vehicles, or scaffolds that promote tissue regeneration. One goal of ours is to design a 100% allograft custom-fabricated human ECM-derived 3D microenvironment that mimics the biological properties of native ECM. Toward this goal, we evaluated the biochemical and functional characteristics of ECM isolated from human placenta, adipose, and cadaveric muscle tissues. In addition, we compared the properties of muscle-derived ECM isolated from young versus old donors. Proteomic analysis showed the presence of proteins involved in matrix remodeling, angiogenesis, cell growth and differentiation. ECM protein variability largely depended on tissue type and donor age. Regardless of tissue origin, ECM-based 3D gels affected cell morphology by reducing cell spreading. Differentiation of adipose-derived stem cells (ADSCs) subjected to ECM sourced from various tissues showed no effect on adipogenic ADSC differentiation. Contrastingly, we found that the presence of young muscle ECM augmented chondrogenic ADSC differentiation, while old muscle ECM favored osteogenic ADSC differentiation. In summary, tissue- and age-related properties of ECM should be considered in developing 3D culture models for basic and translational research.

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Testicular ECM Enhances Human Spermatogonial Stem Cell Survival in Culture

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Successful human spermatogonial stem cell (hSSC) culture could enable stem cell therapies for male infertility. Conditions for expanding rodent SSCs in culture are established and robust, but hSSC cultures are less successful and no published hSSC culture system has yet been independently replicated. Our long-term goal is to establish a feeder cell-free, serum-free culture system for hSSC. Mammalian extracellular matrix (ECM) contains signaling molecules that promote mitogenesis, migration, and/or differentiation of various stem/progenitor cells. Human testicular ECM (htECM), porcine testicular ECM (ptECM), porcine small intestinal submucosa ECM (SIS-ECM), and porcine urinary bladder ECM (UBM-ECM) were prepared, and used to coat tissue culture plates for hSSC culture. In addition to culture of hSSC upon the ECM materials described above, hSSC were cultured on STO mouse fibroblast feeder cells (control), murine laminin or human laminin. UTF1 positive undifferentiated human spermatogonia were quantified by immunocytochemistry at days 0, 7 and 14 in culture. By 7 days the wells with feeder cells showed $\sim$31% the number of starting UTF1+ cells, whereas wells with htECM or SIS showed 60% and 68%, respectively. After passage and continued culture to day 14, recovery of UTF1+ cells from htECM-coated plates was significantly higher than from cultures with STO feeder cells. Homologous species (human), homologous tissue (testis) ECM improves SSC survival in culture and establishes a foundation for development of robust human SSC culture methods.

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Characterization of Mesenchymal Stem Cell-Derived Bone Extracellular Matrix on 3D-Printed Poly(propylene fumarate) Scaffolds for Critical Size Bone Defect Repair

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Current approaches to critical size bone defect repair are dependent on tissue transplantation, which is associated with donor site morbidity and pain. In many cases there is insufficient donor tissue.[1] The development of bone tissue engineered grafts promises to alleviate these limitations.

Previous work in our lab has produced bone-like extracellular matrix (ECM) from bone marrow derived human mesenchymal stem cells (BM-hMSCs) on poly(propylene fumarate) (PPF) thin films.[2] The goal of this project is to characterize bone ECM produced by the same methods on 3D printed PPF scaffolds. 3D micro-CT (μCT) scan images of the scaffold surface have now characterized coverage and thickness of that ECM coating. Additionally, we are collecting histological, nanoindentation, and compression testing data to further characterize the properties of the ECM that coats these 3D printed, porous scaffolds.

Recent micro-CT imaging of a pilot specimen presented an approximately 5 micron thick ECM that completely covered the scaffold. Our goal is to tune our *in vitro* BM-hMSC culturing methods to produce a 30-70 micron uniformly thick ECM coating on these scaffolds. Those methods will also be tuned to insure that this artificially-created bone ECM has mechanical properties that are similar to native bone ECM.

1. Armini AR, *et al*. Crit Rev Biomed Eng. 40, 363, 2012.
2. Mishra R, *et al*. Tissue Engineering. 22, 2016.

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From Cover to Core: Acellular Human Dermis for the Regeneration of Human Heart

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Elasticity of myocardium is mostly due to elasticity of cardiomyocytes and is essential for cardiomyocyte alignment and differentiation. Cardiac decellularized ECM (d-ECM) is emerging as natural scaffold to promote and support myocardial regeneration. It is noteworthy that cardiac d-ECM is obtained through complete removal of cardiomyocytes with loss of elasticity. We hypothesize that decellularized skin might be an easily accessible, viable alternative for myocardium regeneration, as decellularization is unlikely to cause loss of skin elasticity, provided by elastic fibers rather than by resident cells. Skin fragments from patients undergoing plastic surgery were decellularized through novel simple and fast protocol. Decellularized Human Skin (d-HuSk) obtained was assayed in quantitative dye-binding method to measure content of elastin, while elastin distribution was evaluated on histological sections by Paraldehyde Fuchsin Gomori and Weigert Van Gieson stainings. d-Husk was then sectioned and used as scaffold to prepare three-dimensional culture of cardiac primitive cells (CPCs). Then, survival and ability of CPC cultured on d-HuSk to differentiate towards cardiomyocytes was evaluated at gene and protein level. Histological and quantitative analysis provided evidence of effective decellularization, preserved tissue architecture and retention of elastin. CPCs engrafted onto d-Husk, survived, and retained expression of markers specific for cardiomyocytes at gene and protein level. Our study provides compelling evidence that common signals act in cardiac and skin microenvironment to maintain CPC ability to differentiate towards cardiac muscle and that skin holds promise as an alternate biological scaffold for cardiovascular regenerative medicine.

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Low Adhesive Scaffold Collagen Promotes Cell Motility by Regulating Gene Expression in Fibroblasts

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Background: Fibroblasts exist in the connective tissues and are generally separated by extracellular matrices (ECMs). One of functions in fibroblast is synthesis, secretion, and deposition of the ECM proteins such as collagen molecules. In addition, fibroblasts can migrate in connective tissues for remodeling and renewal. We succeeded in developing low adhesive scaffold type I collagen (LASCol) (patent pending), and we found that fibroblasts cultured on LASCol spontaneously form an aggregated sphere body (spheroid), and the resulting spheroid weakly adhered to LASCol. In this study, we report the changes in gene expression of fibroblasts induced by LASCol and the increase in cell motility.

Materials & Methods: The culture dish was coated with LASCol solution or atelocollagen solution. Subsequently, mouse NIH/3T3 cells were cultured on each dish. After 1 h, to confirm spheroid formation, cell crawling was monitored and analyzed by a time-lapse observation. To investigate the cell motility, we measured mRNA expression level of myosin IB (Myo1b), vimentin (Vim), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (Pik3ca), and platelet derived growth factor subunit A (PDGFA) by RT-qPCR.

Results: By time-lapse observation, we showed that NIH/3T3 spheroid formed on LASCol acquired the great ability of rapid migration. The quantity mRNA measured in spheroid clearly increased, as compared with single cell. We demonstrated that LASCol induced the increase of gene expression related in cell motility.

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Processing Tissue-Specific Extracellular Matrix for Use as Electrospun Meshes

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Decellularized extracellular matrix (dECM) has become an attractive platform for complex organ engineering because it provides signaling molecules and native membrane proteins that more closely mimic the cellular microenvironment than commercially available natural polymers. However, there remain many obstacles in modulating the physiochemical and mechanical properties of such materials to achieve the requisite design criteria for tissue scaffolds. The objective of our work is to develop a protocol to decellularize and process ovine donor tissue into bioactive electrospun meshes. Electrospinning allows for manipulation of porosity, pore size, fiber morphology, and mechanical properties with corollary effects on cell adhesion, proliferation, and differentiation. This versatile technology combined with dECM enables the development of tunable natural and hybrid biomaterials for tissue engineering applications.

Previous decellularization protocols were modified for enhanced compatibility with electrospinning conditions. Harvested ovine tissues were thinly sliced and washed overnight in high purity water to remove blood, connective tissue, and microvasculature. After homogenization, samples were treated with Trypsin, Triton X-100, and sodium dodecyl sulfate to remove cellular material and neutralize charges. Finally, samples were dried and milled into a fine powder. This decellularization method effectively removes over 95% of DNA as confirmed by Picogreen assay and allows for appropriate conditions to produce electrospun fibers. DNA, collagen, and GAGs assays have shown promising results in ovine muscle harvested from quadriceps of White Dorper sheep. However, we believe that this method is versatile enough to be implemented in several tissues to produce tissue-specific dECM-laden scaffolds for tissue engineering applications.

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Optimization of Human Heart Decellularization Method

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Extracellular matrix (ECM) is an intricate mesh of collagenous and non-collagenous proteins, whose presence and amount vary according to type of tissue. ECM drew the attention of regenerative medicine scientists as natural scaffold suitable for stem cell delivery into damaged tissues. Although a multitude of protocols and combinations of chemical agents and physical methods have been tested and proved effective in the decellularization of human heart, none of the ones tried in our setting fulfilled the goal of obtaining a structurally preserved cardiac decellularized ECM (d-ECM). While testing already described procedures, we made several adjustments that led to the development of a novel, simpler and robust protocol to decellularize adult human heart. Specifically, we decellularized cardiac samples of the free wall of both ventricles of adult human hearts scaled down to 40x28x6mm, to fit into embedding cassettes used to avoid stirring stress and preserve structure. To shorten the procedure, a combination of SDS, Triton X-100 and antibiotics was used in simple and fast two-step protocol. After decellularization, d-ECM was fixed and processed for histological study or snap-frozen for molecular biology analysis or cytocompatibility test *in vitro*. Histochemistry and immunoistochemistry confirmed the absence of nuclei and the preservation of architecture and composition of d-ECM. Further, while DNA content in d-ECM was well below accepted standards, sGAG, elastin and growth factors were retained and d-ECM scaffolds supported cardiac primitive cell engraftment and survival. Hence, according to our evidence, our protocol is simple, fast, effective and is worth improving for clinical translation.

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Matrix-bound Nanovesicles Within Tissue ECM Rapidly Alter Their Cargo In Response To Tissue Injury In A Rat Muscle Crush Model.

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Volumetric muscle loss (VML), 25% loss of muscle mass or function, represents a significant unmet medical need. Current treatment options include autologous and free grafts which are less than satisfactory and result in donor site morbidity, and experimental stem cell therapy has failed to change the clinical course. Acellular extracellular matrix (ECM) bioscaffolds have been shown to induce functional skeletal muscle regeneration in 13 VML patients who had failed standard of care. Skeletal muscle has greater regenerative capabilities than most other tissues, and the spatiotemporal cellular events following muscle injury are well characterized. However, the signaling molecules that regulate these processes are largely unknown. The importance of the innate immune system, specifically the macrophage component, for skeletal muscle regeneration has recently been recognized. The ability of ECM degradation products to induce a regulatory macrophage phenotype has been well documented. Recently discovered matrix-bound nanovesicles (MBV) within ECM have been shown to strongly influence cell behavior and represent a possible signaling mechanism for supporting skeletal muscle regeneration. In the present study, rat tibialis anterior muscles were subjected to a crush injury and allowed to heal for 14 days. MBV were isolated from the ECM of both crushed and mock-injured counterpart muscles. Results show that, within the first 14 days following injury, MBV cargo is altered when compared to MBV derived from mock-injured muscle. These findings suggest that MBV are rapidly responding entities capable of adapting to injury. MBV represent a bioactive component of ECM that regulate tissue restoration and remodeling after injury.

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Rapid Condensation And Robust Chondrogenesis Of Human Mesenchymal Stem Cells Within Their Own Extracellular Matrix: The Application For Cartilage Regeneration

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Objectives: Mesenchymal stem cell (MSC) derived extracellular matrix (mECM) represents a very bioactive substrate. In this study,

long-term confluent MSC cultures producing mECM were briefly pretreated the culture with trypsin, which allowed MSCs to only partially detach from its mECM and adopt a round morphology *in situ*. We hypothesized that the rounded MSCs would undergo a rapid cellular condensation process upon chondroinduction, which would result in robust cartilage formation with a compact and uniform structure.

Methodology: Human bone marrow derived MSCs (hBMSCs) were isolated and expanded (IRB approved). After a 30s trypsin treatment, 3D bead-like constructs were formed and maintained in chondrogenic medium supplemented with transforming growth factor-β (TGF-β3). Cellular condensation was detected with peanut agglutinin binding. Bovine cartilage explant cultures were utilized to test cartilage repair potential *in vitro*. *In vivo* cartilage formation was tested by subcutaneous implantation of MSC beads into SCID mice (IACUC approved).

Results: MSCs within mECM underwent rapid cellular condensation as early as 1 day after chondroinduction, which was not seen in standard high-density MSC pellet culture until day 4. After 28 days, MSC-mECM displayed a chondrogenesis level similar to MSC pellet, but with significantly reduced hypertrophy. MSC-mECM also exhibited higher mechanical strength (compressive modulus = 620kPa), and showed good integration with cartilage tissues without calcification, which was seen in standard MSC pellet. *In vivo* implantation further showed that MSC-mECM formed a compact, uniform and cartilage-like construct.

Significance: MSC-mECM thus represents an efficacious stem cell-based protocol for cartilage repair without the use of exogenous scaffolds.

Musculoskeletal

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Bone Regeneration At Rat Calvarial Defects Induced By Plasmid MicroRNA-200c

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MicroRNA (miR)-200c actively regulates osteogenic differentiation, bone development, and inflammation. It has a potential use as a therapeutic reagent for bone regeneration.

Objectives: This project is to test the effectiveness of plasmid miR-200c on enhancing osteogenic differentiation and bone regeneration.

Methods: Plasmid DNA encoding miR-200c was transfected into human bone marrow MSCs. The cells with miR-200c overexpression were subsequently exposed to osteogenic differentiation media for up to 4 weeks. The biomarkers of osteogenic differentiation were quantitatively measured at different time points. Additionally, plasmid miR-200c at different doses were loaded into collagen sponge and implanted into calvarial defects at a rat model. The rats were euthanized after 4 weeks. The bone regeneration with different treatments were analyzed using μCT and histology.

Results: The expression of miR-200c in human bone marrow MSCs was confirmed using real-time PCR after the plasmid miR-200c was treated. The biomarkers of osteogenic differentiation, including ALP, Runx2 and OCN, were up-regulated in the cells treated with miR-200c. The bone regeneration measured using μCT in the defects treated with miR-200c are significantly higher than that receiving sponge alone or sponge loaded empty vectors.

Significance: These data demonstrated that miR-200c potently improves osteogenic differentiation of human MSCs and promotes bone formation, which indicating its potential to be used for oral and craniofacial bone regeneration.

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The Effect of SDF1α on Skeletal Muscle Regeneration After Compartment Syndrome Injury

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Skeletal muscle dysfunction can be debilitating to normal life despite its remarkable ability to regenerate after minor injuries. There are a few effective treatments for severe skeletal muscle injuries. SDF1α is a chemokine which promotes migration of stem cells through binding to the CXCR4 receptor and has been suggested to enhance tissue vascularization. In this preliminary study, we tested the effects of SDF1α treatment on the recovery of skeletal muscle regeneration in a rat compartment syndrome(CS) injury model. We have previously demonstrated that this model causes damage to muscular, vascular, and neural compartments as seen in patients (1). Male Lewis rats (~12 months of age) were injured as previously described and divided into either SDF1α or saline treated groups. Immediately after injury, SDF1α at 50 μL (100 ng/μL) or saline were injected into the injured muscle. *In vivo* muscle function (isometric torque) was determined prior to injury and 7 and 14 days after injury via stimulation of the peroneal nerve. Histological assessments and qPCR were used to determine the tissue and molecular effects of SDF1α treatment. Our data showed that SDF1α treatment resulted in a decrease in degenerating fibers at 14 days post-injury and increased pax7 expression and decreased fibrosis at days 7 and 14 post-injury as compared to the saline treated group. Despite not finding any difference in functional recovery with SDF1α treatment, these results are promising. More experiments will be done to confirm these findings.

1. Criswell *et al*. 2012.

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The Effect of Exercise on Skeletal Muscle Regeneration after Compartment Syndrome Injury

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Skeletal muscle injury is a major cause of disability. It is well known that physical therapy can facilitate the recovery of muscle function after injury. However, there is little data on the effect of exercise on the specific physiological and molecular aspects of muscle regeneration. The goal of this experiment to develop and optimize an exercise protocol that can be used in future experiments. Adult male Lewis rats were acclimated to walking on the treadmill using a mild exercise protocol prior to injury. Injury was induced as previously described[1]. Rats were divided into (1) sedentary, (2) flat and (3) decline walking groups. After injury, rats in exercised groups were walked on the treadmill until fatigued, for a total of 27 days. Isometric torque was used as the measure of muscle function at day 7, 14 and day 28 after injury. Tibialis anterior (TA) muscles were collected for histology and molecular analyses. There was no difference in relative muscle weight although body weight decreased in the exercised groups compared with sedentary group. A small increase in function was detected in the decline group at day 28. Marginal differences were found in myogenic and vascular markers by qPCR. These modest differences between the groups are possibly due to the small number of animals used in this pilot experiment. The dose of exercise and training time after injury need to be optimized and sample size will be increased in future studies. 1. Criswell T *et al*., Am J Pathol. 180: 787, 2012.

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Development of Fasciotomy Procedure in a Rat Model of Compartment Syndrome

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Skeletal muscle injuries are a serious cause of disability in the military and civilian populations. Compartment syndrome (CS) is a secondary injury, proceeding any primary injury that results in

swelling and edema within the fascicle compartment of a muscle. This swelling leads to occlusion of the tissue vasculature and, if left untreated, tissue necrosis and loss of limb. The current standard of care for CS is surgical fasciotomy: an incision through the muscle compartments to relieve pressure. This fasciotomy procedure may save the limb, but often leaves a number of patients with scarring and reduced muscle function. Therefore, additional treatments to increase tissue regeneration after fasciotomy need to be explored. Our group previously developed a rodent model of CS for exploring the pathophysiology of the condition and testing potential treatment methods[1]. For this pilot study, we developed and characterized a rodent model of CS followed by fasciotomy and treatment. CS was induced on the hindlimb of adult male Lewis rats as previously described[1]. Fasciotomy was performed on the anterior muscle compartment of adult male Lewis rats with or without CS injury. Functional and histological data from these groups were analyzed. Similar to clinical outcomes, a slight functional improvement was found in animals that had received a fasciotomy after CS. Future experiments will be performed to better optimize this procedure.

1. Criswell *et al*. ''Compression-Induced Muscle Injury in Rats That Mimics Compartment Syndrome in Humans.'' *The American Journal of Pathology*180.2 (2012): 787-97. Print.

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Delivery of Stem Cell Treatment in a Percutaneous Needle Puncture Model of Degenerative Disc Disease in Rabbits

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Various models have been proposed to study pathophysiology of intervertebral disc degeneration, a leading cause of back pain. The purpose of this study was to introduce a minimally-invasive technique via percutaneous needle puncture of annulus fibrosus with subsequent needle injection of treatment in New Zealand white rabbits. Under fluoroscopic guidance, an 18-gauge 9-cm spinal needle was inserted percutaneously into the nucleus pulposus of L5-L6 disc in 12 rabbits. At 4 weeks, a 23-gauge needle was directed through an 18-gauge needle percutaneously into the nucleus, and a microsyringe was used to inject 10-μL amniotic stem cell suspension in 4 rabbits or 10-μl saline in 4 rabbits. No complications were associated with surgery. Weekly x-ray measurements of disc height index (DHI), calculated as the ratio of disc height to sum of height of two adjacent vertebral bodies, demonstrated baseline mean of 0.076, which decreased to 0.038 at 4 weeks ($p < 0.001$). Final DHI at 12 weeks were 0.048, 0.034, 0.037 in the treated, untreated control, and saline control groups, respectively ($p = 0.001$). MRI assessment at 4 weeks demonstrated that needle injury reduced T2 relaxation time by 79% compared to uninjured L4/L5 disc treated, untreated control, and saline control groups, respectively ($p < 0.001$). At 12 weeks, T2 relaxation times were 235%, 110%, and 131% of the 4-week MRI assessment for ($p = 0.009$). The percutaneous needle technique effectively produced disc injury, which improved after stem cell delivery to the injured disc. The injury and treatment procedures are minimally invasive, with minimal risk to the wellbeing of the animal.

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Effect of Matrix Source and Cell Density on Engineering the Superficial Zone of Articular Cartilage

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Articular cartilage is composed of structurally and functionally distinct zones including superficial, middle and calcified zones. The superficial zone is most vulnerable to cell death after injury. Current cartilage tissue engineering strategies are focused on regeneration of the middle zone ignoring the thin yet vital superficial zone. In an attempt to engineer the superficial zone of articular cartilage, hydrogel matrices based on native bovine fetal or adult cartilage (CarMa hydrogel) were synthesized and loaded with human mesenchymal stem cells (hMSCs). CarMa-laden hMSCs were incubated in chondrogenic medium supplemented with BMP-7 and TGF-β1 for 21 days. The effects of matrix source (fetal vs adult cartilage) and cell density on differentiation of hMSCs to the superficial zone chondrogenic lineage were investigated using RT-PCR, Western Blot and histochemical analysis. Results showed that superficial zone protein (SZP) and Collagen type II (Col II) RNA and protein expressions of hMSCs increased over 21 days in both fetal and adult based gel matrices. However, the RNA and protein expressions of SZP and Col II after 21 days were significantly higher in fetal based gels than those in adult based gels. In addition, the SZP and Col II RNA expression of hMSCs in fetal based CarMa hydrogel after 21 days increased by 6.5 and 4.6 folds, respectively, when the cell density increased from 25 to 200 million cells/mL. According to the results, the fetal cartilage based matrix and a high cell seeding density are more suitable for differentiation of hMSCs to the superficial zone chondrogenic lineage.

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Synchronized Vascularization and Osteogenesis using Enzymatically Responsive Nanoparticles

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The on-demand release of morphogens using enzymatically responsive nanoparticles (NPs) is a promising approach for stimulating vascularized osteogenesis. The objective of this work is to engineer biomimetic, enzyme-responsive nanoparticles with tunable degradability in response to cell-secreted enzymes. The secreted enzymes control the release rate of the grafted rhBMP2 within a 3D matrix. First, the level and temporal secretion of plasmin from human mesenchymal stem cells (hMSCs) and endothelial colony forming cells (ECFCs) were determined. Next, plasmin's cleavable peptide and self-assembled peptide (AcGF3) were synthesized as a one sequence using solid phase peptide synthesis. Then, the peptide was conjugated to different molecular weight of maleimide PEG via Michael-like reaction and the ends of the peptide-PEG were functionalized with NHS group. The functionalized peptide-PEG conjugates were dissolved in dimethyl sulphoxide (DMSO) and self-assembled into NPs against aqueous solution. Then, rhBMP2 or VEGF were grafted to NPs through NHS- lysine's ε-amine of rhBMP2. The rhBMP2 release from the NPs was measured by ELISA assay. Nearly 20, 55 and 56% of the rhBMP2 released from the NPs with PEG molecular weights of 2, 5 and 7.5 kDa, respectively, and the released rhBMP2 was enzymatically active by exogenous plasmin. Further, The NPs had the highest Runx2, ALP, and Col I fold expression at day 14 of MSC cultures compared to direct addition of BMP2 and highest extent of mineralization at day 21. These results demonstrate that the release of rhBMP2 in response to enzymes secreted by MSCs can enhance osteogenesis and mineralization.

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Effects of Post-Delivery Placenta Disc (HPH) on Porcine Hyaline Cartilage Matrix Breakdown

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Current therapies for osteoarthritis (OA) do not affect progression from trauma to arthritis. The placental disc synthesizes and regulates chemokines in amniotic fluid. HPH is an acellular preparation of the term placental disc yielding consistent concentrations of its chemokines. We hypothesized that beneficial chemokines in HPH would slow and/or reverse catabolic degradation of cartilage in OA. This study assessed effects of HPH on acute catabolic processes of the osteoarthritic microenvironment in pig chondrocytes and cartilage explants.

Methods: Full cartilage explant discs were collected from the knees of 3 month old pigs. Primary chondrocytes were grown as monolayers. Explants and cells were co-cultured with IL-1α (20ng/ml) and concentrations of HPH of 3.75 or 5.625 mg/ml, respectively, for 48 hours. Explant-conditioned media was collected for glycosaminoglycan (GAG) measurement. Media and cell lysates were immunoblotted for MMP1, MMP3 and MMP13.

Results: Explant media GAG was significantly elevated with IL-1α stimulation. HPH alone did not increase media GAG compared to control. Low dose HPH reduced GAG release by 30% and high dose further decreased GAG concentration by 64% when compared to IL-1 α treatment. ($P < 0.01$) All MMPs were up-regulated with IL-1α stimulation. This upregulation was significantly reduced by treatment of high concentration of HPH. High concentration HPH alone decreased expression of MMP-1and MMP13 compared to controls ($p < 0.01$).

Conclusion: Active degradation of cartilage induced by IL-1α stimulation was ameliorated by HPH application in cultured cartilage explants, and chondrocytes. HPH may have potential as a novel therapeutic application for arthropathy.

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Robust Chondrogenesis of Human Mesenchymal Stem Cells within TGF-β3 Loaded Hydrogels: Significant Contribution of Newly Produced Cartilage Matrix to Mechanical Properties

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Introduction: We previously developed a photocrosslinked (poly-D,L-lactic acid/polyethylene glycol, PDLLA-PEG) scaffold, which is biodegradable, biocompatible, with a Young's modulus within the physiological range of cartilage. In this study, we introduced hyaluronic acid (HA) into PDLLA-PEG scaffolds to supply bioactive motifs and provide a carrier for transforming growth factor-β3 (TGFβ3). We hypothesized that this chondrosupportive scaffold would support robust chondrogenesis of human bone marrow mesenchymal stem cells (hBMSCs) without exogenous TGF β3 supplementation, and that the newly produced cartilage matrix would adequately reinforce the degrading PDLLA-PEG/HA.

Method: hBMSCs were obtained from surgical waste with IRB approval. TGFβ3 release from PDLLA-PEG/HA was performed up to 28 days with PDLLA-PEG as the control. Chondrogenesis was assessed by real time PCR, histology, GAG quantitation and mechanical testing.

Results: TGFβ3 release from PDLLA-PEG/HA was detected for up to 3 weeks, and only for 1 week from PDLLA-PEG. After 4 weeks of culture in TGFβ3-free medium, the Young's modulus of 10μg/ml TGFβ3-loaded PDLLA-PEG/HA scaffolds, containing 20×10^6 hBMSCs, decreased from 600kPa to 380kPa as compared to a decrease from 650kPa to 150kPa in 10μg/ml TGFβ3-loaded PDLLA-PEG control group, suggesting that newly produced cartilage matrix significantly contributed to the mechanical strength of the construct only in PDLLA-PEG/HA group. Interestingly, GAG deposition in TGFβ3-loaded PDLLA-PEG/HA was similar to those not loaded with TGFβ3 but cultured in full chondrogenic medium containing 10ng/ml TGFβ3.

Significance: TGFβ3 encapsulated PDLLA-PEG/HA represents a robust and potentially point-of-care MSC delivery method for cartilage repair, with the capacity of maintaining mechanical property over time.

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Biofabrication of Tissue-Specific Extracellular Matrix Proteins to Enhance Skeletal Myocyte Expansion and Differentiation of Muscle Progenitor Cells

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Skeletal muscle progenitor cells (MPCs) are considered a key candidate for cell therapy in the treatment of skeletal muscle injuries or stress urinary incontinence due to urethral sphincter dysfunction. However, it is challenging to expand functional skeletal muscle cells *in vitro* from a small tissue biopsy because skeletal myocytes often decrease their phenotypic expression in culture conditions. Thus, we sought to develop a better culture system for expansion and differentiation of MPCs to be used for myogenesis *in vivo*. Porcine-derived muscle extracellular matrix (ECM) was generated via decellularization with distilled water, 0.2 mg/mL DNAse or 5% fetal bovine serum, with liver and kidney ECM as controls, respectively. Acellular matrices were homogenized and dissolved. Each ECM solution was combined with a hyaluronic acid-based hydrogel decorated with heparin (ECM-HA-HP). Cell proliferation and myogenic differentiation capacity of human MPCs were assessed when cells grew on each ECM-HA-HP substrate. The skeletal muscle ECM-HA-HP substrate significantly enhanced human MPC proliferation compared to liver or kidney-ECM-HA-HP substrates. Numbers of myofibers and myotubules significantly increased on muscle ECM-HA-HP substrate compared to other gel substrates. Numbers of MPCs expressing specific muscle cell markers (i.e. myosin, desmin, myoD, and myf5) significantly increased when these cells were cultured on muscle ECM-HA-HP substrate. In conclusion, skeletal muscle ECM-HA-HP as a culture substrate is an optimal culture microenvironment similar to the *in vivo* environment. This makes possible the potential use of skeletal muscle-derived ECM gel in 3D bio-printing to enhance the skeletal muscle repair or cell-based therapy for urethral sphincter tissue regeneration.

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In Vitro Bioengineered Model for Studies of Human Muscle Regeneration

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Skeletal muscle injury drives the typically quiescent muscle stem cells (satellite cells) to activate, proliferate, and differentiate [1]. Through this regenerative process, satellite cells (SCs) contribute to new muscle formation. Current engineered human muscle tissues made of expanded progenitors lack regenerative ability and a functional SC pool [2]. In our studies, primary SCs isolated from surgical muscle biopsies were expanded without losing Pax7 expression and incorporated into fibrin-based hydrogels. The gels were cultured in high-serum growth media to encourage cell proliferation followed by low-serum differentiation to stimulate myoblast commitment, fusion, and myotube formation and maturation [2]. Differentiated tissues contained functional myofibers that harbored a pool of SCs in a homeostatic, quiescent state. Regenerative capacity of the engineered muscle was improved by augmenting SC density by lengthening the high-serum culture time and adding freshly sorted primary human SCs into the hydrogel. To model muscle injury *in vitro*, several approaches used in animal models were compared based on their ability to induce loss of contractile function, SC activation, and muscle fiber fragmentation. Cardiotoxin, eccentric contraction (EC), and calcium ionophore treatments, each resulted in significant contractile function decrease. However, only EC and ionophore treatment activated SCs, while only the ionophore significantly fragmented myofibers. This injury model is now applied for studies of muscle regeneration *in vitro*. Herein, we have described the first *in vitro* model of human skeletal muscle tissue with an injury-responsive, functional SC pool.

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Tenogenic Differentiation Of Umbilical Cord-derived and Adipose-derived Mesenchymal Stem Cells

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Objectives: to investigate the tenogenic differentiation of umbilical cord-derived (UC) and adipose-derived (AD) mesenchymal stem cells (MSCs).

Methodology: UC- and AD MSCs were treated with transforming growth factor (TGF)-β1 (10 ng/ml) for the first 3 days and connective tissue growth factor (CTGF) (100 ng/ml) plus ascorbic acid (50 μg/ml) for the next 7 days. The conditioned media were changed every 3 days. The expression levels of tendon-specific genes such as scleraxis, tenascin-C, collagen type I and tenomodulin were measured by quantitative polymerase chain reaction. Protein levels were assayed by Western blotting and immunofluorescence staining of tenomodulin and vimentin were used.

Results: Gene expression levels of scleraxis and tenascin-C were increased in both of UC- and AD MSCs in the presence of TGF-β1 and CTGF. In immunofluorescence staining, tenomodulin and vimentin were expressed in both cells.

Their significance: UC-MSCs can be obtained without ethical issues and efficiently expanded for therapeutic purposes. In addition, they might be a source of allogeneic cell therapy with less immunogenicity. The present study demonstrated that UC MSCs can differentiate into tenogenic lineage, which was induced by growth factors. While AD-MSC has been investigated as a source of stem cell therapy in tendinopathy, UC-MSC could be an alternative candidate for the purpose

Regenerative Medicine Transplantation

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In Body Tissue Architecture (IBTA) Technology For Autologous 3D Tissue Fabrication

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Ideal incubation environment, including germ-free and nourishment or oxygen supply, is regulated in each animal body. Therefore, animal bodies is appropriate for tissue fabrication. Since over ten years ago we attempted in-body tissue architecture (IBTA) technology as a novel concept in regenerative medicine. The technology can produce autologous implantable collagenous tissues, consisting of only recipient's own cells or matrices without any artificial materials, with desired shape, thickness, and robustness by simply embedding molds into subcutaneous pouches of animals within 2 months. Through a series of the tissue fabrication process no cell management protocol is needed. Tubular tissues, named biotubes, obtained from rod-shaped molds, are applied as aortic grafts for almost ten years in a canine model. Small caliber biotubes (0.6 mm) could be function as femoral arteries over 1 year. Growth potential of biotubes was demonstrated when implanted using a beagle juvenile model. The first-in-human study of biotubes was successfully performed for hemodialysis access. Sheet-like tissues, named biosheets, from rod- or plate-shaped molds are applied for reconstruction materials for valvular leaflet, dura, cornea, trachea, esophagus, myocardium, abdominal wall, small intestine, uterus *et al*. All implanted tissues were reconstructed as target native tissues within several months. Four types of heart valve-shaped tissues with complex 3D forms could be obtained as biovalves. Mechanical strength of the leaflet part was equivalent to that of the human pericardium. In a goat model, postoperative echocardiography after surgical replacement of the biovalves showed smooth movement of the leaflets with little regurgitation even under systemic circulation.

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Regenerative Effects of Hyaluronic Acid with Functional Endometrial Stromal Cells on the Regeneration of Damaged Uterine Endometrium

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The inner layer of uterus called endometrium is critical for embryonic implantation and its damage can lead to female infertility. Endometrial stromal cells (EMSCs) are from functional basal layer of uterine endometrium, and hyaluronic acid (HA) is well known for its role during implantation. In this study, we investigated the regenerative effects of murine EMSCs that are pre-mixed with HA hydrogel in an experimentally induced endometrial damage model. EMSCs were harvested from 6-week-old C57BL/6 female mice and *in vitro* decidualized using treatment of 1 nM of estrogen and progesterone and loaded onto HA hydrogel. Recipient animal previously injured by chemical method using ethanol, and the extent of damage was assessed by histopathological staining. Decidualized EMSCs at were injected and the regeneration was confirmed by tissue analyses and development of implanted embryos. At 14 days after injection, cell-treated animals demonstrated significantly regenerated endometrial structure. HA hydrogel provided 3-dimmensional architecture for the attachment and growth of decidualized EMSCs with higher expression of prolactin and CD44 compared to damaged control. This study provides an efficacy of *in vitro* decidualized EMSCs combined with HA hydrogel on the regeneration of damaged endometrium. This strategy for regeneration of endometrium could be used as therapeutic model for uterine factor-derived infertile women (2016R1E1A1A01943455 and 2016R1D1A1A02937287).

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Tagging and Tracking of Neural Progenitor Cells as part of implanted Biosphincters for Treatment of Fecal Incontinence

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Background: Implantation of bioengineered BioSphincters offers a potential treatment for fecal incontinence (FI). It is important to track the implanted BioSphincters following implantation to investigate their integration with the native environment.

Objective: (1) Develop a method for stable lentiviral-transduction of enteric neural-progenitor cells (NPCs) and analyzing their *in-vitro* biological and functional activity as part of the engineered BioSphincters. (2) Assess the fate of NPCs upon *in-vivo* transplantation.

Methods: Lentiviral-transduction for NPCs was optimized. Biosphincters were engineered using IAS smooth muscle cells (SMCs) and tagged NPCs. Biosphincters were characterized *in vitro* and following their transplantation into FI model established in rabbits.

Results: FACS confirmed ~98% GFP-positive NPCs. NPCs viability and proliferation rate remained unaffected after lentiviral transfection. qPCR revealed similar βIII-tubulin expression between tagged (2.94 ± 0.16) and untagged (3.03 ± 0.16) BioSphincters, indicating similar NPC differentiation capacity between tagged and untagged NPCs. Organ-bath studies showed similar functionality of BioSphincters engineered with either tagged or untagged NPCs. Following implantation of tagged BioSphincters, histological analysis revealed tagged neurons in the native circular muscle of the IAS, indicating the survival of the transplanted tagged NPCs.

Conclusion: The NPCs transfection method was successfully optimized. Tagged NPCs were phenotypically and functionally integrated into the bioengineered BioSphincters. The biological and functional activities of the tagged NPCs in the BioSphincters were unaffected after transfection. The transplanted tagged cells displayed

native muscle innervation demonstrated by histological and functional assays. This study provides a method to track cell survival, migration and differentiation following their transplantation.

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Synthetic Peptide Effects on Chondrogenic Differentiation of Stem Cells

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One of the great challenges of using stem cells in cartilage repair is promoting their chondrogenic differentiation in a materials-only approach. Our approach focuses on developing high throughput methods of finding cell-binding peptides with the ability of stimulating chondrogenic differentiation in stem cells. Reducing the binding sequences to a small peptide is attractive because of the advantages peptides have compared to large molecules; peptides are easy to synthesize in large quantities, and their small size reduces the chance of any non-specific binding. Incorporating these peptides in a biocompatible and biodegradable construct that provides the requisite structural integrity for cartilage applications can lead to improving chondrogenic differentiation of scaffolds and promoting clinical translation of arthritis treatments. In our approach, by identifying overlapping sequences in target molecules of interest, as well as exploring the literature for a sequence known to bind to a target receptor, we have identified two proprietary peptide sequences. The chondrogenic potential of these peptides was explored by culturing rat bone marrow-derived mesenchymal stem cells either on surfaces coated with the peptides or on uncoated surfaces with soluble peptide in the medium. Different concentrations of the peptides were explored to elucidate dose effect versus negative (no peptides) and positive (RGD sequence or TGF-β3) controls. The preliminary gene expression results have indicated that the proprietary peptides have the potential to enhance chondrogenic differentiation of rBMSCs and are promising candidates for designing bioactive scaffolds with the capability of improving stem cells chondrogenic differentiation.

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Cell Therapeutics For Hypoparathyroidism: Differentiated Parathyroid Like Cells From Tonsil Derived Stem Cells

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The incidence of thyroid cancer has become epidemic in developed countries. Thyroid cancer has been most common cancer in Korea. Thyroidectomy is well-established and safe procedures, though, collateral morbidities, such as injury of parathyroid glands, are not uncommon. Several different methods have been used clinically in the managing of hypoparathyroidism.; autografting of parathyroid tissues discarded from patients during surgery, daily intake of multiple mega-doses of calcium and vitamin D for a remarkably long time, and daily injection of the synthetic PTH. These managements have several drawbacks. To restore damaged parathyroid function, parathyroid tissue engineering has been rising as new treatment approach. It was previously reported that human embryonic stem cells (hESC) and trans-differentiated thymic stromal cells could be used for the *in vitro* regeneration of parathyroid-like cells. However, these cells have critical limitations for clinical use, including ethical issues associated with the use of hESC and the considerably long time (≥10 weeks) required for trans-differentiation of thymic stromal cells into PTH-secreting parathyroid-like cells. To overcome these limitations, we recently introduced human tonsil-derived mesenchymal stem cells (TMSC) for *in vivo* parathyroid tissue regeneration. TMSC differentiated into parathyroid like cells in 7 to 10 days, shorter than that of thymic cells. Differentiated TMSC(dTMSC) release parathyroid hormone (PTH) which is responsive to extracellular calcium levels. We also developed 'hypoparathyoidism animal models' with rat. dTMSC showed almost complete recovery of survival rates and restored serum levels of PTH and iCa2+ in our animal model (parathyroidectomized rats fed calcium-free diet).

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WITHDRAWN

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Repopulation of Primary Renal Cells for Whole Organ Engineering: Functional Evaluations

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Renal transplantation is the only definitive treatment for end stage renal disease (ESRD), however it is severely limited by the availability of donor organs. Whole organ engineering based on decellularization/recellularization techniques using patient's own cells has been proposed as an alternative approach to renal transplantation. We previously demonstrated that repopulation of primary renal cells within acellular porcine kidney scaffolds leads to cell attachment and organization with maintenance of cellular phenotypes. In this study, we examined whether the recellularized porcine kidney scaffolds possess functional capabilities. Primary cultures of porcine renal cells were expanded, characterized, and seeded into upper pole of decellularized renal scaffolds, followed by bioreactor perfusion culture for 14 days. To evaluate the renal function of the repopulated renal structures formed within the scaffold, the level of the produced erythropoietin (EPO) in culture media as well as EPO mRNA expression, hydrolases activity, and sodium reabsorption were measured. Histological and immunohistochemical analysis demonstrate that the seeded renal scaffolds showed good cell attachment and cell organization. The results of EPO quantification showed that the seeded cells produced the EPO and its level increased over time in the media, confirmed by sufficient amounts of EPO mRNA by the seeded cells. Also, the repopulated renal scaffolds showed hydrolase activity by transporting glutamic acid and leucine from the recellularized cells. In the determination of electrolyte re-adsorption, the repopulated cells were found to uptake sodium. These results provide preliminary data for the generation of a transplantable renal graft as a potential treatment for ESRD.

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Proliferative Capacity of Human Chondrocytes in 3D Bioprinted Cartilage *in vivo* - a Quantitative Analysis

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Cartilage repair and replacement is a major challenge in plastic reconstructive surgery. The development of a process, able to create patient specific cartilage framework, would be a major breakthrough.

Objective: To quantitatively evaluate, *in vivo,* the proliferation capacity and cartilage formation ability in mono- and cocultures of human chondrocytes and human mesenchymal stem cells in a 3D bioprinted hydrogel scaffold.

Methods: 3D bioprinted constructs ($5 \times 5 \times 1.2$ mm) were produced using nanofibrillated cellulose and alginate in combination with human chondrocytes and human mesenchymal stem cells with a 3D extrusion bioprinter. Immediately following bioprinting, the constructs were implanted subcutaneously on the back of 48 nude mice and explanted after 30 and 60 days, respectively and examined morphologically and immunohistochemically.

Results and conclusion: After implantation the constructs retained their mechanical properties and were easy to handle. Constructs with human nasal chondrocytes only showed good proliferation abilities and after 60 days 17.2 % of the surface area was covered with proliferating chondrocytes. In constructs with a mix of chondrocytes and stem cells an additional proliferative effect was observed. The chondrocytes produced glucosaminoglycans and collagen type 2. This study shows a technique to create 3D bioprinted cartilage in a clinically relevant setting with human cells *in vivo*.

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Functionalised Collagen Systems With Bespoke Molecular And Macroscopic Characteristics For Chronic Wound Management

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Chronic wounds are unable to heal via a timely and orderly self-healing process, resulting in an economic burden to healthcare providers worldwide. Despite several chronic wound dressings are commercially available, bespoke clinical solutions enabling wound regulation at both macro- and microscopic scales are only partially realised. To explore this challenge, we have designed a functionalised collagen material that can be injected *in situ* as hydrogel-forming liquid to e.g. spatially-challenging cavity wounds or delivered as textile fabric to e.g. topical ulcers. In both formats, the material building block consists of a type I collagen precursor functionalised with photo-active compounds, e.g. 4-vinylbenzyl chloride. The presence of photo-active adducts is key to achieve a covalent photo-network of collagen triple helices, leading to either direct gelation *in situ* or geometrically-stable fibrous assembly following contact with wound exudate. Other than the material format and macroscopic properties, bespoke synthetic routes have been developed to conveniently manipulate the molecular architecture aiming to achieve drug-free covalent networks capable to manage the activity of upregulated, neotissue-degrading matrix metalloproteinases (MMPs) at the chronic wound microenvironment. This can be realised either in one step, by selecting photo-active crosslink-forming adducts inherently mediating secondary interaction with active sites of MMPs; or sequentially via functionalisation of the collagen precursor with chelating functional groups prior to photo-network formation. Pre-clinical evaluation in diabetic mice has demonstrated the potential of photo-network collagen films in inducing complete healing in a full-thickness wound model and accelerating healing kinetics compared to a polyurethane commercial dressing.

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Efficacy of Keratinocyte Sheet Cultured in Temperature Responsive Dish in Ovine Burn Wound Healing

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Using cultured autologous keratinocyte sheets as a therapy for burn wounds has been investigated for over 30 years. However, certain disadvantages inhibit the use of this technology in the clinical practice. We hypothesized that the enzymatic method (dispase) used for detachment of cultured keratinocyte sheets may have negatively impacted the quality of the sheets, resulting in poor acceptance. A novel technology has been introduced that allows non-enzymatic detachment of the sheets using temperature responsive dishes. In the present study, we compare the quality and efficacy of ovine keratinocyte sheets detached by temperature reduction (T sheet), against enzymatic detachment with dispase (D sheet). For comparison, six full thickness dorsal burn wounds (5X5cm) were induced in sheep. Following debridement, wounds were grafted with ovine cadaver skin. After 3 weeks, rejected cadaver epidermis was replaced with keratinocyte sheets, and then wound healing was monitored for two weeks. Cultured T sheets were stronger and their detachment was easier compared to D sheets. T sheets had better graft acceptance and significantly higher wound re-epithelialization percentage at days 7 and 14 after sheet grafting (95.5 ± 1.3 T vs. 59.1 ± 5.7 D on day 7 and 98.6 ± 1.4 T vs. 81.1 ± 6.0 D on day 14). No significant difference in the epidermal thickness between the groups. T sheets are better accepted than D sheets in burn wounds. The keratinocyte sheet harvesting method using temperature-responsive culture dishes provides better sheet quality and can overcome some of the obstacles of using keratinocyte sheets in therapy.

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Effect of Platelet Derived Growth Factor (PDGF) on Human Foreskin Fibroblast (HFF) Proliferation and Migration in a Novel 3D Wound Assay

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Effect of Platelet Derived Growth Factor (PDGF) on Human Foreskin Fibroblast (HFF) Proliferation and Migration In A novel 3D Wound Assay

Objectives: Chronic wounds are a major problem in the healthcare industry and their prevalence continues to increase with the rise of conditions such as diabetes, heart disease, and obesity (1). Although numerous factors affect mending of the skin, platelet derived growth factor (PDGF) has been identified as having the potential to increase cell migration and proliferation. In this study, the effects of PDGF peptides on human foreskin fibroblasts (HFFs) were investigated *in vitro*.

Methodology: The effects of various concentrations of the PDGF peptide on the growth of HFF cells were studied through proliferation using alamarBlue assays and migration using scratch assays. Various concentrations of the PDGF peptide were tested along with a control conditions with no peptide at or a random peptide sequence.

Results: The PDGF peptide affected cell migration to a greater extent than it did their proliferation. Although lower concentrations of PDGF were somewhat effective in increasing cell growth, at concentrations above some ideal value, PDGF peptide negatively affects cell proliferation.

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3D Bioprinted Skin Tissue Models Using RGD Peptide Conjugated Nanocellulose Bioinks

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3D Bioprinting is a revolutionary technology for converting biomaterials and living cells to tissue models with advanced functionality and versatile design. [1] The use of biocompatible inks in 3D Bioprinters allow for the rapid manufacturing of 3D tissues and organs *in vitro* and the precise control over microarchitecture and functionality of cell-laden constructs. The printing versatility of bioinks together with living cells diminishes the risk for poor cell viability and cell accessibility throughout the 3D network. A typical bioink consist of cells mixed with hydrogels to generate a good distribution of cells inside the 3D scaffold. In the present study, cell adhesive peptide-modified cellulose nanofibril (CNF) bioinks were prepared for 3D bioprinting of human skin tissue models. The CNFs were bioconjugated covalently with a cell adhesive peptide, GRGDSP, in order to promote human dermal fibroblast adhesion and viability in the scaffolds. The degree of peptide conjugation, colloidal stability and chemical composition of the functionalized CNFs were analyzed by chemical characterization methods. The printability of the bioinks with various CNF and alginate ratio was evaluated using a 3D printer under different printing conditions. The influence of peptide modification and bioink composition on fibroblast adhesion, viability and proliferation was studied *in vitro*. The results showed that the adhesion, viability and proliferation of fibroblasts were promoted significantly in the 3D constructs bioprinted with GRGDSP modified bio-inks.

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The Effects of Red Bull on HFF-1 Cells

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Objective: Energy drinks have been popularized due to their proposed stimulatory effects. Studies have shown that increased consumption may have a negative impact on important cellular functions. In this study, the effect of Red Bull Energy Drink on proliferation, morphology and integrin expression of Human Foreskin Fibroblast (HFF-1) cells was investigated.

Methodology: HFF-1 cell proliferation and morphology was examined on ECM (collagen and fibronectin) coated plates and in media containing Red Bull (1% diet Red Bull and 10% Red Bull) at different time intervals. HFF-1 cell migration was investigated on ECM coated Boyden chambers. Integrin expression of HFF-1 cells was studied in 10% Red Bull media over the course of 7 days.

Results: On day 7, proliferation was highest (10.1 fold) on plates coated with 10μg/ml fibronectin. HFF-1 cells were then cultured in media with different concentrations of Red Bull Energy Drink on 10μg/ml fibronectin coated plates. Results showed that cells cultured in media containing 1% diet Red Bull had significantly higher proliferation compared to cells 10% Red Bull media. Next, HFF-1 cell migration was examined on collagen and fibronectin plates. A Boyden Chamber Assay revealed that cell migration appeared to be highest in chambers coated with 10ug/ml collagen indicating an inverse relationship between cell proliferation and migration. Finally, integrin expression was analyzed using FACS analysis of HFF-1 cells in media containing Red Bull on plates coated with 10μg/ml fibronectin. Results showed that β1 integrin expression was increased when cells were exposed to 10% Red Bull over 7 days.

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Wound Healing Potential of Chlorogenic acid and Myricetin-3-O-glucoside Isolated from the Leaves of Parrotia persica

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Medicinal plants have a significant potential to treat wounds caused by trauma, diabetes, ischemic syndromes and other pathological diseases. This is due to their antioxidant, anti-inflammatory and antibacterial characteristics. In this context, polyphenols and flavonoids have received increasing attention due to their low toxicity and potential to alleviate symptoms and inhibit the development of various skin disorders, skin aging, and skin damage, including wounds and burns. In this work, we explored the potential of a flavonoid, myricetin-3-O-glucoside, and a phenolic compound, chlorogenic acid, both isolated from *Parrotia persica* leaves, to promote wound healing and angiogenesis. This was accomplished using *in vitro* scratch and tube formation assays utilizing human epidermal keratinocytes (HKCs), human dermal fibroblasts (HDFs) and human umbilical vein endothelial cells (HUVECs). The assessment of dose response of the compounds demonstrated no cell toxicity between the dosages of 1 μg/ml to 20 μg/ml. We observed chlorogenic acid at 10 μg/ml to be able to accelerate wound closure of HKCs migration by 6-fold as compared to growth media (negative control). On the contrary, myricetin was most effective in promotion of wound closure in assays using HDFs (3-fold increase) and HUVECs (2-fold increase), respectively. Both compounds were able to induce tube formation at an approximately 50% higher rate as compared to growth media control groups. Altogether, our results demonstrate the potential of myricetin and chlorogenic acid to be used in combination in treatment of lesion, bedsores, skin wounds, diabetes ulcers, skin aging and skin diseases.

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Eggshell Membrane Reinforced with Chitosan/poly (vinyl alcohol) electrospun Nanofibers to fabricate a Bi-layered scaffold for Wound Healing Applications

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The objective of the present study is to modify the surface of collagenous microfibrous eggshell membrane (ESM) with electrospun chitosan/ poly (vinyl alcohol) (PVA) smooth nanofibers to fabricate a bi-layered scaffold which is mechanically stable, biocompatible, non-immunogenic and cost effective for wound healing applications. A homogeneous blend of chitosan and PVA was electrospun onto the ESM to decorate it with randomly arranged nanofibers, followed by cross-linking of these nanofibers (256 ± 62 nm φ) to the microfibers (1 ± 0.5 μm φ) using NHS/EDC coupling (EC_PN). The surface topography of the 280 μm thick bi-layered scaffold was explored using FE-SEM and AFM. FT-IR and XPS analysis revealed successful crosslinking between the nanofibers of chitosan/PVA and microfibers of ESM which corroborates to the FESEM and AFM results. The matrix exhibits moderate enzymatic degradation after 30 days, 80% wettability, tensile strength of 7.2 MPa and substantial anti-microbial activity. Additionally, MTT assay demonstrates excellent cell viability whereas Rhodamine-DAPI studies confirm superior cell adhesion and proliferation of human dermal fibroblast (hDF) cells. Furthermore, the *in vivo* analysis of the samples presented enhanced wound healing characteristics when deployed over a full thickness wound on a rat model. Moreover, histopathological examination of the treated wounds at periodic intervals revealed fast epithelization and collagen deposition in the extracellular matrix. Owing to its excellent bi-layered microstructure, physicochemical and biocompatible properties, along with its superior wound healing efficacy, the nano/micro architectured EC_PN mats could be a potential wound healing matrix for clinical skin regeneration.

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The Effect of Manuka Honey on Neutrophil Cytokine Release

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While the role of neutrophils has been classically considered phagocytic, recent studies show an ability to regulate healing through cytokine release. Neutrophil behavior can be separated into two microenvironment-induced phenotypes: pro-inflammatory ''N1s'', which fight infection but delay wound healing, and anti-inflammatory ''N2s'', which promote tissue repair. Due to the importance of the N1/N2 balance in directing wound resolution, there is growing interest in regulating neutrophil phenotype. Clinical evidence has demonstrated the ability of Manuka honey to reduce wound inflammation and improve healing. Given this evidence, it is hypothesized that Manuka honey will modulate neutrophil phenotype to aid in wound resolution. In this study, Manuka honey's effect on neutrophil phenotype was measured using a differentiated human promyelocytic leukemia cell (dHL-60) model. After differentiation to a neutrophil-like phenotype, non-polarized N0s, inflammatory-polarized N1s, and anti-inflammatory-polarized N2s were exposed to Manuka honey-supplemented (0-20%) media for 3 and 24 hours. Trypan blue was used to assess the honey's *in vitro* cytotoxicity, and levels of 17 released cytokines were measured using a magnetic bead-based immunoassay. A significant ($p < 0.05$) cytotoxic effect was seen at honey concentrations of 5% and higher. Low and intermediate concentrations of honey (0.5% and 3%) lowered the production of inflammatory molecules for the N1 phenotype while raising their production by the N0s and N2s, and the highest concentration of honey (20%) suppressed cytokine release via cytotoxicity. These results indicate that Manuka honey modulates neutrophil cytokine release, which may facilitate enhanced wound healing.

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3D Bioprinting of a Human Skin Tissue Model

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Skin is the largest organ of the human body and the first barrier to outer environment. In case of injury, for example burn wounds, the barrier is compromised and the body is more exposed to the damaging environment. For larger injuries, skin transplants might be needed. However, this cause additional pain and suffering for the patient. 3D bioprinting can be used for biofabrication of human skin grafts with autologous cells. The printed skin can be transplanted with perfect fit to the patients wound, and thereby solve the problem with lack of skin for transplantation.

In this study, we focus on a bioink based on fibrin, a native human protein active in the wound healing process, mixed with nanocellulose and alginate modified with RGD. Human dermal fibroblasts (HDF) were mixed with fibrin bioink or nanocellulose bioink (control) and 3D bioprinted. The printed constructs were cultured *in vitro*. Human epidermal keratinocytes (HEK) were seeded in pure fibrin on top of the constructs and then cultured in an air-lift system.

Data shows that cell viability was high in both groups. At 14 days post-printing, most of the HDFs in fibrin bioink displayed a stretched morphology while HDFs in control showed mostly a rounded morphology. Collagen production and the experiments with HEKs are still under evaluation. Since HDFs are stretching out when attaching to the environment, the fibrin bioink seems to mimic the dermis better than control bioink. Therefore, fibrin-based bioinks may be a good scaffold when creating biofabricated skin.

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Development and Characterization of Protein-Based Degradable Membranes for Stratification of Cellular Co-Cultures

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We developed a dual-chambered bioreactor (DCB) to stratify cell populations and mimic the multilayered structure of skin. Furthermore, by employing membranes with varying permeability to regulate transport between the chambers, we aim to control molecular cross-talk between engineered dermal layers. We produced keratin membranes via ultra-violet crosslinking, hypothesizing that the amount of energy projected during curing (4 to 200 mJ/mm^3) defines the network's crosslinking degree, and thus membrane permeability. Crosslinking was assessed by sol fraction, swelling with phosphate-buffered saline (PBS) and minimum essential medium (MEM), mechanical properties, and changes in differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA) profiles. Our approach produced membranes with various degrees of crosslinking and permeability, where increasing the amount of crosslinking energy results in (1) decreased sol fraction ($75.2 \pm 0.1\%$ to $56.4 \pm 0.1\%$), (2) slower PBS and MEM uptake with higher swelling ($1274 \pm 208\%$ to $1729 \pm 229\%$) and longer stability periods before degradation (3 to 28d), (3) higher compressive modulus (2.4 ± 0.6kPa to 18.8 ± 3.1kPa), and (4) significant alteration of TGA and DCS profiles with an additional energy peak ($36.1 \pm 4.3\%$ to $65.9 \pm 18.8\%$ change). In the DCB, one chamber contained human mesenchymal stem cells (hMSCs) with adipogenic media; the other, normal human dermal fibroblasts (NHDFs) with NDHF media. After 7d, the adjacent co-culture allowed NHDF proliferation and hMSC adipogenic differentiation and proliferation. The membrane allows co-culture separation and its crosslinking degree regulates transport before starting to degrade. Overall, modular DCBs using degradable membranes can regulate gradients and stratification, and can be used to fine-tune interactions between cell populations in tissue engineered constructs.

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Enhancement of Wound Matrix Stability through Microdeformation Inducing Microgels

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Fibrous proteins like fibrin, fibronectin, and collagen play important roles in wound repair by providing 1) physical and biochemical cues to direct cell migration into the wound bed and 2) mechanical strength to the surrounding tissue. The mechanical integrity of the fibrous networks is compromised in chronic wounds due to excess protease activity and fibroblast senescence. The premise of this study is to develop wound targeting ultra-low crosslinked (ULC) pNIPAm microgels that interact specifically with these fibrous networks and improve the mechanics of wound-associated matrices by inducing micro-deformations within the fibrillar networks. ULC microgels are highly deformable and previous studies have shown that, when coupled to fibrin-binding antibodies, ULC microgels induce deformation within the fibrin network through a Brownian Wrench Mechanism. Here, we coupled fibrin-binding peptides to ULC microgels and incorporated them into a fibrin network. Cryo-SEM demonstrated that fibrin-targeting particles promote more network collapse than the non-targeting particles. Network collapse was found to correlate with an increase in network density, ECM stiffness, and enhanced network mechanics and stability. Current work is also investigating the utility of this approach with fibronectin and collagen-specific peptides. These particles may prove useful in the treatment of chronic wounds.

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Evaluation Of The Effects Of Air Pollutants On Diabetic Wounds

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Abstract: Although air pollution containing fine dust particles is gaining attention worldwide, little is known about the effects of such pollutants on diabetic wounds. Air pollutants from diesel exhaust particles (DEPs) cause inflammation, resulting in the increased expression of pro-inflammatory cytokines and chemokines, which attract monocytes and T cells to the sites of inflammation. We evaluated the effects of air pollutants on diabetic wounds.

Materials and Methods: Fibroblast cells were derived from streptozotocin-induced diabetic rats. Cell Counting Kit-8 (CCK-8) assays were used to determine cell viability. The expression of pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), cyclooxygenase-2 (COX-2), and interleukin-6 (IL-6), was evaluated by reverse transcription polymerase chain reaction and Western blot analysis.

Results: The proliferation of DEP-treated fibroblasts decreased with time. The mRNA expression of TNF-α and COX-2 in DEP-treated fibroblasts increased in both normal and diabetic fibroblasts, where IL-6 expression remained unchanged. The protein expression of TNF-α, COX-2, and IL-6 in DEP-treated fibroblasts increased compared to samples not exposed to DEP.

Conclusions: DEPs regulate the expression of pro-inflammatory cytokines such as Il-6, TNF-α, and COX-2, which may impede diabetic healing *in vitro*.

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Soft Tissue

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Dual Method Verification of Adipogenesis in Cultures Containing an Adipose Derived Delivery System for Adipose Restoration

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Hydrogels derived from adipose derived extracellular matrix (AdECM) have shown potential in its ability to generate new adipose tissue *in vivo*. The goal of this study was to evaluate quality and efficacy of a composite adipose derived delivery system (CADDS) containing adipogenic factors encapsulated in polymeric microspheres to increase adipogenesis in human adipose-derived stem cells (ASC) culture before examining volume retention in an immunocompetent rat model. Two separate methods of adipogenesis quantification were used to determine if the CADDS scaffold increased adipogenesis in adipose-derived stem cell culture. These two methods compared Oil Red O staining with UV spectrophotometric measurements. Oil Red O stained lipids from the 7 and 14-day *in vitro* study of ASC differentiation demonstrated increased adipogenesis of the adipogenic factor-loaded CADDS when compared to the empty CADDS. Quantification using Image J of Brightfield images demonstrated significantly increased adipogenesis at day 7 in conditions containing CADDS with adipogenic factor-loaded microspheres. A similar trend with the CADDS experimental groups using spectrophotometry was observed, at both 7 and 14 day time points. Next steps involve the analysis of the composite adipose-derived scaffold *in vivo* using a rat model to study volume retention, immune response, and neotissue formation.

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Co-culture of Adipose-derived Stem Cells and Human Umbilical Vein Endothelial Cells for Vascularized Adipose Tissue Engineering

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Adipose tissue is highly dependent on vascular networks for survival post transplantation. Therefore, engineered adipose tissue requires an extensive vascular network for survival and function. Adipose-derived stem cells (ADSCs) are a promising cell source for the engineering of adipose tissue because of their relatively high availability and efficient differentiation into mature adipocytes. In addition, ADSCs can exhibit pericyte characteristics when co-cultured with endothelial cells to promote vascular formation. This study aims to develop a 3D vascularized construct of mature adipocytes that can mimic *in vivo* adipose tissue by co-culturing ADSCs and human umbilical vein endothelial cells (HUVECs). Spheroids containing ADSCs and HUVECs were encapsulated in fibrin hydrogels and cultured for 2 or 3 weeks. The culture conditions were optimized in terms of cell ratio, fibrinogen concentration, medium content, adipogenic factors, as well as the timing of differentiation. Adipogenic differentiation was assessed by Bodipy staining. Vascularization was assessed by CD31 immunostaining. The results show that mature adipocytes congregate around the spheroids in the engineered adipose tissue. Vessel-like structures sprout from the surface of the spheroids and branch through the adipocytes forming a vascularized adipose unit.

Stem Cells

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Regulation of Senescence of Mesenchymal Stem Cells by the Hippo Pathway Effectors TAZ/YAP

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Stem cells such as mesenchymal stem cells (MSCs) harbor a promising role in regenerative medicine. However, the longer ex vivo passage of MSCs is usually accompanied with senescence, and it is still remained to be determined the mechanism underlying the senescence. MSCs senescence is accompanied by some striking morphological changes. Transcription co-activator YAP plays an important role in the regulation of organ size. A growing body of evidence has shown that YAP could be regulated by mechanical signals through F-actin. Here, we found that with the increasing passages of MSCs, the cells showed with decreased F-actin polymerization and inactivated of downstream protein YAP, observed by YAP and F-actin staining, as well as a positive relationship between the inactivity of YAP and β-gal staining. Taken together, our date indicated that important role of YAP and F-actin in the regulation of cellular senescence. It may provide significant insight to the understanding of stem cells aging and pave the way for the clinical application of MSCs.

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Adult Stem and Progenitor Cell Heterogeneity in Human Bone and Adipose Tissues

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Advancing the understanding of the heterogeneous population of native stem/progenitor cells (Connective Tissue Progenitors/CTPs) in different tissues is key to define the ideal cell source to use in cell therapy.

The aim of this study was to compare sources and characterize the biological features of human CTPs derived from bone and adipose tissues in primary culture and after expansion to passage-2 (P2).

Cancellous bone and subcutaneous adipose tissues were collected from 8 patients in IRB-approved protocols. Three cell-fractions were isolated per patient: MS, bone-derived marrow-space; TS, bone-derived trabecular-surface; and AT, adipose tissue-derived cells. We assessed CTP prevalence (P_{CTP}), colony metrics, cell counts using Colonyze™ software; and phenotypic characteristics using flow cytometry to determine percentage of cells expressing classical-MSC surface-markers: CD73, CD90, CD105, as well as CD146, E-cadherin, Ep-CAM, hyaluronan, Oct3/4, Sox-2, Nanog, SSEA-4, SSEA-3, Cripto-1.

Mean P_{CTP} and cell density in TS-fraction were significantly higher ($P = 0.0003$ and $P = 0.0002$, respectively) than in MS. Mean total cells/10^6 cells plated was significantly different between tissue sources, with AT>MS ($P = 0.01$) and TS>MS ($P < 0.0008$). All P2 cell sources expressed classical-MSC markers >95%. However, large variations were observed between patients and tissue sources in all other markers. The prevalence and biological potential of CTPs are different from one patient and one tissue to another. The heterogeneity in stem/progenitor populations is an untapped opportunity for improving the performance of culture-expanded cells. Lack of variation in classical-MSC markers limits their value. Greater emphasis needs to be placed on establishing critical quality-attributes based on other markers that will be predictive of future biological behavior and therapeutic potency of stem/progenitor cells.

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Effect of Low Level Laser Therapy on the Proliferation of Adipose Tissue Mesenchymal Stem Cells

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The use of mesenchymal stem cells (MSCs) has been widely investigated in the different areas of tissue engineering and regenerative medicine. The literature shows that the higher the proliferation of MSCs, the greater the regenerative and cicatricial capacity of the tissues. In this context, we can highlight the low level laser therapy (LLLT), which presents itself as as an effective therapy in the proliferation of several cell types, however the application is dependent on several parameters, such as wavelength, energy, power and time[1]. Objective of this study was to evaluate the action of LLLT in MSCs with different energies. Was used adipose tissue mesenchymal stem cells (ADSC) were divided into three groups, one control group (GC) and two groups irradiated by LLLT, GL1 with 0.56J and GL2 with 1.86J, at 24, 48 and 72 hours. An InGaAIP laser was used, with 660nm, power of 40mW. The ADSC were seeded in 24-well plates, intercalated to avoid dispersion of laser light. For the evaluation of cell proliferation and viability the MTT and trypan blue tests were used. The results showed that both energies accelerated cell proliferation, 41% and 56%, when compared to GC, and that the GL2 group presented a higher proliferation (15%) when compared to GL1. Thus we can conclude that LLLT has the capacity to accelerate the proliferation process of ADSC.

Acknowledgments: FAPESP- **1** Mvula B, Moore TJ, Abrahamse H. Effect of low-level laser irradiation and epidermal growth factor on adult human adipose-derived stem cells. Lasers Med Sci 33-9, 2010.

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Generation Of Chondrocytes With Reduced Hypertrophy From Human Mesenchymal Stem Cells By Modulating The Erk1/2 Signaling Pathway

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Objective: The initiation of hypertrophy concomitant with chondrogenic differentiation of adult mesenchymal stem cells (MSCs), characterized by high level expression of genes such as collagen type X (COL10) and matrix metalloproteinases-13 (MMP-13), represents a major barrier for clinical application of MSCs for cartilage repair. During endochondral ossification, chondrocyte hypertrophy is a highly regulated process, involving signaling by the extracellular signal-regulated kinases(Erk)-1/2 pathway. We hypothesized that MSC hypertrophy is similarly regulated, and that optimal Erk1/2 inhibition in human MSCs would generate chondrocytic cells with low hypertrophy potential, without significantly compromising chondrogenic differentiation.

Methodology: Human bone marrow derived MSCs (hBMSCs) were obtained with IRB approval. hBMSCs were seeded in hyaluronic acid(HA), previously shown to partially inhibit hypertrophy, and cultured in full chondrogenic medium for 21 days. Erk1/2 inhibitor (PD98059) was added at day 3, 7, 10, or 14, and lasted for the rest of culture.

Results: Early introduction of Erk1/2 inhibitors significantly compromised both chondrogenic (collagen type II, COL2, and aggrecan, AGG) and hypertrophy gene expression. However, after 7 days of chondrogenesis, the addition of PD98059 resulted in significantly increased COL2 (2.5-fold) and AGG (5-fold) expression, and decreased COL10 (2-fold) expression. Safranin O staining and immunohistochemistry (COL2 and COL10) further showed that PD98059 treatment (starting from day 7) resulted in higher GAG and COL2, but significantly less COL10 production, compared to untreated group.

Significance: Our findings represent a novel method to generate chondrocytes with reduced hypertrophy potential from human

MSCs, which may be applicable for the clinical repair of joint cartilage defect.

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Differential Response of Amnion and Adipose MSCs to Low Grade Inflammation - Implications for Mitigating Osteoarthritis

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Osteoarthritis (OA) is a disease of the synovial joint marked by chronic, low-grade inflammation leading to cartilage destruction. Strategies to combat OA progression include intra-articular injection of mesenchymal stromal cells (MSCs), however identification of an optimal MSC source has yet to be identified. We recently demonstrated that human amniotic MSCs (hAMSCs) mitigate OA progression more effectively compared to adipose MSCs (hADSCs) in human OA cartilage/synovium explant co-cultures. However, the mechanisms responsible need to be elucidated. We hypothesized that hAMSCs produce more anti- and less pro-inflammatory cytokines compared to hADSCs when cultured in low-grade inflammation. To test this, we used a simplified model of hAMSCs and hADSCs cultured independently for 48 hours in media containing an OA-mimetic pro-inflammatory cocktail (IL-1β: 10 pg/ml + TNF-α: 500 pg/ml) or control media. Media was analyzed for pro-/anti-inflammatory mediators and prostaglandin E_2 (PGE_2) using a cytokine array and ELISA, respectively. Results demonstrated hADSCs produced significantly ($p < 0.05$) more interleukin (IL) −6, IL-8 and PGE_2 compared to hAMSCs in control media. Under inflammatory conditions, hADSCs produced significantly ($p < 0.05$) more PGE_2 (a catabolic mediator of cartilage homeostasis) than hAMSCs, and demonstrated increasing production of MCP-1 and IL-8 (macrophage chemoattractants) with time; the opposite trend was found in hAMSC cultures. This demonstrates that hAMSCs produce lower concentrations of soluble mediators that can contribute to OA joint damage compared to hADSCs. Funding: NIH(NIGMS:5P20GM103444-07)

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CD264: A Predictor of MSC Regenerative Potential

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The regenerative potential of marrow-derived mesenchymal stem cells (MSCs) exhibits significant variation in their regenerative potential, particularly among older patients. The objective of this study is to identify a cell-surface marker whose expression is predictive of the *in vitro* proliferation and differentiation potential of MSCs. This study evaluates surface expression of decoy TRAIL receptor CD264, *in vitro* regenerative potential and metrics of cellular aging for marrow MSCs from 12 donors, 20-60 years old. Male and female donors were age-matched. When CD264(+) cell content was 20% to 35%, MSC cultures from young (20-40 yr) and older (45-60 yr) donors proliferated rapidly and differentiated extensively. Older donor MSCs containing less than 35% CD264(+) cells had a small size and negligible senescence despite the donor's advanced chronological age. Above the 35% threshold, CD264 expression inversely correlated with proliferation and differentiation potential. When CD264(+) cell content was 75%, MSCs were enlarged and mostly senescent with severely compromised regenerative potential. There was no correlation of the older donors' chronological age to either CD264(+) cell content or the regenerative potential of the donor MSCs. CD264 was upregulated after p53 and had a similar expression profile to that of p21 during serial passage of MSCs. No sex-linked differences were detected in this study. The strong inverse correlation of CD264(+) cell content to the *in vitro* regenerative potential of MSCs has possible application to predict the therapeutic potential of patient MSCs, and to standardize the composition and efficacy of MSC therapies.

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Nerve Fibers Restoration In A Pudendal Nerve-transected Rat Model Of Stress Urinary Incontinence After Sphincteric Adipose Stem Cells Injection In Combination With Platelet-rich Plasma

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Objective: To assess whether adipose stem cell (ADSC) plus platelet rich plasma (PRP) could promote nerve fibers formation in a stress urinary incontinence (SUI) rat model.

Methodology: Thirty five female inbred Wistar rats were used in our study. Animals were divided into seven groups (five animals per group): continent (C), sham (S), PNT (D), PNT+PBS injection (P), PNT+PBS+ADSC injection (PA), PNT+PRP injection (R) and PNT+PRP+ADSC injection (RA). Twenty five females rats underwent bilateral pudendal nerve section (PNT) to induce SUI. ADSCs were purified from fat tissue of a 4-week-old inbred male Wistar rat, labeled CM-Dil and injected into the urinary sphincter in twelve ó clock position with 70 microliltres of PBS or PRP. Four weeks after injection, cystometry was undertaken in all animals and leak point pressure (LPP) measured to assess urethral resistance function. All groups were sacrificed after cystometry, urethra sections were submitted for histology, immunohistochemistry assessment.

Results: LPP was increased significantly in R, RA and PA animals after implantation ($P < 0.01$), but was not different from group C and S. Histological and immunohistochemical examination demonstrated increased numbers of surviving ADSCs increased muscle/collagen ratio as well as increased nerve fibers density arround stem cells location in RA compared to PA animals (CM-Dil +).

Significance: PRP may potentially improve the action of transplanted ADSC to restore the histology and function of the urethral sphincter by increasing the amount of nerve fibers arround ADSC in a SUI rat model.

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Development Of An Antibody Immobilizing Device For Label-Free Selective Capture And Collection Of Stem Cells

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For tissue engineering and cell therapy, purification of stem cells is one of important technologies. Generally, stem cells were labeled by fluorescent and magnetic modified antibody and purified by FACS and MACS system, respectively. In the present study, for label-free capture and collection of target stem cells, we developed an antibody immobilizing device and investigated the capture and release of target stem cells. Polyethylene meshes were modified by grafting of poly (acrylic acid) (PAAc) and biotin was introduced into the PAAc graft. The desthiobiotin-antibody (anti-mouse CD45 antibody) was immobilized on the film through desthiobiotin-avidin interaction. The immobilization of CD45 antibody was confirmed by immunostaining. Mouse bone marrow cells (CD45 positive) were seeded on the meshes with and without immobilizing CD45 antibody, the cellular adhesion of cells was observed. When biotin conjugated water-soluble polymers were used as dissociation agents, the captured cells were completely released, indicating that the exchange reaction occurred due to the different binding constant of desthiobiotin and biotin to avidin. From these results, it was suggested that target cells could be selectively

captured and collected by using the antibody immobilizing device via desthiobiotin-avidin interaction.

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Human Urine-Derived Stem Cells Lessen Inflammation and Fibrosis within Kidney Tissue In a Rodent Model Of Aging-Related Renal Insufficiency

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Introduction: Kidney function declines with age. Most older patients with cardiovascular diseases have some degree of impaired renal function. Cell-based therapy is a promising alternative method of the treatment of chronic renal insufficiency. Our goal of is to determine whether human urine-derived stem cells (USCs) can prompt renal tissue remolding in a rodent model of age-related kidney insufficiency.

Methods: Urine samples were obtained from healthy men (n = 6, 28-35y). Human USCs were isolated and expanded at passage 5. Eighteen male SD rats (50-62w) with renal insufficiency were divided into 3 groups (G). G1, animals received 5 intravenous cell implantations of 2 x10^6 cells/0.2 ml serum-free medium /rat/time period weekly; G2, as in G1 but intraperitoneally delivered; G3, as in G1 but serum-free medium alone. Healthy male SD rats at the same age were controls.

Results: ELISA assay showed that cultured USCs secreted >20 kinds of trophic factors. Immunocytochemical staining showed that ED1 (inflammatory marker), alpha-smooth muscle actin (myofibroblast marker), and collagen deposition were significantly less within the medulla or cortex in G1 and G2 rats, compared to G3. Cell therapy with human USCs significantly inhibited inflammation and fibrosis in renal interstitial tissue, and reduced collagen deposits around renal vessels and the basement membrane of renal tubule and glomerular tissue *in vivo*.

Conclusions: Renal fibrosis is potentially reversible and therapeutic use of USCs can reverse specifically targeted decrease of influx of inflammatory macrophages, interstitial fibrosis formation and collagen deposition through their paracrine effects

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Whole Transcriptome Comparison between Multipotent Adult Stem Cells (MASCs) and Two Families of Mesenchymal Stem Cells (MSCs)

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There are several different varieties of adult stem cells, which include mesenchymal stem cells (MSCs), and multipotent adult stem cells (MASCs). MASCs are found in several tissues in post-natal animals and able to differentiate into phenotypes of all three dermal lineages, with an apparent unlimited proliferation potential. We hypothesize that MASCs have a unique gene expression profile in comparison to MSCs. MASCs were isolated from human neonatal foreskin and RNA-seq was performed on cells that were passage 20. We then compared the gene expression of the MASCs to published databases of genes expressed by bone marrow-derived mesenchymal stem cells (BM-MSCs) and placenta-derived mesenchymal stem cells (PL-MSCs). The NIH Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to perform gene functional annotation clustering and pathway enrichment All three stem cells do not express Oct-4, Sox-2, and Nanog We have identified 507 genes that are expressed in MASCs but not in MSCs, and 520 genes that were expressed in the MSC lineages but not the MASCs. MASCs expressed epiregulin, a gene required for sustained cell growth by telomerase; genes for early neural development (ROBO2, FRMPD4, PCDH9); development of germline cells (LHX9 and IGF2BP3); early neuroectodermal development (RSPO2 and ZIC1); development of endodermal phenotypes (NKX6-1 and ONECUT1); and genes associated with cardiac development (TBX5). The first 3 developmental pathways, as well as genes involved in germline development, may help explain the wide differentiation potential of MASCs. Expression of epiregulin may help explain the apparent unlimited proliferation potential of MASCs.

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Effect of Anisotropy on Fate Specification of Human Mesenchymal Stem Cells

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Human stem cells hold the potential of almost unimaginable medical breakthroughs for the treatment of a variety of diseases. However, their use as a therapy is hampered due to the limited understanding of the mechanisms by which cells integrate environmental stimuli. Efforts to understand extracellular biophysical cues have demonstrated the critical role of cell geometrical parameters and mechanical signals in determining the ultimate fate of stem cells. The goal of this contribution was to dissect the interplay between cell shape anisotropy and matrix stiffness in stem cell lineage specification. To accomplish this goal, microcontact printing was employed to create isotropic and anisotropic hydrogels of varying stiffness moduli. Human mesenchymal stem cells (hMSCs) were confined to the hydrogels at a single cell level and were given the choice to differentiate along adipogenic and osteogenic routes. Our results demonstrated that on soft matrices, anisotropy exerted a negligible effect and the cells primarily differentiated to adipocytes. On the contrary, cell shape anisotropy enhanced the effect of increased stiffness guiding stem cells to differentiate along osteogenic lineage. The insight gained from this project provide a rational basis for designing stem cell culture systems that will facilitate their use in research and clinical settings.

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Transplantation of Human Urine-Derived Stem Cells Transfected with Pigment Epithelium-Derived Factor to Protect Erectile Function in a Rat Model of Cavernous Nerve Injury

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Introduction: The aim of this study was to investigate whether intracavernous injection of urine-derived stem cells (USCs) or USCs genetically modified with pigment epithelium-derived factor (PEDF) could protect the erectile function and cavernous structure in a bilateral cavernous nerve injury-induced erectile dysfunction (CNIED) rat model.

Methods: USCs were cultured from the urine of six healthy male donors. Seventy-five rats were randomly divided into five groups (n = 15 per group): sham, bilateral cavernous nerve (CN) crush injury (BCNI), USC, USCGFP+, and USCGFP/PEDF+ groups. The sham group received only laparotomy without CN crush injury and intracavernous injection with PBS. All of the other groups were subjected to BCNI and intracavernous injection with PBS, USCs, USCsGFP+, or USCsGFP/PEDF+, respectively.

Results: The USC and USCGFP/PEDF+ groups displayed more significantly enhanced ICP and ICP/MAP ratio ($p < 0.05$) 28 days after cell transplantation. Immunohistochemistry and Western blot analysis demonstrated that the protection of erectile function and the cavernous structure by USCsGFP/PEDF+ was associated with an increased number of nNOS-positive fibers within the penile dorsal nerves, improved expression of endothelial markers (CD31 and eNOS) and a

smooth muscle marker (smoothelin), an enhanced smooth muscle to collagen ratio, decreased expression of transforming growth factor-1 and decreased cell apoptosis in the cavernous tissue.

Conclusion: The paracrine effect of USCs and USCsGFP/PEDF+ prevented the destruction of erectile function and the cavernous structure in the CNIED rat model by nerve protection, thereby improving endothelial cell function, increasing the smooth muscle content, and decreasing fibrosis and cell apoptosis in the cavernous tissue.

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Urine-Derived Stem Cells: A Potential Bio-marker for Renal Insufficiency

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Introduction: Kidney tissue biopsy is most commonly used to diagnose renal insufficiency after renal transplantation and other kidney diseases. However, this invasive approach causes severe complications such as arteriovenous fistula and hematuria. The need for accurate and noninvasive biomarkers for early prediction of acute kidney injury and chronic kidney disease is clear. Our goal is to investigate whether patient-sourced urine-derived stem cells (USCs) as a potential biomarker to address this need.

Methods: We collected 72 urine samples from 24 male patients (average age 60-75y) with renal insufficiency and 30 urine samples from 10 heathy men (60-75y) as controls. We used these samples to quantify the numbers of cells shed off into urine, numbers of cell clone s, and cell phenotypes.

Results: USCs (70%) from patient had weaker regenerative capacity in cell proliferation, less secretion of paracrine factors and telomerase activity, and lower renal tube epithelial differentiation potential compared to healthy controls. Patient samples also had high levels of inflammation factors and oxidative stress. Protein levels of LC3, ATG3, 5 and 7 for autophagy markers and pmTOR/mTOR, pRaptor/Raptor, p-ULK-1 for mTOR signaling molecules were lower in patients' USCs. In addition, miRNA-next-generation sequencing showed that the top 10 up-regulated (including miR-146a) and down-regulated miRNAs (including miR-193b) among changed miRNAs with higher than 1000 reads in the patients' USCs.

Conclusions: Regenerative performance of USCs in paracrine effect and repair capacity declines with age. Specific miRNA changes identified in this study might lead to novel tools for diagnosis and prognosis of renal insufficiency.

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Assessing the Osteogenic Potential of Adipose-derived Stromal Vascular Fraction Cells

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Critical-sized craniofacial bone defects are challenging to treat due to their geometric complexity. Scaffold-based technologies that deliver osteogenic stem cells have shown remarkable regeneration but are hampered by the need for extensive *in vitro* manipulation prior to implantation. To address this, we explore the bone forming potential of the stromal vascular fraction (SVF) obtained from lipoaspirate. SVF contains a sub-population of adipose-derived stem cells (ASCs), which can be effectively induced to deposit mineralized tissues. In this study, we determined whether the heterogeneity of SVF inhibited or enhanced bone formation in 3D-printed scaffolds relative to passaged ASCs from the same donor. SVF and ASCs were suspended in a fibrin hydrogel at 20,000 cells/μL and cast in a porous 3D-printed osteoinductive scaffold a 30:70 mixture of decellularize bone matrix:polycaprolactone. Cell seeded scaffolds were cultured for three weeks in control media (low glucose DMEM +10% serum) or osteoinduction media (control media + osteoinductive supplements), harvested and analyzed biochemically for calcium and DNA content. We observed that both populations successfully mineralized the scaffold demonstrating robust bone formation properties of SVF. However, SVF deposited significantly less calcium than ASCs in both control media (443.5 ± 6.9 μg vs. ASC: 525.2 ± 12.1 μg, $p < 0.05$) and osteoinduction media (489.6 ± 13.9 μg vs. ASC: 603.3 ± 8.0 μg, $p < 0.005$). Ongoing studies will assess whether this reduction in mineralization is due to the smaller fraction of osteoprogenitors in SVF or the inhibition of osteogenesis by other subpopulations in SVF as well as examining the bone forming potency in critical sized cranial defect models.

Tissue Chips & Tissue Organoid Models

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Primary Patient Tumor Organoids for Personalized Drug Treatment

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Recently, there has been an increased focus on personalized drug treatments for cancer as the influence of genetic mutations on chemotherapy efficacy has become better understood. However, success rates in the clinic are still low. Precision medicine could benefit from personalized models in which therapies identified through genetic screening can be tested prior to administration to the patient. We have hypothesized that 3D organoid biofabrication technologies allow for patient tumor cells to remain viable for personalized drug screening, which can compliment precision medicine genetic screening. To test this, we have biofabricated primary patient tumor organoids using extracellular matrix hydrogels for maintenance of tumor biopsy-derived cell population viability *in vitro* to carry out chemotherapy drug screens. We have been able to carry out this work with six successful patient samples, including high grade appendiceal, low grade appendiceal, and peritoneal mesothelioma tumors, yielding quantitative treatment results. This has included the survival and drug testing of cancer cell types previously undocumented in relation to successful cell culture. The development of this application has allowed for drug screens with multiple treatments yielding statistically significant conclusions to which chemotherapies are most effective. These results have been gathered using quantification of live/dead cell staining and MTS proliferation assays. Maintenance of cancer type phenotypes were additionally confirmed using IHC antibody staining. With further development we hope to be able to use patient specific organoids for prediction of the most effective treatments for individual patients in the clinic.

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WITHDRAWN

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Three-dimensional (3D) Quadruple Cultured Brain on Chip for Organophosphate Toxicity Screening

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Organophosphate-based compounds (OPs) represent a significant threat to warfighters and civilian populations. There is a pressing need to develop *in vitro* testbed to rapidly screen large numbers of organophosphate compounds (over fifty thousand potential combinations existed). Here we report on high content brain-on-a-chip, a three dimensional, two-compartment, organotypic microphysiological system which can mimic *in vivo* environment of neurovascular (blood compartment with endothelial cell) and nerve system (brain compartment with neuroblastoma, astrocyte, and microglia) with high throughput capability and further screen neurotoxicity of a set of organophosphate compounds. We demonstrate its utility and reproducibility in measuring OP effects on endothelial barrier integrity, brain AChE activity and cell viability. We found that the OPs cross the blood brain barrier (BBB) and rapidly inhibit AChE activity, which results were correlated with *in vivo* data.

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Tissue-Engineered Human Skeletal Muscle Model of Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease that primarily targets the joints, but also affects other tissues including skeletal muscle. Disability rates from RA reach 40% from 5-10 years after diagnosis. Skeletal muscle contributes to RA disability, but the underlying mechanisms are not well understood. Since animal models and cell culture systems are imperfect replicates of human disease and do not recapitulate biologic and physiologic features of human skeletal muscle, we use engineered, electrically-responsive, contractile human skeletal muscle constructs (myobundles) to model RA *in vitro* and identify features of the disease phenotype. To establish the *in vitro* system as a model to test therapies for RA in skeletal muscle, we are testing the hypothesis that (1) myobundles derived from cells of RA patients show reduced capacity for repair and differentiation and reduced force production and (2) the decrement in differentiation and function is due to select myokine and cytokine production. We expect that pro-inflammatory cytokines and myostatin will be elevated in RA. Myostatin is particularly important since it not only is a negative regulator of muscle differentiation, but may promote tissue fibrosis during chronic inflammation. To study the role of myostatin, we are using the small molecule LDN-193189, which, at a dose of 0.05 μM, blocks myostatin and subsequent downstream signaling events and promotes myogenesis in 2D and 3D culture.

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In Vitro Three-dimensional Vascularized Tissue Platform

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The vascular network structures are general and essential circulatory system that functions to transport blood or lymph throughout the body. These structurers have a major role in the regulation of metabolic activity, development, healing, immune response and the progression of many diseases. Therefore, the mimicking of the vascular network structures has potential uses in applications of various parts such as tissue regeneration and drug screening. In this talk, *in vitro* 3D vascularized tissue platform will be introduced, which based on compressed collagen tube and lung tissue derived decellularized extracellular matrix made of human hMSCs, human lung fibroblasts and HUVECs in microfluidic chambers. To establish the 3D vascularized tissue system, at first, we fulfilled formation and characterization of the compressed collagen tube and ldECM hydrogel, respectively. In the 3D vascularized bed system which was combined the compressed collagen tube and ldECM hydrogel, we could find that microvascular network was formed in ldECM hydrogel and anastomosis from the compressed collagen tube to microvascular network in the ldECM hydrogel. Therefore, this unique experimental platform can be used to investigate angiogenesis and anastomosis and applied to drug screening.

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Tissue-engineered Skeletal Muscle Actuator Having Long-term Drivability

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The living muscles have excellent characteristics of lightweight, high flexibility, and remarkable efficiency for driving energy. We have made a three-dimensionally cultured tissue-engineered skeletal muscle actuator having native-like contractive property driven by electric stimulation. In this work, effective electric stimulation for long-term drive of the actuator has been studied. Two artificial tendons made up of acellular porcine blood vessel were incorporated at the both ends of the actuator to handle firmly. After insertion of two artificial tendons into pins on the culture substrate, C2C12 cells embedded within collagen gel solution were placed between two tendons. The construct was then cultured in differentiation media to enhance differentiation of the C2C12 cells to myotubes. Three weeks after incubation, continuous electric stimulation was applied for 1 hour to the tissue-engineered muscle actuator to induce twitch or tetanus. The isometric twitch force of the tissue-engineered muscle actuator was not affected by continuous twitch induced by single electric stimulation. However, it gradually decreased by continuous tetanus induced by electric stimulation of more than 15Hz frequency. The isometric twitch force recovered soon after the end of continuous tetanus when the frequency of electric stimulation was low, however not recovered enough when the frequency was high. These results suggest that the frequency of electric stimulation to the tissue-engineered muscle actuator is important for its long-term drive.

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3-deimensional Choroidal Neovascularization Model Using Endothelial Cell invasion

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Purpose: Accumulation of A2E and Exposure of blue light are a factor of marcular degeneration. These are related with angiogenesis between retinal pigment epithelial (RPE) cells, endothelial cells and choroid. We developed 3D retinal-vascular mimetic system reflecting angiogenesis induced by A2E-blue light damage.

Methods: Human RPE cells and peripheral micro-vascular endothelial (PMVE) cells were co-cultured in a microfluidic channel. hRPEs were pretreated with 10 μM A2E, and then the cells were exposed with/without blue light (the channel of RPE cells were exposed to 400 ± 10 nm for 30 seconds). We examined the chemical and optical stimulus on 3D retinal-vascular mimetics system, and then evaluated cell invasion, Vascular endothelial growth factor (VEGF) secretion and activation of the signal mediators.

Results: In the 3D retinal-vascular mimetic system between RPE and PMVE cells, PMVE cell were invaded into RPE cells in hypoxia (21.0 ± 3.93 cell/unit area), A2E (22.3 ± 4.26 cell/unit area), and A2E with blue light (36.3 ± 5.02 cell/unit area) conditions for 24hrs. The secretion of VEGF by RPE increased A2E with/without blue light and hypoxia conditions by ELISA assay.

Conclusions: In this study, data indicated A2E-blue light induced the secretion of VEGF and the invasion of PMVE cells in 3D co-culture model. We established new 3D retinal-vascular mimetic model, an angiogenesis model related with CNV, would be able to detect invasion of endothelial cells under a disease condition. This model would be to employ for a candidate selection before a pre-clinical trial.

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Drug and Environmental Toxin Screening in Bioengineered Liver and Cardiac Tissue Analogs

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Modern drug testing often progresses from 2D cell culture to animal models without an intermediate 3D *in vitro* human-derived testing platform. This can often lead to proposed pharmaceuticals progressing either too fast through animal model testing, only to notice serious toxic side effects human trials or worse, the general population. Countless drugs have reached the public only to be recalled later after causing toxicity and death in humans, resulting in massive associated costs. To address this shortcoming, we employed bioengineered 3D cardiac and liver organoids to screen a panel of FDA recalled drugs that caused adverse effects to these respective organs, and charted the response of the organoids to these compounds. These drugs, from multiple drug companies and designed to treat a wide range of ailments, all reached the general public and were found to cause serious adverse effects in the general population. Tests were performed that ATP activity, LIVE/DEAD viability and cytoxicity staining, and cardiac organoid beating activity. Results showed that the 3D organoids had responses that matched the listed reasons that each drug was recalled. Additionally, in further analysis, known environmental toxins were also obtained and tested in these organoids, further demonstrating the capability of these organoids to be deployed in toxicity testing. Taken together, these results show the potential application of human-based 3D organoids to be applied in a variety of toxicity screening applications, such as drug development and environmental toxin detection.

Unlocking the Potential of Gelatin and Collagen in Regenerative Medicine

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Effect of ECM protein coating and multi-layering on Smooth Muscle Cell Differentiation from Adipose Stem Cells

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For bladder tissue engineering, human adipose-derived stem cells (hADSCs) are an attractive cell source that can be differentiated into smooth muscle cells (SMCs). The objective of this study was to investigate the effect of ECM proteins on differentiation of hADSCs into SMCs in a 3D multilayer. hADSCs were coated with fibronectin and gelatin (FN-G) in a layer-by-layer fashion and seeded onto cell culture inserts with FN-G coated membranes under growth media. After two days, the media were changed to smooth muscle inductive media medium (SMIM: DMEM, 2.5 ng/mL TGF-β1, 5 ng/mL PDGF-BB) and the cells were cultured for 10 days under standard culture conditions (37°C, humidified, 95% air, 5% CO2). Changes in cell morphology and SMC phenotypic marker expression were examined via histology and immunofluorescence. SMCs pre-differentiated in a flask then cultured on a membrane formed a monolayer. However, membrane differentiated SMCs resulted in a multilayer construct. As hADSCs differentiate into SMCs, they became less elongated and more fusiform in shape, attributing to the larger cell layers. Both groups displayed SMC phenotypic markers, suggesting differentiation from hADSCs into SMCs. The results of this study concluded that differentiation of SMCs from hADSCs on the membrane resulted in a multilayer construct that expressed SMC phenotypic markers that can further be developed to form a bladder tissue biomimetic patch.

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Poster Session 2

Monday, December 4, 2017, 6:30 PM - 8:30 PM

Biofabrication and Bioreactors

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A Controlled Atmosphere Reduces Biofabrication Contamination Risk

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Microbial contamination can bring biofabrication production processes to a halt, costing valuable time and samples. The largest source of bioburden in a biomanufacturing environment is personnel, even when they are dressed in cleanroom garb. The next largest sources are air and surface contamination. Using bioprinters in BSC can reduce contamination risks during the bioprinting step, however there is still risky room air present in a BSC, and the rest of the production process is prone to contamination as well. The Xvivo barrier isolator can separate the entire process from the risks of personnel and room air with a soft, flexible glovefront. Filtered, tanked, medical grade gases provide a completely controlled and aseptic environment. We decided to study the reduction in risk for bioprinted constructs associated with the separation of the room air from the bioprinting environment. With the null hypothesis that the environment would make no difference, we printed test samples either inside or outside of the Xvivo System using the INKREDIBLE 3D Bioprinter by CELLINK. We also performed environmental monitoring of the chamber and bioprinter using touchplates and an air sampler. We incubated the constructs in a highly permissive color-changing TSB broth. We found that enclosure with the Xvivo System effectively prevented contamination of the constructs and the equipment. Rejecting our hypothesis, we concluded that separation of the process from room air reduces contamination risks for both biomanufacturing equipment and the cell and tissue products.

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Crosslinkable Amine Coating of Poly(L-Lactic Acid) for Bone Tissue Engineering Scaffolds

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Introduction: Materials for tissue engineering have seen increasing attention since the properties of the scaffolding structure affect cell adhesion and proliferation, host immunogenic response, and stem cell differentiation. Poly (L-lactic acid) (PLLA) is especially popular in bone tissue engineering for its mechanical strength and biodegradability, but its chemical structure limits its usefulness in establishing cell extracellular matrix (ECM) and addressing other biochemical needs. Efforts to activate PLLA scaffolds by incorporating amine groups through various processes have indicated positive effects on cell adhesion and proliferation at the cost of sub-surface material integrity or nonhomogeneous modification of scaffolds. Other techniques have depended on non-FDA-approved chemicals for tissue engineering applications or costly equipment. In this study, we propose a simple wet chemistry modification approach that only uses approved materials for PLLA amine-coating and seek to demonstrate its homogeneity, surface-specificity, and potential for specialized subsequent crosslinking modification to bioactive molecules with known effects on surface-adherent cells.

Conclusions: This study produced a simple wet chemistry technique for biologically activating PLLA scaffolds that acts

homogenously on 2D films by introducing primary amine groups which can be crosslinked for further directed modification. Further experiments will demonstrate the surface specificity of the modification, potential for 3D scaffold applications, negligible effects on topography, and direct effect on adherent stem cells.

References: Alvarez-Barreto, J.F., Shreve, M.C., Deangelis, P.L., and Sikavitsas, V.I. Tissue Engineering 13, 6, 2007.

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Engineering Personalized Constructs for Intervertebral Disc Regeneration

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Lower Back Pain associated to intervertebral disc (IVD) degeneration is estimated to affect up to 80% of the population at some time in their lives, presenting a huge socio-economic impact in industrialized countries, since it is one of the main causes of medical visits, work absenteeism and hospitalization (1). One possible strategy addresses total IVD substitution/regeneration which should comprise personalized approaches by means of using reverse engineering, i.e. combining imaging techniques (e.g. MRI and micro-CT) and 3D-bioprinting technology. The implantation of custom-made implants closely mimicking native IVD and possessing an appropriate size, shape, mechanical performance, and biodegradability can improve recovery time after surgery and help to restore spine biofunctionality. Hydrogels have become especially attractive as matrices for developing a wide variety of tissue engineered tissues and organs (2). Nevertheless, one of the main disadvantages of processing hydrogels is the difficulty to shape them in predesigned geometries even when Rapid Prototyping technologies are used. The difficulties are mostly related with the difficulty in controlling the gelation event. In this work, a two-stage strategy is proposed. In the first stage, human IVD datasets (MRI or CT) are adequately analyzed for developing accurate 3D models that mimic the native IVD sub-compartments. In the second stage, 3D anatomical scaffolds are printed and characterized thoroughly *in vitro*, in terms of physico-chemical, mechanical and biological performances.

References:

1. Silva-Correia J, *et al*. Biotechnol Adv, 31, 1514, 2013.
2. Pereira DR, *et al*. J Tissue Eng Regen Med, 7, 85, 2013.

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Computational Model of Perfusion for 3D Printed Bone Tissue Engineering Scaffold *In Vitro* Culturing

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Our work with bone marrow-derived human Mesenchymal Stem Cells (hMSCs)-seeded, 3D printed porous poly(propylene fumarate) (PPF) resorbable scaffolds has led us to ask: As these scaffolds grow in size, will there be a limit beyond which static culture will prove insufficient? The goal of this study is to insure that our *in vitro*, pre-implantation, scaffold culturing always results in a scaffold that is fully coated with bone ECM (extracellular matrix). We expect that that the size limit will be determined by scaffold permeability, nutrient infusion and turnover levels, waste product removal, and cytokine delivery. To model and test our model we have chosen hMSC-seeded, cylindrical porous PPF scaffolds with a Schoen's gyroid pore geometry. Our models of these cylindrical scaffolds compute time-dependent velocity and pressure profiles using COMSOL® (Los Angeles, CA). The model assumes homogeneous distribution of cells on the scaffold surface. Results indicate that we now have an adequate description of the interaction between porous scaffolds and the flow of cell culture media. We have found that perfusion rates are highly dependent on pore distribution within the scaffold. Our next experiments will answer the simple question: With fixed pore geometry, and cylindrical scaffolds and bioreactors where the diameter does not change, at what length is flow required to allow full hMSC coating of the scaffold to mature and produce the desired bone extracellular matrix prior to implantation.

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3D Bioprinting of Biomimetic Skeletal Muscle Tissue Constructs

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Bioengineered skeletal muscle tissue can be a promising solution to achieve functional recovery of volumetric muscle injuries. However, the conventional methods have been limited to fabricate a volumetric muscle tissue with the functional cellular organization and accelerated vascularization and nerve integration. In this study, we utilized 3D bioprinting strategy to create volumetric skeletal muscle constructs that mimic the native skeletal muscle organization and function. To facilitate long-term tissue survival and accelerate innervation *in vivo*, human neural stem cells (hNSCs) were combined with human muscle progenitor cells (hMPCs) in the 3D bioprinted muscle constructs. The introduction of hNSCs enhanced the cell viability and tissue maturation, which includes highly aligned myotube formation *in vitro*. The implanted bioprinted muscle constructs developed highly oriented myofibers with the integration of host vasculature and nerve, and muscle mass and function were improved in a rat tibialis anterior (TA) excisional model. Our results demonstrate that creation of biomimetic engineered muscle tissue constructs using the 3D bioprinting system is feasible and that this muscle construct can contribute to the restoration of muscle function.

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Optimizing The Tissue Engineering Of Tubular Organ Structures By Bioprinting

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Normal 0 false false false EN-US JA X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Cambria; mso-ascii-font-family:Cambria; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Cambria; mso-hansi-theme-font:minor-latin;} Optimizing the conditions for cells to self-assemble into functional structures *in vitro* may help to engineer tissues and organoid. To facilitate self-assembly one can employ the technology of bioprinting; a robust and accurate way to arrange cells and supporting biological materials. Bioprinting is distinct from 3D printing in that post-printing maturation comprises a critical part of the tissue or organoid formation. After printing, it takes time for the structure to mature via self-organization processes, in particular tissue fusion, and become fully functional. Quantitative information about the maturation process is critical to assure that the final product has the appropriate properties for use. In particular, the possibility to accelerate this process may allow for earlier implantation. Tubular organ structures form a large portion of the human body and play vital role in it. Here we report on the optimization of the tissue engineering process of fabricating tubular bio-printed biological structures, which, among others, allows for predictive quantification of the maturation process. We prepared convenient bioink particles, spherical and cylindrical aggregates and assessed their biomechanical properties such as apparent tissue surface tension (ATST) and characteristic fusion time. The fastest fusion of

bioink particles and, consequently construct maturation, was achieved using cylindrical bioink. This finding has important implications for the time needed to fabricate vascular conduits for regenerative purposes.

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Digitizing a Sea Sponge to Print a Living Filter

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For safe human travel to deep space, NASA's Technology Roadmap calls for ''innovative physical manufacturing processes which combine the 'digital thread' that integrates modern design and manufacturing.'' Our objective is to 3D print a living filter by replicating the anatomical architecture of a sea sponge (Cinachyra spp.) using a combination of noninvasive medical imaging and biofabrication. The methodology defines four tasks: (1) Characterize the essential attributes that make the sea sponge of interest to NASA, those being filtration and self-regeneration. Filtration absorbs organic waste, and self-regeneration replenishes ''stock material'' in resource-scarce outer space. (2) Digitization via microCT scanning and analysis of a sponge specimen reveals the sponge's anatomy is a heterogeneous composite of open pore space, low- and high-density cell-laden matrix. (3) Fabricate a living sponge replicate by 3D printing a sponge cell suspension loaded into a multi-material bioprinter. (4) Compare the 3D printed sponge to that of the original, natural sponge. Our results demonstrate our objective is feasible. Surface-area-to-volume topologies and minimum feature size derived from microCT data facilitated pervasive nutrient diffusion, thereby maintaining cell viability. Post-printing, cells proliferate and begin to function as designed, absorbing organic debris from effluent. Inclusion of the symbiotic bacteria living on the sponge's surface will be critical to increasing the printed sponge's metabolism. The finding's major significance is the demonstration of a successful, first iteration proof-of-concept living filter. Future work to genetically engineer the sponge cells, as well as it's bacteria, may enhance the filter's selectivity for toxins.

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Cell-laden Bioprinting Hydrogel Depending on Diffent Compositons For Soft Tissue Engineering

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Bioprinting techniques, which can construct three dimensional structures with biocompatible materials and cells, have gained great attractions for various biomedical applications, such as tissue engineering and drug screening studies. For a successful bioprinting, bioinks are critical to ensure the processibility of printing and the viability of the encapsulated cells. Alginate is the most popular bioink for such purposes owing to its biocompatibility and mechanical properties. However, the influence of its composition on bioprinting with cells has not been well explored. In this study, we prepared the different compositions of alginate bio-inks by varying concentrations of high molecular weight alginate and low molecular weight alginate (by varying the ratios of high and low molecular weight alginate and their total concentrations). We found that bioinks containing 3 wt% alginate permitted good shapes and controls of bioprinting. Our bioprinting with fibroblasts and *in vitro* culture studies revealed that the printed scaffolds supported cell viability and growth up to 7 days. Interestingly, the bioinks prepared with high and low Mw alginate at 2:1 ratio resulted in the better cell growth compared to those with other compositions. Our study will benefit the design and applications of alginate-based bioinks for bioprinting platforms in tissue engineering.

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Formulation of Photoinitiator Free Gelatin Based Bioink and its Application to Tissue Printing

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Current major challenge in 3D printing of biological tissues is lacking of proper printable biomaterials, so called bioinks. Development of 3D printable bioinks for safely and efficiently printing tissue substitute is on high demand. Most of hydrogel based bioinks include photoinitiator to be crosslinked by either UV or visible light to obtain mechanically stable gel. However, use of crosslinking chemical has concerns for its potential harm to biological substances. Our study aimed to formulate and optimize a gelatin-protein complex bioink without any crosslinking chemical still having proper rheological, chemical and biological properties. We investigated its applicability and efficiency to print soft tissue especially skin. This new hydrogel is designed to enhance angiogenesis and effective wound healing. The formulation was investigated *in vitro* and *in vivo* for its printability, stability, cell viability, migration, invasion and angiogenic property. The study results indicated a potential of this bioink as a 3D printing material since they are easy to print, gelling, not toxic, stable, and cost effective.

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An Ex Vivo Bone Bio Reactor System

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Recent approaches in materials and manufacturing processes have allowed the development of intricate implant surfaces to facilitate bony attachment. However, refinement and evaluation of these new design procedures is hindered by the cost and complications of animal studies, particularly during early iterations in development process. To address this issue, we have designed and validated an *ex-vivo* bone bioreactor culture system to facilitate empirical testing of candidate structures and materials. In this study, we investigated mineralization of a titanium wire mesh scaffold under both static and dynamic culturing using our *ex vivo* bioreactor system. Cancellous cylindrical bone cores were harvested from bovine metatarsals and divided into five groups under different conditions. After incubation for 4 & 7 weeks, the viability of each bone sample was evaluated using Live-Dead assay and microscopic anatomy of cells were determined using histology stain H&E. Matrix deposits on the scaffolds were examined with scanning electron microscopy (SEM) while its chemical composition was measured using energy-dispersive x-ray spectroscopy (EDX). The viability of bone cores was maintained after seven weeks using our protocol and *ex vivo* system. From SEM images, we found more organic matrix deposition along with crystallite like structures on the metal samples pulled from the bioreactor indicating the initial stages of mineralization. EDX results further confirmed the presence of carbon and calcium phosphates in the matrix. In conclusion, we can say that the bone bioreactor can be used as an alternate tool to *in-vivo* for bone ingrowth studies on new implant surfaces or coatings.

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In Silico Approach For Bioprinting

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A very complex design (blueprint) will be fundamental to reach a 3D bioprinted organ in the future. Since many biological and mechanical aspects are involved the project of an organ encompasses an over wide range of variables. *In vitro* and *in vivo* experiments require the investment of large sums of money and time, besides being specific and complex. A viable alternative is to create the blueprint by means of *in silico* (computational) simulations, which is not only faster, but allows a broader flexibility in the choice and tailoring of parameters to be studied. This paper proposes using predictive probabilistic methods and energy calculations - applied to two frameworks: mechanical and biological. The mechanical would use finite element analysis to observe mechanical behavior such as hydrostatic pressure, elasticity, and fluid flow. While the biological would use complex systems of cellular interaction to analyze behaviors such as cell division, diffusion and chemotaxis of the basic units that make up the organ. A possible application, which is presented here, is a simulation of the process of osteogenesis - the formation of bones. Osteogenesis occurs with the differentiation of mesenchymal cells into osteoblasts that produce the bone matrix which can simulate for instance the expected exponential growth of the number of cells of the spheroid due to mitotic behavior or the total volume of the spheroid. The integration of mechanical and biological bias software brings to the *in silico* approach a variety of parameters that aid the decision to best apply biofabrication methods.

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Microtissue Sliding Technique for Biofabrication

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One approach to overcoming a grand challenge of tissue engineering, the fabrication of thick tissues with high cell densities, is modular tissue engineering. Modular tissue engineering involves building macrotissues using microtissue building parts in a layer-by-layer approach. Part movement and placement is a critical component of constructing macrotissues. As such we wanted to determine if we could slide microtissues as a placement mechanism. Microtissues were formed by seeding cells into nonadhesive micro-molds, where cells self-assembled scaffold-free parts in the shape of toroids and honeycombs. To determine if toroids and honeycombs could slide on a non-adherent surface in liquid media, we constructed a sliding setup using a goniometer. Microtissues were placed at 15°, 20° and 30° and the time recorded to travel 10mm. Both toroids and honeycombs slid in the non-adherent liquid environment at all three angles tested, with the velocity increasing as the angle increased. In addition, honeycombs slid at a faster velocity than the toroids. We aim to harness the ability of microtissues to slide as a technique to biofabricate thick macrotissues of a high cell density.

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Rapid Generation of Three-dimensional Large Tissues using Standing Wave

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Background and Objective: Scaffold-free tissues are essential for advancing regenerative medicine and drug discovery. As one of the promising methods for generating scaffold-free tissues, an acoustic standing wave has been employed in previous studies. However, they use special and complex devices to generate tissues, which are generally in micrometer size [1]. We propose a novel method to rapidly generate millimeter-sized tissues in a clinically ubiquitous cell culture dish using an acoustic standing wave.

Method: We fabricated a cell trapping device in which a ø60 dish was set above a Langevin transducer via degassed water in an acrylic pool. Note that the distance between the dish and the transducer was adjusted to generate a 110.7 kHz acoustic standing wave. By injecting C2C12 myoblast into the dish, the cells were trapped at the nodal position of the acoustic standing wave and were cultured for 3 hr to generate a tissue. The size and the cell proliferation of the generated tissue was evaluated. For evaluating the cell proliferation, the tissue was dispersed into single cells by trypsinization and cultured for 7 days.

Results and Conclusion: The maximum diameter and thickness of the generated tissue after 3-hr trapping were 6.5 mm and 2 mm, respectively. Moreover, the cell proliferation for 7 days was comparable to that of cells with general culture in a dish. In short, the proposed method rapidly generates a large scaffold-free tissue in a clinically ubiquitous cell culture dish. [1] Evander M, Nilsson J. Lab on a Chip. 12, 22, 2012.

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3D Biofabrication with Advanced Solutions Life Science

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The 3D biofabricating technology of the BioAssemblyBot®, which is the world's first 6-axis robotic 3D bio-printer, precisely creates cell systems and microenvironments at high resolutions enabling *in vivo*-like cell behavior in a prescribed manner. Further, users can implement both additive and contour 3D printing approaches as well as take advantage of interchangeable end-effectors or tools that allow for the incorporation of an array of different tasks and materials in a single fabrication project. The design, visualization, and analysis of precise and reproducible 3D computer models are done through our software program, Tissue Structure Information Modeling (TSIM®). An example case of our entire fabrication workflow from our Lab Innovations team is to generate liver tissue mimics containing a pre-determined self-organization based upon design parameters. In creating dense 3-dimensional tissue systems, we leverage both TSIM® and the BioAssemblyBot® to generate multiple columns within multi-well plate formats that serve as regions of nutrient and gas exchange, thereby eliminating the concern for hypoxia that is commonly seen in larger tissue aggregates. When hepatocytes and other non-parenchymal cells are placed surrounding these cavities, they self-organize per the overall pattern provided. This system can then be used to conduct drug-screen assays that provide results more meaningful than 2-dimensional assays. Therefore, Advanced Solutions Life Sciences provides an enabling platform in which tissue microenvironments can be designed while also providing the permissive environment in which natural biological processes can occur. Altogether resulting in more *in-vivo* like tissue mimics.

Biomaterial Scaffolds

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Dehydration Methods Affect Properties of Urinary Bladder Matrix Derived Particulate

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Extracellular matrix (ECM) biomaterials can be dehydrated and milled to make a dispensable particulate for medical applications. For example, ACell's MicroMatrix® is made of lyophilized, milled Urinary Bladder Matrix (UBM) and is used clinically in various wound management applications, particularly for wounds with tunneling/undermining or irregular contours. Altering the ECM dehydration process is a non-chemical means of impacting the properties and behavior of the resulting UBM particulates. In this study, UBM sheets were dehydrated using different drying methods (lyophilization and vacuum pressing) to produce distinct UBM particulate for comparison. Assessment of the particulates included the determination of bulk particulate densities, particle size distributions, scanning electron microscope imaging, and relative enzymatic (proteinase K) degradation profiles. The particulate produced from vacuum pressed

sheets were much denser and less fibrous, with marked morphological differences, compared to the particulate produced from lyophilized sheets, and the vacuum pressed sheets also showed a slower enzymatic degradation rate *in vitro*. These results reveal that altering dehydration and processing methods of ECM materials, prior to milling, can result in particulates with distinct characteristics which could have clinical benefits. Specifically, a more persistent and denser ECM particulate in wound applications could reduce the frequency of necessary interventions such as dressing changes.

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Functionalized Gold Nanorods Affect the *in Vitro* Neurogenic Potential of Human Mesenchymal Stem Cells

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AbstractA novel layered structure was developed using amine functionalized gold nanorods (AuNRs-SH-PEG-NH2). The designed structure was used successfully to investigate the ability of AuNRs to act as a bioactive scaffold. The adherence of cellular proteins was enhanced by functionalizing gold nanorods via thiol linkage to an amine-terminated polyethylene glycol from which the amino groups are intended to interact with the cellular proteins. The gold nanorod scaffold was characterized using transmission and scanning electron microscopy, ultra violet-visible spectroscopy, Zeta-potential, and X-ray photoelectron spectroscopy. Neurogenic potential of adipose tissue - derived human mesenchymal stem cells (hMSCs) was evaluated using the *in vitro* expression patterns of Vimentin, S100β, and glial fibrillary acidic protein (GFAP). The expression pattern and localization of Vimentin confirmed the mesenchymal origin of stem cells and tracked morphological changes in hMSCs during differentiation. Since, S100β and GFAP co-localize in astrocytes, their expression patterns were used as indicators for neural differentiation and results suggested that this process was enhanced when the cells were seeded on the AuNRs in the absence of neural supplements in the media. The study indicates that the scaffold design and the surface properties of AuNRs could affect the neural differentiation of hMSCs and hence, might prove beneficial for neural tissue engineering scaffolds.

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Investigation on the Effects of Acid and Alkali Treatment on Decellularized Tilapia Fish Skin

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In the pressing need of an effective wound dressing whose raw material is easy to get, available in abundance, low-cost, safe from transmissible diseases, environmental-friendly, and high cultural acceptance, decellularized nile tilapia fish skin is one of the best choice that fulfills the mentioned benefits. Not to mention the novelty of the material itself when currently there is only one commercial product in the market that produces decellularized fish skin. However, the currently available methodology of decellularization has a limited biocompatibility with L929 fibroblast cells. Therefore, pretreatment using ascorbic acid and sodium hydroxide was added in the hope to improve the biocompatibility of the scaffolds. However, instead of improving the biocompatibility between the scaffold and the L929 cells, these treatments result in an improved mechanical property (softer scaffold) that plays an important role in a real clinical study by preventing abrasion due to friction between an overly stiff wound dressing with the wounds.

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A Novel Nanocomposite Containing Graphene as a Platform for Stem cell delivery and Bone Regeneration

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The potential of graphene-based nanoparticles (GNPs) has recently gained significant attention in biomedicine, especially in tissue engineering. In this study, we investigated the osteoinductive and osteoconductive effects of a composite consisting of agarose and low oxygen content graphene (LOG) nanoparticles on adult mesenchymal stem cells (MSCs) *in vitro* and *in vivo*. We showed that adult goat MSCs were viable in the presence of 0.1 mg/mL LOG and retained their stem cell properties. A 3D scaffold made from agarose was used to encapsulate MSCs and GNPs. Scanning electron microscopy demonstrated the cell morphology and adherence of MSCs to GNPs in the 3D form. The GNPs and MSCs in the 3D scaffold were xenogenically implanted into a rat unicortical tibial bone defect. The combination of MSCs and GNPs resulted in improved active bone formation and increased mineralization. These results strengthen the applicability of GNPs as an adjunct treatment for bone tissue engineering.

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Molecular Assessment of Collagen Denaturation in Decellularized Tissues Using a Collagen Hybridizing Peptide

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Decellularized extracellular matrix (ECM) derived from tissues and organs are emerging as important scaffold materials for regenerative medicine. Many believe that preservation of the native ECM structure during decellularization is highly desirable. However, because effective techniques to assess the structural damage in ECM are lacking, the disruptive effects of a decellularization method and the impact of the associated structural damage upon the scaffold's regenerative capacity are often debated. Using a novel collagen hybridizing peptide (CHP) that specifically binds to unfolded collagen chains, we investigated the molecular denaturation of collagen in the ECM decellularized by four commonly used cell-removing detergents: sodium dodecyl sulfate (SDS), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), sodium deoxycholate (SD), and Triton X-100. Staining of the detergent-treated porcine ligament and urinary bladder matrix with carboxyfluorescein-labeled CHP demonstrated that SDS and Triton X-100 denature the triple helical collagen molecule while CHAPS and SD do not, although second harmonic generation imaging and transmission electron microscopy (TEM) revealed that all four detergents disrupt collagen fibrils. Our findings from the CHP staining were further confirmed by the circular dichroism spectra of intact triple helical collagen molecules in CHAPS and SD solutions, and the TEM images of CHP-conjugated gold nanoparticles binding only to the SDS and Triton X-100 treated collagen fibrils. CHP is a powerful new tool for direct and reliable measurement of denatured collagen molecules in decellularized tissues. It is expected to have wide applications in the development and standardization of the tissue/organ decellularization technology.

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Evaluation Of A Novel Nanocomposite For Osteogenesis

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The use of biomaterials for regenerative purposes has been a widely pursued area of research. Among these pursuits is the development of a biomaterial capable of stimulating growth and proliferation of osteocytes for bone repair. Design of an effective material for replacing and restoring function of damaged bone has been an evasive and complex challenge, largely attributed to the highly dynamic nature of the organ. Implementation of components that mimic those found in natural bone allow for construction of a biomimetic scaffold with a favorable environment for osteogenesis. The investigated material embodies this concept and is a multicomposite of a polymer, nanohydroxyapatite and cancellous granules of bone mineral substitute. The multicomposite scaffold was tested *in vitro* and subsequently *in vivo* using MC3T3E1 cells for cell adhesion, proliferation and osteogenic differentiation. *In vitro* analyses demonstrated modulation of gene expression in exposed cells with characteristics similar to those exhibited during bone remodeling. The *in vivo* analyses using a rat unicortical tibial bone defect model, displayed endogenous cell migration and new bone formation with no signs of inflammatory reaction. Results suggest the potential to translate this strategy into a large animal model.

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Cytotoxicity After Hydrogen Peroxide Gas Sterilization Of Graphene-based Biomaterials

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Graphene is increasingly the focus of studies aimed at determining the use of this material for biomedical applications. Complex biomaterials including graphene may offer scientific advancement of tissue regeneration strategies with a wide range of applications including peripheral nerve regeneration. We conducted studies designed to assess the suitability of novel graphene-polymer biomaterial designs as candidates for regeneration of damaged peripheral nerves. Specifically, graphene based materials crosslinked with polyethylene glycol (PEG) is being studied for suitability in biomimetic peripheral nerve scaffolds. These *in vitro* studies utilized PC12 immortalized cell lines co-cultured with various iterations of graphene-PEG. Initial studies revealed failure of cellular adhesion and rapid cytotoxicity with cell death. This complication lead to a systems analysis of the components, steps, and protocols associated with the *in vitro* system. System analysis revealed that the use of hydrogen peroxide gas to sterilize the biomaterials within X hours prior to cell culture experiments resulted in generation of cytotoxic radicals. Additional studies are required to more fully assess the factors associated with cytotoxicity and determine protocols for safe and effective sterilization of carbon based biomaterials. This may have a profound effect on future manufacturing processes and hospital protocols for preparation of graphitic scaffolds for implantation in the body.

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Nanofibrous Scaffolds Produced by Electrospinning, Rotary-Jet Spinning and Airbrush for Orthopedic Tissue Regeneration

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Nanofibrous Scaffolds Produced by Electrospinning, Rotary-Jet Spinning and Airbrush for Orthopedic Tissue Regeneration

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Abstract: Biomaterials-based three-dimensional scaffolds are being extensively investigated in orthopedic tissue engineering. Polycaprolactone (PCL) is a bioresorbable polymer with potential applications for bone and cartilage repair. In this study, polycaprolactone fibers (with and without hydroxyapatite nano particles (nHAp) and carbon nanotubes (CNT)) were produced using three different methods: electrospinning, rotary-jet spinning and airbrush. The scaffolds were characterized using contact angles, atomic force microscopy, differential scanning calorimetry, scanning electron microscopy, and transmission electron microscopy and were subjected to cell culture, bacterial assays and mechanical (tensile) testing. The biological and material properties were studied to understand how the various fabrication techniques and nanoparticles affect fibroblasts, gram positive and gram negative bacteria growth on samples. Experiments showed no toxic effect on cells and a significant decrease in bacterial density (in range of 4% for electrospinning to 80% for rotary-jet spinning) by adding nHAp and CNT to the PCL scaffolds without using growth factors or antibiotics.

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Preclinical Evaluation of Bioengineered Collagen Composite Constructs

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Biomaterial options for tissue repair and regeneration are limited; integration and vascularization remains a critical challenge in tissue repair. Current biologic meshes used in soft tissue repair are derived from different tissue sources and are generally sold as decellularized tissues. We report the pre-clinical evaluation of collagen based bioengineered constructs through comparison with a commercial product in a rat model of abdominal full thickness defect. The commercial product is a biologic mesh derived from bovine pericardium (Veritas). Bioengineered constructs, were prepared from porcine skin extracted collagen type 1. After purification, the collagen was formed into sheets that were physically bonded to form a mechanically robust construct that was subsequently laser micropatterned with pores. The collagen construct was then embedded in an RGD-functionalized alginate gel that serves as a bioactive interface (collagen-alginate construct). Overall, the performance of the bioengineered constructs was similar to that of the commercial product with comparable integration strength at 8 weeks. These designed collagen bioengineered constructs demonstrate promise for a variety of load bearing applications in tissue engineering.

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Fabrication of Alginate/hydroxyapatite Microsphere as Substitute Material for Bone Tissue Regeneration

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We fabricated alginate/hydroxyapatite hybrid microsphere for bone tissue regeneration which has a potential to utilize as bone substitute materials. The microspheres were prepared with mixture of alginate solution and hydroxyapatite via ionic cross-linking. We investigated Bone Morphogenetic Protein 2 (BMP-2) loading capability and release behavior of the microsphere as a carrier for induction of osteogenic differentiation. We also investigated bone tissue regeneration efficacy of encapsulated cells which were stimulated by released proteins from hydroxyapatite. The osteogenic potential was estimated by histological and immunohistochemical analysis, quantification of ALP, Calcium and osteogenic marker gene expression (e.g., type I collagen and osteopontin). We could

effectively tailor BMP-2 loading capability and release behavior by regulation of alginate/hydroxyapatite weight ratio in microsphere. In addition, we could successfully induce osteogenic differentiation of encapsulated cells by released BMP-2. These alginate/hydroxyapatite hybrid microspheres might be approached for cell and protein delivery carrier for various tissue engineering applications.

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Fabrication of Plasma Treated Chitosan Nanofiber Scaffolds for use as Tissue Engineering Applications

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Chitosan has been demonstrated to be an invaluable material in the fields of tissue engineering because of its good biocompatibility, biodegradability, and non-toxic properties. To improve the initial cell adhesion and infiltration of chitosan nanofiber scaffolds, we studied the synergetic effects of the plasma treatment and ultrasonication, respectively, in chitosan nanofiber scaffolds for tissue engineering applications. The basic characterizations of modified chitosan nanofiber scaffolds such as surface properties, mechanical properties, and wettability were performed. The contact angles also decreased as the duration of the plasma treatment on chitosan nanofiber scaffolds was prolonged. In addition, the cell study *in vitro* confirmed that the plasma treatment improves the initial cell adhesion, proliferation and infiltration of human dermal fibroblast cells. After 6 hours of cell culture, the cell on plasma treated nanofiber scaffolds showed enhanced cell adhesion and spreading than untreated nanofibers. To investigate the effect of modified nanofiber scaffolds on cell viability, we seeded HDFs on chitosan, sonicated chitosan, and plasma and sonicated chitosan nanofiber scaffolds after 1, 4 and 7 days of culture. Collectively, the results showed that such plasma treatment could greatly improve the biocompatibility, wettability and infiltration of chitosan nanofiber scaffolds, and thus increase the potential for tissue engineering applications.

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Osteoinductive and Proangiogenic Bioactive Glass Silk Composite Scaffolds towards Resorbable and Vascularized Bone Grafts

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Successful reconstruction of large volume segmental bone defects necessitates the use of resorbable and proangiogenic scaffolding matrix. Vascularization forms the main essence of bone formation and bone remodelling during healing process. Herein, we investigated the use of copper doped bioactive glass silk composite matrices for mediating osteogenesis, angiogenesis and bone resorption. Two silk varieties namely, mulberry (*Bombyx mori*) and non-mulberry (*Antheraea assama*) were studied as scaffolding matrix. Degummed silk microfibers functionalized with copper doped bioactive glass were used as reinforcements in the silk matrices to augment the mechanical properties in the composite matrices. The composite matrices enhanced the osteogenic potential of human mesenchymal stem cells (hMSCs) in comparison to pure silk matrices. The functionalized silk microfibers also improved cell attachment and migration of hMSCs and endothelial cells, in co-culture. The incorporation of Cu^{2+} in sol derived bioactive glass helped in stabilization of nuclear HIF-1α resulting in upregulation of angiogenic specific markers (VEGF, angiopoietin-1 and CD-31) through CXCL12/CXCR4 signalling. The hypoxic scenario that is prevalent in the micro-millieu upregulated MMP-9, cathepsin and tartarate resistant acid phosphatase in human osteoclasts, thereby substantiating the effectiveness of these composite scaffolds in mediating bone remodelling.

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Bioprinting Three-Dimensional Scaffolds with Modified Gelatin in Dynamic Culture Condition for Bone Tissue Engineering Application

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3D bioprinting is the combined printing of cells along with biomaterials to get a cell loaded construct as a whole. Gelatin is temperature sensitive, self-supporting for layer-by-layer deposition and has the ability to gel rapidly on the printing surface which makes it a suitable biomaterial for extrusion bioprinting[1]. Modified form of gelatin is used to achieve better polymerisation. Dynamic culture techniques are known to enhance osteogenic differentiation[2]. The aim of this study is to achieve enhanced osteogenesis on a 3D bioprinted scaffold having mesenchymal stem cells (MSCs) and modified gelatin in pure form or with the addition of hyaluronic acid, which would be cultured in dynamic conditions. MSCs were obtained from lipoaspirate by enzymatic digestion. Modified gelatin at a concentration of 10% or with the addition of 0.5% hyaluronic acid was mixed with 0.5% photoinitiator. The 3D construct was printed using a 3D-printer (Biobot, USA) at a speed of 4mm/s and pressure of 20psi. The 3D bioprinted scaffold was checked for cell viability using FDA/PI staining. Cell proliferation study, osteogenic differentiation potential, degradation studies and mechanical tests are also evaluated. Our preliminary results show good cell viability on 3D printed scaffolds. Dynamic culture condition result in better osteogenic differentiation. We anticipate that the findings of this study will be used to achieve enhanced osteogenesis using our combination of modified gelatin hydrogel and dynamic condition.

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Comparison Of Three Types Electrospun ECM/PEUU Scaffold For Abdominal Muscle Regeneration

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Tissue-derived extracellular matrix (ECM) materials have been used as surgical mesh devices in numerous preclinical and clinical area. Our group have been using electrospun biodegradable poly (ester urethane) urea (PEUU) as a regeneration material and synthesized ECM/PEUU scaffold for soft tissue regeneration in various areas, including cardiac area and abdomen. In this study we prepared a model rat with bilateral partial-thickness abdominal wall muscle defects (1×1 cm) and examined the difference in terms of muscle regeneration by comparing a group in which the ECM was derived from dermal tissue, small intestine and urinary bladder. Each groups were sacrificed 2, 8 and 16 wk after the implantation and immunohistological and rtPCR assessments were performed. The data showed dermal ECM group could retain the space to be filled with regenerated muscle tissue in 8 and 16 wk time point. The other groups were also seen regenerated muscle tissue infiltration but they couldn't retain the space to be replaced completely. And dermal

ECM group also increased mRNA for muscle regeneration factors. To supply grows factors and to retain space are the main tasks for tissue regenerative scaffold. The dermal ECM/PEUU scaffold could match to play these rolls.

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Incorporation of Growth Factor Peptide Mimics into GelMA Bioinks

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Despite the growth of the 3D bioprinting field in recent years, the diversity of currently available biomaterials that can be bioprinted remains limited compared to traditional construct fabrication techniques. Therefore, the goal of this project is to expand the diversity of this palette though the incorporation of growth factor-mimicking peptides into established bioink systems for enhancing bioink bioactivity and recapitulating complex extracellular matrix microenvironments such as tissue-specific niches. We hypothesize that the incorporation of VEGF and FGF mimetic peptides into 3D-printable bioinks via covalent cross-linking mechanisms will enhance the bioactivity of these bioinks. Therefore, two bioinks based on GelMA were developed through the incorporation of acrylated growth factor mimetic peptides. Either a VEGF mimicking AcQK peptide or a FGF mimetic F2A4-K-NS peptide was incorporated via blending into GelMA at several different concentrations. Bioink printability and bioactivity was analyzed through a tubulogenesis assay (VEGF-mimetic bioink) or the activation of FGF receptors and downstream signaling cascades such as ERK1/2 (FGF-mimetic bioink) The printed constructs were also stained with LIVE/DEAD Cell Viability Assay and histology performed on days 1 and 7 to visualize cell distribution and ECM deposition. Analysis of both vessel formation and activation of FGF receptors were positive and dependent on the concentration of the incorporated peptide. These results demonstrate that VEGF or FGF-2 mimetic acrylated peptides can be incorporated within bioinks, printed, and exhibit a positive biological response form the incorporated cells.

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WITHDRAWN

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Decelluarlized Corneal Lenticule Embedded Compressed Collagen: A Biocomposite For Limbal Epithelial Stem Cell Carrier

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Collagen-based limbal epithelial stem cell (LESC) carrier is one of the promising ways to treat clinically the limbal stem cell deficiency (LSCD). The carrier possesses adequate biological properties for LESC expansion and delivery to the diseased area. While previous studies have tried to utilize compressed collagen as LESC carrier in the meaning of chemical-free approach, only the physical entanglements of collagen fibrils in the compressed collagen cannot provide suitable mechanical strength and long-term stability required in a surgical application. To overcome the limitation, this study fabricated the LESC carrier in the form of biocomposite by integrating decellularized corneal lenticule (dCL) inside the compressed collagen. The corneal lenticule mechanically support the compressed collagen inside the biocomposite. The bicomposite was obtained by compression/dehydration of collagen gel embedded with dCL. *In vitro* and *In vivo* tests verified that the biocomposite can effectively promote the corneal epithelium reconstruction with successful LESC expansion on the biocomposite, comparable to the compressed collagen.

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Development of 3D Bioactive Composite Scaffold for Bone Regeneration

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Currently, metallic implants have been used in fixation devices and supporting structures for orthopaedic applications. Due to high stiffness difference between bone and metallic implant, stress shielding may occur. This results in bone resorption, leading to poor integration of implant into the surrounding bone and implant loosening, hence reducing the implant life time. To avoid this issue, there is an increasing need for materials that are bioactive, mimicking the structural anisotropy of autologous bone. Calcium phosphate is similar as bone material, but too brittle to use. In this study, a bioactive composite with a high content of ceramic (up to 60% in weight) was developed by using biodegradable poly(ethylene oxide terephthalate)/poly(butelene terephthalate and β-tricalcium phosphate (TCP). TCP is already applied in the clinic, for being osteoinductive and degradable *in vivo*. 3D composite scaffolds with controllable TCP content were fabricated by additive manufacturing (AM). The scaffolds exhibited a fully interconnected porous network with controllable porosity and pore size. The TCP content directed the hydrophilicity of composite. Degradation experiments showed a release of calcium and phosphate ions, which may be an important factor for composite bioactivity. Further experiments will be performed to examine the role of the released ions for (stem) cell proliferation and differentiation. With AM, the resulting scaffolds could mimic the structures and properties of both cancellous and cortical bone. The creation of additive manufactured patient-specific implants using this bioactive composite with designed properties will provide the basis for improved bioactivity that is required for a successful bone regeneration.

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Placenta Derived Extracellular Matrix for Osteochondral Tissue Engineering

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Introduction: Repair of osteochondral defects requires simultaneous restoration of both cartilage and bone. In this context, ECM derived from placental tissues (PEMS) is an attractive option as it contains abundant extracellular matrix components coupled with well-preserved endogenous bioactive molecules.

Materials and Methods: The decellularized placental extracts mixed homogeneously, poured into moulds of required dimensions, freeze-dried for 72 h to obtain PEMS. Three osteochondral cylindrical defects (4 mm in diameter and 5 mm deep) with a sterilized stainless-steel drill bit was made. Out of 3 cylindrical defects in an animal, one was left untreated (Empty Defect), one was implanted with cell-free PEMS (CFP) and one with cell-seeded PEMS (CSP) (n = 5). For CSP, Human Amniotic Membrane-derived Stem Cells were cultivated in PEMS for 7 days before implantation in the defect site. Rabbits were sacrificed 60 days post-implantation and

specimens were retrieved for semi-quantitative histomorphological analysis.

Results: From the histomorphometric analysis, it is evident that the defects filled with CSP supported superior osteochondral regeneration compared to CFP and ED after 60 days of implantation. Moreover, from the histological grading scores, the CSP scored higher than the CFP ($p < 0.05$) and the control group ($p < 0.05$).

Discussion & Conclusions: We developed biomimetic human placenta-derived extracellular matrix sponges for osteochondral tissue engineering. The ability of CFP and CSP to mediate early osteochondral repair in rabbit models is demonstrated.

References: Rameshbabu A.P. *et al. Journal of Materials Chemistry B*. 4, 613, 2016.

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Injectable Collagen-Chitosan Microspheres for Cutaneous wound healing

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Introduction: Wound injuries occurred from skin carcinoma, diabetic ulcer and burn can be categorized as non-healing wounds. *Cell-microsphere constructs* not only provide amiable microniches but also serve as a vehicle to deliver cells or growth factors directly at the dermal wound area where it may accelerate the granulation tissue formation by facilitating cellular migration, efficient transport process. In present study, collagen-chitosan microspheres (ColCMs) were synthesized and its application was observed.

Methods: ColCMs were synthesized in water-in-oil emulsion and following characteristics were performed. SEM, degradation, cytotoxicity, cell proliferation and growth factor release study was investigated. Further, *in vivo* wound healing study by injecting the microspheres at intra-dermal region (wound size 10 mm) in a rat model.

Results & Discussion: The resulting ColCMs were spherical and $\sim$150 μM in size. The constructs were significantly prominent with excellent cytocompatibility compared to tissue culture plate. At day 5, proliferating as well as migrating primary rat fibroblasts were visible under SEM by forming bridge between adjacent microspheres. Significant cell proliferation rate was also confirmed by higher DNA content and fluorescence microscopy (Rhodamine-DAPI). Injected cell seeded ColCMs showed faster wound closure rate than control (PBS) group. Histological findings revealed improved neo-vascularization, re-epithelialization and dermal tissue regeneration compared to control.

Conclusion: Therefore, 3D constructs is very efficient thus can be used as injectable scaffold for dermal wound healing. *In vitro* stem cell culture onto those microspheres in bioreactor will be explored further for advance.

Reference: Kodali A., Lim T., Leong D., Tong Y. Macromol. Biosci., 14(10):1458-68. 2014.

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Chitosan Hollow Fibers For Tissue Engineering

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The search for an ideal vascular graft with ideal material has been an extensive research interest from the last century. With the recent development in the field of tissue engineering, bioresorbable vascular scaffolds comprising of Poly lactic acid (PLA) and Polyglycolic acid (PGA) are being explored extensively but polymers of natural origin have not been utilized yet to full potential. In our study, we have chosen to develop hollow fibers or tubes of chitosan by spinning process. Chitosan is a natural amino polysaccharide polymer, composed of glucosamine and N-acetyl glucosamine obtained by N-deacetylation of chitin polymers, naturally derived from shells of crustaceans, shrimps. Chitosan have functional groups of free amine and hydroxyl which can be further modified to have desired tailor-made properties.

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Printability Metrics for Bioink Standardization

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In recent years, bioinks with diverse compositions and applications have been developed. However, the development of novel bioinks and blends has greatly outpaced the standardization of printability analysis. The standardization of what constitutes good printability is critical for consistency in the fabrication of existing and new bioink blends. It is necessary to determine how printability metrics can be rapidly quantified and utilized for comparison between different bioink compositions. Additionally, for better predictively of printability, determination of how varying bioprinting parameters such as pressure, speed, viscosity, temperature, and nozzle size and shape relate to the resulting filament size, uniformity, and properties. To this end, we have developed standardized protocols for the analysis and comparison of distinct bioink compositions. These protocols seek to collect reliable data from which printability of bioinks can be predicted based on printing pressure, nozzle shape and size, temperature, viscosity, and translation rate. Additionally, developed image analysis algorithms that seek to remove the ''human element'' from the analysis of printability and better quantify filament shape or construct structural fidelity than the common used 'eye-test'. At the moment, the algorithms can rapidly determine how filament diameter changes over the filament length, permitting the quantification of irregularities in filament shape at the beginning and end of the lines where the printing nozzle is rapidly translating. Additionally, grid structures and angles between the filaments can be quantified to determine regions of imperfections in a printed pattern and where parameters must be adjusted to result in a better print.

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Evaluation of 3D Bioprinted Human Skin Analogs Comprised of Distinct Bioinks

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Recently, three-dimensional (3D) bioprinting has drawn attention as an attractive technique for the rapid and reproducible fabrication of constructs that recapitulate striated tissue structures such as skin. The objective of this study was to utilize bioprinting to fabricate multilayered constructs for ultimate application as an engineered skin construct. Several bioinks/growth factor combinations were evaluated, including GelMA, GelMA-Heparin: TGF-β_3, CELLINK-RGD-TGF-β_3, and CELLINK-RGD-fibrin. The resulting constructs possessed dimensions of 10 by 10 by 1.6 mm and consisted of 4 layers each with a height of 0.4 mm and spacing between each filament of 2 mm to allow nutrient diffusion. After the bioprinting process, the cell-laden skin constructs were crosslinked and incubated in 3D cell culture medium under standard culture conditions (37°C, 5% CO_2 and 95% relative humidity). The constructs were maintained in culture for 4 weeks with a media change every 2-3 days. Human epithelial keratinocytes (HEKa) were seeded on top of the skin constructs on the following day and the 3D skin constructs cultured at the air-liquid interface (ALI) for 4 weeks. At day 14 and 28, samples were collected for histological, immunohistochemical and gene expression analysis collagen I production, and cell morphology.

Differences in dermal tissue maturation were evaluated between the different bioinks. Future studies will focus on the generation of more complicated dermal constructs with additional layers that contain additional cell types and growth factors to better mimic native dermal tissue.

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Osseointegration Of Chitosan Coated Porous Titanium Alloy Implant By Reactive Oxygen Species-mediated Activation Of The Pi3k/akt Pathway Under Diabetic Conditions

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Chitosan coated porous titanium alloy implant (CTI) is demonstrated a promising approach to improve osseointegration capacity of pure porous titanium alloy implant (TI). Since chitosan has been demonstrated to exhibit antioxidant activity, we propose CTI may ameliorate the ROS overproduction, thus reverse the poor osseointegration under diabetic conditions, and investigate the underlying mechanisms. Primary rat osteoblasts incubated on the TI and the CTI were subjected to normal serum (NS), diabetic serum (DS), DS + NAC (a potent ROS inhibitor) and DS + LY294002 (a PI3K/AKT-specific inhibitor). *In vivo* study was performed on diabetic sheep implanted with TI or CTI into the bone defects on crista iliaca. Results showed that diabetes-induced ROS overproduction led to osteoblast dysfunction and apoptosis, concomitant with the inhibition of AKT in osteoblasts on the TI substrate. While CTI stimulated AKT phosphorylation through ROS attenuation, thus reversed osteoblast dysfunction evidenced by improved osteoblast adhesion, increased proliferation and ALP activity, and decreased cytotoxicity and apoptotic rate, which exerted same effect to NAC treatment on the TI. These effects were further confirmed by the improved osseointegration within the CTI *in vivo* evidenced by Micro-CT and histological examinations. In addition, the aforementioned promotive effects afforded by CTI were abolished by blocking PI3K/AKT pathway with addition of LY294002. These results demonstrate that the chitosan coating markedly ameliorates diabetes-induced impaired bio-performance of TI via ROS-mediated reactivation of PI3K/AKT pathway, which elicits a new surface functionalization strategy for better clinical performance of titanium implant in diabetic patients.

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Novel Heterogeneous Polycaprolactone/human Serum Albumin Electrospun Membrane For Tissue Engineering With Improved Properties

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Development of electrospun membrane with improved mechanical properties, proper biodegradation, and excellent biocompatibility simultaneously is the great challenging task. In this background, we have prepared the heterogeneous polycaprolactone/human serum albumin (PCL/HSA) membrane by blending the solution of the different mass weight ratio of PCL to HSA using electrospinning technique. Physio-chemical and biological properties of the as-fabricated membranes were evaluated. FE-SEM images revealed that all PCL/HSA mats were composed of interlinked nano-nets along with conventional electrospun fibers while nano-nets were not found for pristine PCL mat. PCL-HSA membranes showed excellent mechanical properties (3 fold tensile stress compared to pristine PCL), improved biodegradation, enhanced mineralization and extraordinarily supported the cells early adhesion and proliferation compared to pure PCL membrane. These results indicate that the nano-nets supported PCL/HSA membranes with the ability to induce progressive cell growth could be promising in tissue engineering applications.

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Development of Biological and Mechanical Evaluation of Porous poly(para-phenylene) for orthopedic implants

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Poly(para-phenylene) (PPP) is a novel high strength polymer that has shown promise as an orthopaedic biomaterial. However, its biocompatibility has not been previously reported. The objective of this study is to evaluate the biological and mechanical behavior of solid and porous PPP compared to polyether-ether-ketone (PEEK, current orthopaedic material). Experiments were performed in four groups for comparison: 1) Solid PPP, 2) Solid PEEK, 3) Porous PPP and 4) Porous PEEK. Material cellular compatibilities were assessed by measuring mouse pre-osteoblast (MC3T3 cells) proliferation and osteogenic differentiation. To measure osseointegration, bilateral drill holes were made in the tibial metaphysis of rats, and a 2 mm × 6 mm cylindrical scaffold was placed in the defect. Bone ingrowth was quantitatively assessed by microcomputed tomography. Finite element simulated pushout tests were performed to predict interfacial strength of the implants after bone ingrowth. MC3T3 cells were able to grow on each scaffolds and the proliferation rate was not significantly different in solid materials. Cells grown on porous scaffolds showed a significant increase in ALP activity and calcium deposition compared to cells on solid scaffolds. Porous PPP showed 9.4 folds and 1.4 folds increase in bone volume to solid implants and porous PEEK respectively. The finite element model showed approximately 50% higher interfacial strength for porous PPP implants than porous PEEK. Histological assessment suggested porous PPP explants showed cellularized and matured bone formation throughout the entire porous structure. These results indicate that porous PPP has the potential to accelerate bone fusion in the clinical setting.

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3D Printing and Characterization of Bioceramic Tissue Scaffolds using Mask-Projection Micro-stereolithography

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Introduction: The need for orthopedic bone tissue engineering scaffolds is a significant issue that persists in the biomedical community. In 2014, the number of orthopedic surgical operations exceeded 600,000 in the United States [1]. Current commercial treatments often use bone cement paste implants to fill defects. However, there is a need to design and create customized porous scaffolds to exactly fit the geometric dimensions of the bone defect while governing bone tissue regeneration. Additive Manufacturing (AM) is a tool that offers design freedom to fabricate complex structures from computer aided design (CAD) models or CT-scans specifically tailored for the patient.

Methodology: In this study, a vat photopolymerization (VP) additive manufacturing process is employed to fabricate bioceramic scaffolds from a suspension comprised of commercial photocurable resins and dispersant containing Calcium Phosphate (60 wt%). Green parts underwent thermal treatment up to 1150 C to remove the photocurable binder, enabling calcium phosphate particles to fill vacated voids and densify into a final structure. Dimensional

shrinkage, surface morphology, pore size, and part density after sintering were evaluated.

Results: Porous cylindrical calcium phosphate scaffolds were successfully 3D printed and sintered. Thermal treatment induced shrinkage values of 12.6%, 8.7%, and 7.2% for the length, outer diameter and inner diameter, respectively. Scaffold pore size was 281 ± 28 μm and relative density was 97.1%. Future work will focus on osteoblast biocompatibility and cell response of these 3D printed constructs.

Reference: 1. Parikh, S. N. (2002). Bone graft substitutes in modern orthopedics. *Orthopedics*, *25*(11), 1301-1309.

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Blended Electrospinning: New Environments for Liver Bioengineering

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A combination of increasing liver disease incidence and a shortage of organs available for patient treatment and research[1] has led to a need for liver 'organoids'. Biofunctionalization of electrospun polymer scaffolds with cell-derived extracellular matrix [ECM] has been shown to influence hepatocyte function[2], and with this in mind we created blended protein/polymer scaffolds - directly incorporating bioactive proteins into electrospun polylactic acid [PLA] fibrous scaffolds.

Scaffolds were fabricated containing each of the following; Collagen I, Fibronectin, Laminin-521 and decellularized whole human liver ECM. THLE-3 cells were seeded onto the scaffolds and assessed for survival and function at 1/3/7 and 14 days using scanning electron microscopy [SEM], mechanical and biochemical quantification, histology, and gene expression analysis.

Initial results indicate that the incorporation of proteins into the scaffold influences cell survival and function, with alterations in albumin production and gene expression found between conditions.

Directly incorporating bioactive molecules such as proteins into scaffolds provides a method of creating tailored, consistent environments for liver bioengineering and the investigation of cell matrix interactions. Blended protein/polymer scaffolds represent a viable method of providing a bioactive environment for hepatocytes.

References:
1. Williams, R. *et al*. Lancet 384, 1953-1997, 2014.
2. Grant, R. *et al*. Tissue Eng. Part A [2017].

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A Tunable Poly(Diol Diacid) System for Drug Delivery and Tissue Engineering Applications

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Introduction: A scaffold material with easily tunable mechanical properties is of great utility as scaffold mechanical properties have been shown to affect cell differentiation. Additionally, biomaterials can be utilized to release antibiotics to combat infection. Terminal diols can be reacted with diacids to form a polyester. By altering the length of the diol and the saturation of the diacid, matrices with tunable mechanical properties and drug release kinetics can be synthesized.

Methods: Polymers were synthesized using decanediol in a 1:1 molar ratio with a diacid. The diacids fumaric acid (FA) or succinic acid (SA) were added in molar ratios of 1:0, 1:1, 1:9, 1:19, and 1:29. After purification, the resulting polymers were evaluated via NMR and GPC. After photocrosslinking with n-vinylpyrrolidone, the swelling and sol fraction in an aqueous and organic solvent, compressive moduli, and clindamycin release from these matrices were measured.

Results: Polyester chains were capable of being formed with all 5 FA:SA ratios as evidenced by NMR and GPC. However, the ratio of FA:SA incorporated into the polymer was lower than the initial monomer feed. All 5 polymers formed solid constructs after crosslinking; however, the group with the lowest FA:SA ratio was soluble in chloroform, indicating a lack of crosslinking. Alternating ratio of FA:SA significantly altered mechanical properties, swelling and sol fraction, and drug release from matrices ($p < 0.05$).

Conclusions: By altering the FA:SA ratio in the initial monomer feed, the crosslinking density of the polymer can be controlled, impacting mechanical properties and drug release.

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Synthetic Production of Cell Derived Extracellular Matrix for Liver Bioengineering

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An increasing incidence of liver disease and a shortage of organs available for clinical transplant[1] has led to a push for lab grown liver 'organoids'[2]. Part of the challenge of developing such an organoid lies in the creation of 3D scaffolds. Biofunctionalization of electrospun polymer scaffolds with cell-derived extracellular matrix [ECM] has been shown to influence hepatocyte function[3]. We selected a single ECM protein; Fibronectin, and selectively biofunctionalized an electrospun polylactic acid [PLA] scaffold.

A fibronectin vector [Protein Structure Initiative:Biology Material Repository] was transfected into confluent 5637 bladder epithelials, seeded on electrospun PLA scaffolds and cultured for 7 days before decellularization. The resulting synthetically derived ECM-PLA scaffolds were re-seeded with HepG2s, and cultured for 3/5 days before scanning electron microscopy [SEM], mechanical and biochemical quantification, histology, and gene expression analysis were performed.

SEM demonstrated retention of both native and synthetically derived ECM proteins. Gene expression and albumin production was significantly altered between conditions. Differences in mechanical properties confirmed the presence of an altered ECM on the vector treated constructs.

Transfected cells can be harnessed to produce customized ECMs for liver bioengineering. These results demonstrate that synthetically derived ECMs represent a viable method of biofunctionalizing electrospun polymer scaffolds.

References:
1. Williams, R. *et al*. Lancet 384, 1953-1997, 2014.
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Display of BMP-2 from PCL Micro-Fibers within Fiber-Reinforced Collagen Composites

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Bone is the second most transplanted tissue after blood, and while the iliac crest is the primary source of autologous tissue, continuing concerns of tissue availability, donor site morbidity, and cost have motivated the development of alternative tissue sources. Traditional biomaterials for orthopaedic reconstruction continue to be mechanically robust and bio-inert polymers, ceramics, and alloys. They can integrate with tissues but do not degrade; hence, they are effective at replacing bone function but suffer from mechanical fatigue and implant loosening. The past decade, though, has seen the emergence of degradable, drug-eluting, bioactive and biomimetic ''smart'' scaffolds that have potential for guiding bone regeneration.

The purpose this this project is to establish a novel framework for bone regeneration built around BMP-2-tethered fiber-reinforced hydrogels. Here, sparse fiber layers are prepared by electrospinning blends of polycaprolactone and heparin, to which the osteogenic morphogen BMP-2 are adsorbed or covalently tethered. This thin osteogenic layer - which does not impede cell migration or proliferation - is then combined into a larger collagen gel to produce a model 3D composite for bone regeneration. In this presentation we examine how the mechanical and biochemical properties of the composite affect viability, proliferation, and osteoblastic differentiation of mesenchymal stem cells. We believe this model system has promise for the fundamental studies (e.g., mechanotransduction, cell-cell interactions, foreign body responses) as well as development of a pedicle for bone transplantation.

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EGF-Enriched Collagen Coated PDLLA Electrospun Scaffold for Use as a Skin Substitute

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Bioengineered skin substitutes are an alternative life-saving approach for treating several clinical situations, such as major traumas and burns. In the search for a model that could be used not only as a protection but also stimulate tissue healing, the current study has aimed to produce and characterize an EGF enriched biomaterial using PDLLA polymer and Type 1 Collagen. Scaffolds were constructed by the coaxial electrospinning technique and divided into the following groups: 1) PDLLA, 2) PDLLA/EGF (coaxial fiber with EGF core) and 3) PDLLA/EGF/Collagen (a PDLLA/EGF scaffold with collagen coating). The sScaffolds were evaluated by scanning electron microscopy (SEM) with fiber diameters for group 1 of 1.293μm ±0.320, 1.235μm ±0.48 for group 2 2 and 1,219.±0.42 for group 3. All the groups showed similar pore sizes being 7,75μm, 7,410μm and 7,314μm for groups 1, 2 and 3, respectively and core-shell relation was confirmed by confocal microscopy. Fourier transform infrared spectroscopy (FTIR) analysis suggested the presence of collagen in the fibers from group 3 with a strong peak in 1540-1660 cm^{-1} region. Coating of the scaffolds with collagen decreases contact angle measurements (WCA), which what suggests increased hydrophilicity for group 3. In conclusion, a stable core-shell fiber EGF-enriched scaffold was developed for application in skin tissue engineering, which demonstrated better hydrophilicity with collagen coating. Financial support: FINEP, CNPq, FAPERGS and Stem Cell Research Institute.

Cardovascular

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Automated Micro-Fabrication of a Small Diameter Vascular Graft Mimicking the Structure and Mechanical Properties of Human Vessels

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Design strategies and fabrication of small diameter vascular grafts has been converging towards tissue engineered grafts in order to produce a biologically compatible graft that can overcome post-surgical complications due to mechanical mismatch between the graft and native tissue. We have developed an automated micro-fabrication technology for the standardized fabrication of cellularized multilayer cylindrical constructs with native-liked mechanical behavior. The biofabrication technique combines the deposition of cell-laden layers by a technology developed in house, and the intercalated deposition of reinforcing nanofibers by an adapted solution blow spinning device. The versatility of this automated technique allowed us to resemble the pattern of different concentric cell types, and the structural configuration of collagen and elastin fibers in native vessels. Encapsulated cells showed a homogenous distribution and well-defined concentric patterns across the constructed graft. Immunogenicity studies indicated that the combination of materials chosen did not cause rejection in the studied animals. Finally, mechanical analysis shows that the engineered graft's stress-strain curve and compliance resembles that of a human coronary artery. In conclusion, this study demonstrated the feasibility of an automated system for easy and rapid fabrication of suitable vascular grafts.

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Generation of Microchannels Using a Rapid Subtractive 3D Printing System as a Template For Tissue Vascularization

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One of the most challenging limitations with engineered tissues is the lack of a microvascular system that can provide adequate oxygen and nutrients to cells. One promising solution for this limitation is generating a template microchannels within the scaffold to enable transport of nutrients. In this study, we used a femtosecond laser-based method for rapid 3D subtractive printing at a high rate of 2,500 mm/s to generate microchannel within cell containing scaffolds composed of 0%, 1%, 2%, and 3% poly(ethylene glycol) diacrylate/collagen composite hydrogels. Compressive properties, optical clarity, swelling, degradation and cell viability via live/dead assay were measured to characterize the scaffolds. All blend conditions tested were transparent enough to allow microchannel generation. Mechanical tests confirmed that these hydrogels are a good model capable of generating channels. Different laser power was used to show control over channel diameter. The average channel width for all hydrogel was increased from 27.8 ± 5.1 to 45.6 ± 7.7 μm when the laser energy increased from 1.7 to 3.5 μJ. Importantly, these channels can be generated at a high rate with our technique, which is 2500 and 50 times faster than current inkjet and subtractive printing methods, respectively. Cells seeded within hydrogels were viable except for the ones that were very close to the channels (<10 μm). Viability can be improved by optimizing the optical system and using controlled photoablation. Overall, with the data that was collected, we validated this laser technique as a viable rapid technique for generating channels down to micrometer size.

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Engineering Vascularized Grafts for Myocardial Regeneration

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Tissue engineered cardiac 'patches' have the potential to restore functionality to the infarcted myocardium. With the high metabolic demands of cardiomyocytes, there is a need to engineer grafts with embedded capillary-like vascular networks that will anastomose with host and improve blood flow to the graft. In this study, we sought to engineer aligned, electrophysiologically connected, vascularized cardiac grafts by culturing cardiomyocytes, adipose-derived stem

cells (ASCs), and endothelial cells on 1 cm × 1 cm electrospun fibrin microfiber sheets. We tested two fibrin concentrations, 0.75% and 2.0%, that correlated with stiffnesses of 50.0 ± 11.2 and 90.0 ± 16.4 kPa, respectively. When only cardiomyocytes were cultured, we observed slightly better functional properties on the softer vs. stiffer substrates: peak conduction velocities (CVs) were 19.8 ± 3.5 vs. 14.8 ± 2.8 cm/s, maximum captured rates (MCRs) were 4.15 ± 0.67 vs. 3.56 ± 0.53 Hz, and the spontaneous contraction forces measured 16.52 ± 42.28 vs. 10.68 ± 3.37 mN. However, there were minimal functional differences in response to substrate stiffness with tri-cultures: CVs were 15.7 ± 2.7 vs. 16.7 ± 3.2 cm/s and MCRs were 4.00 ± 0.93 and 4.35 ± 0.86 Hz, while vessel networks measured 100 ± 20 vs. 108 ± 18 mm per field of view in the 50 kPa vs. 90 kPa substrates, respectively. We sutured both groups of sheets epicardially onto the left ventricular of live rats. Upon harvesting at Day 7, we found significant cell survival and graft integration. Ultimately we have shown that fibrin microfibers support the development of densely vascularized cardiac tissue patches and have significant potential as therapeutic grafts.

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WITHDRAWN

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In vitro assessment of a PLGA/Gelatin Tubular scaffold toward a Vascular Substitute.

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In this preliminary report, a fully constructed PLGA-gelatin tubular scaffold was evaluated toward its *in vitro* cellular functionality

Materials and Methods: PLGA-Gelatin dissolved in Hexafluoroisopropanol was electrospinned in a dynamic cylinder collector. SEM, FTIR, AFM were performed to evaluate morphology. Beside, cell Viability, Cytotoxicity, Cell Adhesion and Extracellular Matrix (ECM) Synthesis were analyzed.

Results: 2 μm PLGA-Gelatin fibers circularly oriented, generated a 6 mm lumen tubular scaffold with 0.45 mm thick and Vascular Smooth Muscle Cells was seeded on it. Cell Viability was determined above of 98% and no cytotoxic effects was observed. Collagen I, Collagen III, Fibrilin 1 and Elastin fibers replaced PLGA/Gelatin ones.

Discussion and Conclusions: Scaffold were analyzed by their morphological and cell-functionality properties, correlating topography, composition, degradability, concluding that is creditable its future employing in vascular grafts.

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A Biofunctional Vascular Scaffold for Replacing Small-Diameter Blood Vessels

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Diseases of the vascular system affect a large number of patients and have resulted in a clear clinical need for the development of functional vascular substitutes. We have previously fabricated a vascular scaffold by electrospinning of poly(ε-caprolactone) (PCL) combined with collagen [1]. In this study, we hypothesized that multiple bioconjugation of endothelial progenitor cells (EPC)/endothelial cell (EC) specific antibodies and anti-thrombogenic agents onto the vascular scaffold could facilitate *in situ* endothelialization while preventing blood clotting. The biofunctionalized scaffolds conjugated with EPC/EC-specific antibodies and heparin were able to achieve effective EPC/EC capturing and anti-thrombogenesis. *In vivo* experiment showed that these vascular scaffolds maintained a high degree of patency and structural integrity without eliciting a histologic inflammatory response over the course of 6-month period in sheep. Moreover, the matured EC coverage on the lumen and smooth muscle layer were observed at 6 months after implantation. We demonstrated that the generation of the biofunctional vascular scaffold, along with existing tools for selection and directed immobilization of EPC/EC-specific antibodies and anti-thrombogenic agent, could provide the necessary components for successful tissue engineering of small diameter blood vessels.

Acknowledgments: This study was supported by the Armed Forces Institute of Regenerative Medicine (X81XWH-08-2-0032).

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Glycocalyx Regeneration to Inhibit Endothelial Dysfunction and Atherosclerosis

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Overview: We tested the idea that repairing damaged extracellular glycocalyx (GCX) can reverse dysfunctional permeability across the endothelial cell (EC) layer, impaired EC-to-EC transport relevant to the loss of vascular tone control, and atherosclerosis progression.

Methods: In cell culture and atherosclerotic animal experiments [1] we studied EC with intact GCX, heparinase III enzyme degraded GCX (to disrupt GCX heparan sulfate the most abundant GCX component), mechanically degraded GCX, or regenerated GCX (by replacing and stabilizing GCX heparan sulfate). We assessed cell culture permeability of fluorescent polymer-coated gold nanoparticles [2]. EC-to-EC transport was evaluated by quantification of Lucifer yellow dye spread via gap junctions in cultured EC monolayers [3]. Magnetic resonance imaging and histological analysis were performed to visualize EC- and GCX-dependent atherosclerotic plaque progression in the mice.

Results: The results demonstrated that GCX repair with exogenous HS, sometimes requiring a co-factor, leads to nanoparticle

permeability that is similar to healthy conditions, while degraded GCX is six times more permeable. GCX repair also restores EC-to-EC transport that was lost by GCX degradation. The effect of GCX repair on atherosclerosis progression in animals is still under investigation.

Conclusion: This work will lead to new therapies targeted at the GCX to address glycocalyx- and endothelial-dependent aspects of vascular disease.

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3D Bioprinted Functional and Contractile Cardiac Tissue Constructs

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Bioengineering of a functional cardiac tissue construct composed of primary cardiomyocytes has great potential for myocardial regeneration and *in vitro* tissue modeling. However, its applications remain limited because the cardiac tissue is a complex and highly organized structure with unique physiologic, biomechanical, and electrical properties. In this study, we aimed to develop a contractile cardiac tissue construct with cellular organization, uniformity, and scalability by using a three-dimensional (3D) bioprinting strategy. Primary cardiomyocytes were isolated from infant rat hearts and suspended in a fibrin-based composite hydrogel bioink. This cell-laden hydrogel was sequentially printed with a sacrificial hydrogel and a supporting polymeric frame through a 300-μm nozzle by pressured air. Bioprinted cardiac tissue constructs had a spontaneous synchronous contraction in culture, implying *in vitro* cardiac tissue development and maturation. Progressive cardiac tissue development was confirmed by immunostaining for α-actinin and connexin 43, indicating that cardiac tissues were formed with uniformly aligned, dense, and electromechanically coupled cardiac cells. Bioprinted cardiac tissue constructs exhibited physiologic responses to known cardiac drugs (epinephrine and carbachol) regarding beating frequency and contraction forces. In addition, Notch signaling blockade significantly accelerated development and maturation of the bioprinted cardiac tissues. Our results demonstrated the feasibility of bioprinting functional cardiac tissue constructs that could be used for tissue engineering applications and pharmaceutical purposes.

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Manipulating Fibroblast Mechanical Memory with Phototunable PEG Hydrogels

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Activation of valvular interstitial cells (VICs) to myofibroblasts and their persistence play a key role in the progression of aortic valve stenosis (AVS). In healthy valve tissue, VICs transiently activate to myofibroblasts following injury. However, chronic exposure to stiffened mechanical environments prevents reversal of myofibroblasts to a quiescent state, resulting in persistently activated myofibroblasts. This *in vivo* time-dependent response to stiffness implies VICs possess a mechanical memory of their past environments. To study how VICs acquire their persistent phenotype, phototunable polyethylene glycol (PEG) hydrogel platforms were used that mimic physiological stiffnesses. With light, the hydrogel modulus may be reduced *in situ* from a pathological stiffness that activated myofibroblasts, to a soft condition where the quiescent VIC phenotype can be maintained. With this system, we identified a time-dependent mechanical dose on stiff hydrogels that triggers VICs to transform from transiently activated myofibroblasts to persistently activated myofibroblasts. After a short time period (<5 days), activated VICs can return to a quiescent state when switched from stiff to soft elastic moduli. However, after VICs are exposed to stiff environments for a long period of time (>7 days), the cells no longer can return to quiescence. The signaling pathways associated with mechanical memory will be identified with transcriptome analysis and potential mechanisms will be validated using siRNAs and/or pharmacological inhibitors. Understanding how VICs maintain mechanical memory will potentially lead to a much-needed non-surgical treatment for individuals suffering from AVS.

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Viability Assessment Of Engineered Heart Constructs: Effects Of 3d Printing And Use Of Bioluminescence For Longitudinal Monitoring

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In the fields of cell biology and tissue engineering, 3D printing has quickly become a popular and effective method for creating macroscopic tissue constructs. Yet, there are still significant concerns that bioprinters decrease cell viability due to heat and mechanical stresses on cells during printing process. To address these issues, we compared the viability of primary neonatal rat cardiac fibroblasts and myocytes before and after an extrusion-based 3D printing. Analysis of cell viability was conducted with microscopy, immunocytochemistry, and bioluminescence imaging (IVIS Lumina K machine Perkin Elmer, Waltham, MA). To evaluate the effect of the 3D printing process on cell proliferation and viability, 3D printed cardiac fibroblast constructs were compared to cardiac fibroblasts cultured in monolayers. Likewise, 3D printed cardiac myocyte constructs were compared to cardiac myocytes cultured in monolayers. We have also evaluated, side-by-side, two viability assays for non-invasive longitudinal monitoring of engineered heart tissue. The first one is a standard spectrophotometric assay based on LDH release. The second relies on luciferin bioluminescence and involves infection of tissue construct with recombinant adenoviral vector encoding firefly luciferase (AdCMVLuc). The bioluminescence-based approach was found to be a simple-to-use and highly effective way to monitor construct viability. It also provided quantitative information about the distribution and proliferation of viable cells within the construct.

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Metabolic Assessment of Therapies for the Development of Drug-Eluting Stents

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Craniomaxillofacial Tissue Regeneration using 3D-printed Poly(propylene fumarate) Tissue Engineered Bone Grafts

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Craniomaxillofacial injuries represent 25% of U.S. military injuries in Afghanistan and Iraq. Additive manufacturing (3D Printing) allows fabrication of patient-specific tissue engineered grafts. The resorbable polymer, Poly(propylene fumarate) (PPF), can be 3D-printed and degrades into non-toxic byproducts. Ring-opening polymerization (ROP)-synthesized PPF was used for its controlled molecular mass and molecular mass distribution which enables tuning of degradation and mechanical strength. ROP PPF also improves the UV-based 3D-printing of these implants which was done on an EnvisionTEC (Dearborn, MI) Perfactory® P3 3D-printer. The implant shape was derived from a Computer Aided Design file that fills a mandibular segmental defect seen in a 3D Computed Tomography scan. The 3D printed porous implant was seeded with bone marrow-derived human mesenchymal stem cells (*h*MSCs) and cultured for three weeks to facilitate bone extracellular matrix (ECM) deposition. *h*MSC to osteoblast differentiation was confirmed by increased alkaline phosphatase expression after 18 days of *in vitro* differentiation. ECM mineralization and coverage throughout the implant was shown by scanning electron microscopy and an Alizarin Red S Assay. A segmental mandibular defect was then created in a canine model where the implant was affixed with a titanium bar and screws. CT scans taken every four weeks show substantial neo-bone formation.

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Controlling Tissue Patterning in Scaffold-free, Engineered Dentin-Pulp Complexes

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A major challenge in organ engineering is generating multiple tissues with proper spatial organization. Recently, we have shown that scaffold-free constructs (SFCs) formed from human dental pulp stem cells (hDPSCs) self-assemble into organized dentin-pulp complexes. Understanding the mechanisms facilitating this patterning will enhance engineered organ design. We hypothesize that transforming growth factor beta 1 (TGFb1) induces the formation of a dentin-like structure on the periphery of the constructs, similar to processes seen naturally during reparative dentinogenesis, and that altering TGFb1 concentration gradients across SFCs will modulate tissue patterning. Scaffold-free engineered dentin-pulp complexes were formed by culturing hDPSCs in 24-well dishes in osteogenic differentiation medium. The hDPSCs formed a tissue sheet that contracted into a spherical tissue composed entirely of the cells and their endogenous matrix. SFCs were assessed after static culture +/- TGFb1 or after exposure of a linear gradient of TGFb1 using a microfluidic device. In the presence of TGFb1, SFCs formed a dentin-pulp complex with a distinct peripheral dentin-like tissue characterized by localized mineral deposition and the expression of dentin proteins. Whereas, SFCs formed without TGFb1 lacked this dentin structure. Gradients of TGFb1 across the SFCs altered patterning such that a dentin-like tissue formed in the direction of higher TGFb1 concentration. In this study, we developed methods to generate engineered, multi-tissue structures with controllable spatial organization. The ability to control patterning in engineered constructs will lead to improved methods for full organ regeneration.

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Which Non-invasive Source Of Mesenchymal Stem Cells Have The Best Osteogenic Potential To Be Used In Bone Tissue Engineering For Cleft Lip And Palate Patients?

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The closure of alveolar clefts is a major challenge in rehabilitation of patients with complete CLP and it is being currently investigated

the effectiveness of mesenchymal stem cells (MSC) to be used in alveolar bone reconstruction. Studying new non-invasive sources of the MSC can open fronts in regenerative medicine.

Purpose: In attempt to provide the best non-invasive source of mesenchymal stem cells to be used in alveolar bone tissue engineering for CLP patients.

Methods: The strains were obtained from deciduous teeth, orbicular oris muscle fragments and fragments of umbilical cord. They were characterized by flow cytometry and induced to osteogenic differentiation. The quantification of the calcium deposition measured by colorimetric assay.

Results: All cell strains reacted positively to mesenchymal markers (CD29, CD90, CD105, CD166, CD73) and they were generally negative for CD45, CD34 and CD31. After the *in vitro* differentiation under appropriate conditions, these cell populations can undergo osteogenic cell differentiation. The best osteogenic potential were observed at dental pulp stem cells and orbicular oris muscle. Using real time PCR, we test the expression profile of some neural crest genes in these cells, we observed the high expression level of PAX3 gene in dental pulp stem cells, and orbicular oris muscle derived MSC.

Conclusion: the non-invasive sources of MSC with the best osteogenic potential are dental pulp stem cells and orbicular oris muscle, we suspected that they have the best osteogenic potential, because these cells can retain part of these neural crest genetic markers.

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Clinically Relevant Bioprinting Workflow for Bone Tissue Construct Design And Validation

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Three-dimensional (3D) bioprinting is emerging as a promising enabling technology for tissue engineering applications. In this study, we developed a workflow to take 3D medical images and utilized them for bone construct design, bioprinting, and *in vivo* validation. Medical imaging and 3D bioprinting strategy converged to allow the fabrication of a structure with complex shape and inner architecture based on patient anatomy and biomimicry. Bone constructs were made of a composition of poly(ε-caprolactone) and β-tricalcium phosphate (PCL/TCP) and fabricated on the Integrated Tissue-Organ Printing (ITOP) system [1]. We examined bone regeneration in a critically sized defect at 4 and 8 weeks with the printed bone constructs. The results showed the increased bone density and volume and new bone formation and maturation with time. We demonstrated that the printed bone construts were able to organize into mature tissues of their specific characteristics *in vitro* and *in vivo*. We validated the concept that patient-specific anatomy could be translated to 3D bioprinting strategy through medical imaging and image processing software with strong clinical relevance.

Acknowledgments: This study was supported by the Armed Forces Institute of Regenerative Medicine (X81XWH-08-2-0032).

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Effect of hPDLSCs Transplantation on Periodontal Regeneration in Rats with Ligature-induced Periodontitis

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Cell therapy using human periodontal ligament stem cells (hPDLSCs) has been indicated to be clinically useful in chronic periodontitis. This study investigated immunomodulation and tissue regeneration of hPDLSCs in periodontitis model. Rats received silk ligature around the maxillary 2nd molars on both sides for 5 weeks. hPDLSCs were injected into each palatal side of maxillary 2nd molars. The immunological effect of hPDLSCs on gingiva and spleen was determined by flow cytometry for isolated T lymphocytes. The frequency of regulatory T cell (Treg) increased approximately 1.5-fold ($P < 0.01$) in the spleen and 1.9-fold ($P < 0.001$) in the gingiva at 7 days post-injection of hPDLSCs compared to ligature only group. Regenerative efficacy of hPDLSCs was estimated by μCT and histological analysis. The periodontal tissue regeneration was shown significantly at 8 weeks after the treatment of hPDLSCs. These results suggest that hPDLSC could upregulate Treg and increase periodontal regeneration. *Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MSIP) (NRF-2015R1A2A2A01004589)

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Effect of Estrogen Deficiency on Loaded and Non-loaded Area of Temporomandibular or Knee Joint

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Low level of estrogen has regarded as a main contributing factor of temporomandibular joint (TMJ) arthritis in young women patients. However, there is lack of evidence about the occurrence of arthritis in knee joint (KJ) related with estrogen deficiency. This study aimed to investigate the effect of estrogen deficiency on the loaded and non-loaded bone area of TMJ or KJ. Total of 21 SD rats were allocated into three groups, the sham-surgery group, the ovariectomy (OVX) group and the estrogen replacement group following OVX. At 12 weeks after OVX, all groups were scarified. Changes in the bone area of KJ and TMJ were analyzed using micro-CT. We analyzed the bone areas in TMJ and KJ each, which were compartmentalized into three areas on loaded, middle and non-loaded area. BMD and three-dimensional micro-CT parameters were compared between TMJ and KJ. Non-loaded area of TMJ showed significant decreases of BV/TV and BMD in OVX rats, which was recovered with the estrogen replacement. However, there was no difference in BV and BMD either in loaded area of TMJ or in all areas of KJ in both loaded and non-loaded OVX rats. Middle area of TMJ in OVX rats with or without loading showed a significant decrease in BV/TV, but no difference in BMD. These results revealed that OVX-mediated estrogen deficiency led to a significant decrease of bone formation and quality in non-loaded bone area of TMJ and no influence on bone area of KJ, suggesting that TMJ is more sensitive to estrogen deficiency.

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Efficacy of the Collagen Sponge Combined with rhBMP-2 and Alendronate on Bone Regeneration

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Inspired by the osteogenic cell differentiating and osteoclast suppressing capabilities of alendronate (ALN), we manufactured a composite type of ALN-loaded collagen sponge (ALN-CS) which controls the early detrimental effect of high-dose recombinant human bone morphogenetic protein (rhBMP)-2. This study aimed to evaluate ALN-CS as a high-dose rhBMP-2 carrier by investigating its initial biomolecular effect and efficacy on intramembranous

ossification at 1, 4, 8, and 24 weeks using a rat calvarial defect model compared with nonloaded CS. The *in vitro* rhBMP-2 release in the ALN-CS exhibited a lower burst followed by a sustained release despite lack of calcium, compared to that in CS alone. *In vitro* Characterization showed that osteoblast differentiation and mineralization of mesenchymal stromal cells were more enhanced with ALN-CS. The ALN-CS-BMP group showed higher expression of bone-forming/resorbing markers *in vivo* than the CS-BMP group at 1 week. However, osteoclasts activation in the ALN-CS-BMP group was significantly reduced over CS-BMP group. ALN-CS-BMP promoted early and dense ossification at the initial defect, with 100% greater bone mass, 20% greater bone density, and less fatty marrow tissue than CS-BMP, which continued during the whole healing period. However, CS or ALN-CS alone failed to show complete defect closure even at the 24-week healing interval. Our results demonstrate that ALN-CS has remarkable advantages over CS alone in high-dose rhBMP-2 delivery, with potent suppression of resorption, early and dense ossification with less fatty marrow formation, which highlights its great clinical potential as a rhBMP carrier for bone regeneration at intramembranous ossification site.

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Application of Novel Osteogenic Agent, Oxy133 for Alveolar Cleft Osteoplasty in a Rodent Model

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Objectives: Bone morphogenetic proteins (BMPs) have played a central role in the development of regenerative therapies for bone reconstruction, including applications in cleft and craniofacial surgery. However, the high cost and significant side effect profile of BMPs limits their broad application. Osteogenic oxysterols, naturally occurring products of cholesterol oxidation, are a promising alternative to BMPs. The osteogenic capacity of these inert and relatively inexpensive molecules has been documented in rodent and rabbit models of localized bone formation. We studied the impact of Oxy133, a novel oxysterol analogue, on *in vivo* bone regeneration in a rodent cleft model.

Methodology: A critical-sized alveolar cleft (7x4x3 mm) was generated according to the previously published method and one of the following treatment options randomly assigned to each defect: collagen sponge incorporated with 1, 10, or 20 mg of Oxy133, BMP-2, or no treatment. The maxilla was harvested for histologic and radiographic analyses.

Results: The defect treated with collagen sponge incorporated with 20 mg Oxy133 and BMP-2 demonstrated robust bone regeneration. Additionally, a lower dose of Oxy133 (1 mg) showed substantially increased bone formation as compared to no treatment. Histological studies of 20 mg Oxy133 group revealed almost complete bridging of the alveolar defect with architecturally mature new bone without any inflammation.

Significance: Collectively, these radiographic and histologic data demonstrate that Oxy133 supports new bone formation in a cleft palate model in the absence of any additional osteogenic growth factors. Oxy133 may therefore represent a viable alternative to BMP-2 in bone tissue engineering paradigms.

Matrix & Extracellular Matrix Biology in Regenerative Medicine

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Evaluation of Native and Non-native Bio-inks For 3D Printing of Human Tissues

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A variety of human skin models have been developed for applications in regenerative medicine and efficacy studies. Typically, they employ material of animal origin, which besides poorly representing physiology of the human tissue microenvironment, can also potentially lead to adverse immune responses. The use of recombinant extracellular matrix proteins can overcome these limitations. In parallel, there is a growing interest in employing 3D bioprinting platforms for tissue engineering given the possibility for precise cell positioning, flexibility, reproducibility and high throughput. Here we present our work on combinatorial screening of skin matrix components for the development of bio-inks to be used with primary human skin cells for 3D bioprinting of skin. The screening of the basal membrane proteins (collagen IV, laminin and fibronectin) demonstrated that certain protein combinations increase proliferation of keratinocytes compared to the control (no protein coating) while others mitigated cell proliferation. In the investigation of the dermal components (collagen I and III, elastin and hyaluronic acid), the primary influence on the rate of fibroblast proliferation was attributed to the source of the collagen type I (rat tail, human and bovine). We will present our results on the influence of the specific bioinks on skin cells from different donors (adult and neonatal skin origins) as well as the influence on biological and mechanical properties of the skin constructs generated with these bioinks. Our approach highlights the relevance of the bioinks to the development of 3D bioprinted Reconstructed Skin Models that are suitable for clinical translation.

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Influence of the Cell Microenvironmental Dimensionality on The Signaling Pathways in Chondrogenic Differentiation of Human Adipose Derived Stem Cells

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To design a novel stem cell niche for cartilage tissue engineering, many researchers have tried to investigate how cells interpret microenvironmental signals. Yet most of these signaling studies have been performed in two-dimensional (2D) environments. Although 2D *in vitro* studies are useful to explain general principles about the regulation of cellular signaling pathways by biochemical and biophysical cues, they do not recapitulate phenomena in 3D microenvironments. It is not clear whether regulation of TGF-β3/Smad and MAPK signaling pathways during chondrogenesis in 3D microenvironments are comparable with those in 2D environments. In this study, we compared 2D and 3D TGF-β3/Smad and MAPK signaling pathways in human adipose derived stem cells (hADSCs) using tissue culture polystyrene plates for 2D and photocrosslinkable hyaluronic acid (HA) hydrogels for 3D. We found that chondrogenesis in 3D was enhanced compared to 2D and phosphorylation of Smad2 increased with TGF-β3 treatment and phosphorylation of p38 and JNK increased when hADSCs were encapsulated in HA hydrogels. In addition, effect of TGF-β3 conjugation in HA hydrogels on these signaling pathways and cartilage regeneration were further studied. Taken together, these results showed that TGF-β3/Smad signaling was dependent on the addition of the biochemical cue, TGF-β3, while MAPK signaling of p38 and JNK was dependent on the biophysical cue, dimensionality. Additionally, our results suggest that the interplay of biochemical and biophysical factors on cellular signaling pathways should be considered when designing novel biomaterials for tissue engineering.

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Role of Extracellular Matrix in organ specificity and differentiation

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The instructive properties of the extracellular matrix (ECM) depend on matrix architecture (spatial cues), structural matrix proteins (chemical

cues) and on functional matrix components such as growth factors (functional cues). To investigate the tissue specificity of these cues, we used ECM from different organs and determined their effect on human induced pluripotent stem cell (iPSC) - derived mesodermal cells. Using sections of ECM (600 μM thick) derived from human kidney, heart and liver we showed organ specific ECM instruction of iPSC - derived precursor mesoderm: heart ECM triggers differentiation into cardiomyocytes, kidney ECM triggers differentiation into renal cells and liver ECM does not trigger differentiation of mesodermal cells under identical cultivation conditions. Cell identity of ECM - instructed cells was determined by PCR and immunocytochemistry and showed expression of nephron cells on renal matrix and beating cardiomyocytes on heart ECM expressing relevant markers. The iPSC-derived mesoderm cells on kidney ECM did not show any cardiac markers, and on heart ECM, no renal markers were detected. The endodermal derived liver ECM did not induce any renal, cardiac or liver markers in mesodermal cells. The slice ECM allows to easily observing cell phenotypes on different ECMs under the microscope or using high content screening devices.

Conclusion: Our results shows that our decellulrized matrix has the ability to instruct IPS derived- mesodermal cells to differentiate into their respective tissue cells and that specific matrix (organ specific) cues exist.

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Improved Decellularized Lung Scaffold Recellularization with ECM Hydrogel Coating

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Decellularized tissue has shown great promise as a whole lung replacement but current approaches have incomplete cell coverage. Insufficient attachment is partly due to direct exposure of the lung basement membrane to harsh decellularization chemicals causing functional ECM to be removed. The goal of this research is to add a tissue specific ECM hydrogel to the airway surfaces to increase cell attachment. Pig/mouse lung decellularization and hydrogel formation were done according to established protocol [1]. SDS-PAGE, western blotting, and rheometry were used to optimize and characterize hydrogel parameters. The hydrogel was applied as a thin ECM coating to decellularized mouse lungs before seeding with human epithelial cells using a programmed rotating device. Control decellularized lung scaffolds were reseeded with cells alone. Seeding efficiency was then assessed by histology and DNA quantification. ECM hydrogel characterization shows that lung specific ECM profile is preserved through processing with some protein fragmentation and mechanical properties similar to native lung tissue. Recellularization using a slow rotation set up and ECM hydrogel coating significantly increased cell attachment and distal lung coverage compared with cells alone. Improvement of recellularization of decellularized scaffolds is an important first step towards whole lung regeneration.

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Protein Nanoparticles from Porcine Lung Shift Macrophages to Pro-Regenerative Phenotype

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Severe lung diseases can only be cured through lung transplantation; however, lung transplants are limited by availability and donor compatibility. Engineering a transplantable lung has several hurdles to overcome still such as ensuring cells are located in the appropriate niches. Scaffolds based on extracellular matrix (ECM) proteins are regularly used in clinical applications for other tissues. Initiating self-repair of diseased lungs may be possible if clinicians can distribute healthy ECM scaffolding to the diseased regions of the lung. We hypothesize that nanoparticles formed from ECM proteins will induce pro-regenerative effects in macrophages. We decellularized pig lungs, leaving only a protein scaffold. The remaining proteins were digested in acid and electrosprayed to form nanoparticles. The formed particles were sterilized and added to bone-marrow derived monocytes from C57BL6 mice. The formed nanoparticles had an average size of 222 nm. The macrophages exposed to nanoparticles increased CD206 25% over unactivated macrophages. Additionally, CD11b was decreased in the macrophages exposed to nanoparticles by 11% compared to unactivated macrophages. These results may provide a novel method to distribute healthy ECM to diseased regions of lung tissue which may begin repair in certain lung diseases. This work was supported by NSF GRFP, and NSF CAREER CMMI-13516.

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Combination of Amnion and Fibrin as a Potential Hybrid Matrix for Guided Wound Regeneration Applications

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Amniotic membrane (amnion/AM) has inherent anti-inflammatory, anti-fibrotic, angiogenic and strong analgesic nature, whereas fibrin is a natural wound healing scaffold. Effect of both fibrin and amnion on chronic wound healing has been studied independently. This study analysed the efficiency of the hybrid matrix of amnion and fibrin (AM-F) by long term culture of dermal-like fibroblasts for accelerated and guided wound regeneration. Adipose-derived mesenchymal stem cells (ADMSCs) (IEC & IC-SCRT approved) pre-differentiated to fibroblasts were used for *in vitro* experiment, after confirming specific marker expressions for fibroblast-specific protein-1 (FSP-1) and extracellular matrix (ECM) molecules like fibrillin-1, collagen I, collagen IV and elastin after 7d and 14d of induction. Lineage commitment was evident by 7d and these cells were transferred to hybrid matrix for long term culture of 20d and 40d. ECM quantification of the decellularized matrix after induction showed higher ECM proteins as compared to control (ADMSC-grown matrix). Hence the highly regulated fibroblast properties of induced ADMSC makes it an good candidate for skin tissue engineering. To estimate the benefits of AM-F matrix, proliferation assay by ^{3}H-thymidine uptake, ECM expression studies, collagen & elastin quantification after 20d and 40d of culture were performed. Results confirmed matrix effect on ECM synthesis by ADMSC-derived fibroblasts. Wound healing efficiency was analyzed by treating 4 cm^2 size burn wounds in rabbit model. Healing in terms of reduction in wound size, epithelialisation, collagen organization and angiogenesis in 28 days was remarkably high in wounds treated with AM-F matrix as compared to fibrin alone or sham wounds.

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A Partial Least-Squares Model of Cardiac Reprogramming Predicts Reprogramming Outcomes for Different Microenvironments

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In recent years, *in vivo* cardiac reprogramming has emerged as a promising paradigm-shifting strategy for heart regeneration. The reprogramming efficiencies reported, although low (~1%), were sufficient to achieve small improvements in ischemic heart function. We hypothesize that further functional improvement may be attained by engineering the cellular microenvironment. We investigated key

microenvironmental influences on reprogramming using a tractable polyacrylamide cell culture platform to independently vary the modulus, ligand density, and ligand identity. The dedifferentiation and cardiogenic efficiencies observed were modulated by the three parameters, each in a unique fashion, thus generating a multi-dimensional response landscape. This complex biological response necessitated the use of a systems biology analysis, which connected the extracellular input signals to the reprogramming outcomes through cell-integrated signals. Observations of the YAP signaling axis and cell generated traction forces, two established mechanotransduction signals, in fibroblasts reprogrammed on the polyacrylamide platform were consequently used in the derivation of a partial least-squares (PLS) regression model. The resulting two principle component PLS model predicted the outputs of the training data with low residuals, and was then successfully able to predict reprogramming outcomes for cells cultured on a different substrate system. The inclusion of additional signals in this data-driven model may further improve its predictive power, and thereby help in the selection of biomaterial platforms that can improve cardiac reprogramming efficiencies *in situ*.

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The Role of Complex Mechanical Stimuli on Differentiation of 3T3/L1 Fibroblastic Preadipocytes and the Lipid Accumulation

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Cells, especially those of fat and muscle, are constantly subjected to mechanical stress during various physical activities. Understanding the role of the physical activity and the resultant mechanical stresses are critical for various cellular activities in the body, such as controlling cell growth, migration, differentiation, apoptosis, and wound repair. Adipogenesis process, which controls the overall metabolism through secretion of adipokine hormones, is known to be effected by the mechanical environment in the form of both static and dynamic stimulation. In this study, we report on our understanding of the role of regular (up to 10%) and cycle-by-cycle variable (0-75% variability with mean stress amplitude maintained at 10%) stretch on the differentiation of 3T3/L1 fibroblastic preadipocytes cultured on flexible membranes, in a custom-built biaxial cell stretching system [1]. Prior to stretch, cells were plated on the flexible membrane, and were allowed to proliferate to reach the appropriate confluency. They were then treated with both MDI and mechanical stretch for 45 hours and were allowed to differentiate for 5 more days prior to any evaluation. Oil Red-O was used to evaluate the cell differentiation through comparing the number of differentiating cells, the number of lipid droplets per cell, and the size range of the lipid droplets. The results showed excessive inhibition of differentiation for applied variable mechanical stretch.

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Development and Characterization of 3D Printed Pericardium Scaffolds to Promote Remodeling in Vascular Wound Applications

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A permanent, living replacement for vascular wall injury would improve outcomes of major surgical reconstructions. An implantable material that achieves this endpoint can be engineered by incorporating extracellular matrix (ECM) molecules into structurally-supportive synthetic hydrogels. These components together can promote endothelial cell growth as well as modulate the inflammatory action from macrophages, key indicators of long term incorporation of the degradable scaffold. We have developed a 3D-printable and degradable scaffold by combining polyethylene glycol (PEG) acrylate and homogenized pericardium matrix (HPM). HPM provides tissue specific variety of structural proteins including collagen, fibronectin, and glycosaminoglycans. HPM and PEGacrylate formed a hybrid hydrogel with significantly distinct modulus between 0.5 and 20 kPa. Tissue specific cell growth on hybrid hydrogels culminated with a co-culture of rat aortic endothelial cells (RAECs) and rat macrophages (RMCs). ECs cultured on HPM hydrogels laid more matrix with more VWF staining compared to cells on PEG scaffolds. RMCs developed an M2 phenotype in response to low amounts (0.03%, w/v) of HPM in culture, but responded with inflammatory phenotypes to high concentrations (0.3%, w/v). In co-culture, both M1 and M2 macrophages were detected, along with a combination of both inflammatory and healing cytokines. This result may not be pathogenic, as some inflammation is important to early wound repair, with a dynamic shift to an M2 dominated environment at later time points. Finally, models of neonatal vasculature were manufactured using DLP 3D printing, which demonstrated the utility of this approach to building specific structures for use in congenital heart defect reconstruction.

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Derivation and Cellular Response Towards a Porcine-Derived Vocal Fold Lamina Propria Extracellular Matrix Hydrogel

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Due to their anatomical location, vocal folds are highly susceptible to injury from external and internal stressors that can lead to irreversible damage and changes in function. As the structure and composition of the vocal folds are heavily linked to their unique function, we hypothesize that a vocal fold-derived extracellular matrix (ECM) would be the ideal scaffold in a regenerative approach to vocal fold repair. Our group has previously described a porcine-derived vocal fold lamina propria ECM (VFLP-ECM)[1]. In order to optimize the delivery modality of the VFLP-ECM, we have developed an injectable hydrogel form of the ECM scaffold and have studied the effects of tissue specificity using human vocal fold fibroblasts (hVFF) and human peripheral blood-derived macrophages (hPB-Macrophages). Both cell types play unique roles during the inflammatory and wound healing response at the site of vocal fold injury. In the present study, we compare VFLP-ECM with other ECM hydrogels (such as collagen, heart, bladder) in their ability to activate and modify gene expression of hVFFs and hPB-macrophages. This information will help us tailor the VFLP-ECM hydrogel to modulate the environment present during vocal fold injury.

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Reference: 1. Wrona *et al*. Derivation and Characterization of Porcine Vocal Fold Extracellular Matrix Scaffold. Laryngoscope 126, 4, 2016.

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Elucidating Interactive Signaling of Biochemical and Mechanical Cues on MSC Chondrogenesis in 3D Using Gradient Hydrogels

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Stem cell niche is a multi-factorial environment including biochemical and mechanical cues. While extensive studies have highlighted the important role of biochemical or mechanical cue alone on stem cell fate regulation, little is known about how interactive niche signaling modulate stem cell fate in 3D. The goal of this study is to employ a 3D gradient hydrogel platform we recently developed for high-throughput screening of interactive niche signaling on MSC fate. PEG-norbornene (PEGNB) and linear dithiol (SH) were used to control stiffness, and methacrylated chondroitin sulfate (CS-MA) and hyaluronic acid (HA-MA) were chemically incorporated as biochemical cues. Hydrogel with stiffness gradient higher than $\sim$20 kPa generally inhibited neocartilage deposition, and varying biochemical cues did not lead to noticeable differences. When encapsulated in hydrogels with lower stiffness gradient (0.5 - 10 kPa), chondroitin sulfate led to most robust cartilage matrix formation by mesenchymal stem cells (MSCs) compared to that of hyaluronic acid. Finally, we have utilized such platform for optimizing scaffold design with stiffness ranging from 0.5 kPa to 15 kPa and chondroitin sulfate concentration ranging from 3% to 8%. Such design supported robust MSCs proliferation and matrix deposition while induced distinct zonal-dependent tissue formation that mimics native cartilage zonal properties. Such 3D gradient hydrogels could provide a powerful tool for elucidating the complex interactions between multifactorial niche cues in a high-throughput manner, and facilitate rapid identification of optimal niche cues that led to desirable stem cell fate and tissue formation.

Musculoskeletal

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Creation of a Novel Ubiquitous tdTomato Rat and Tracking of Cells in an Implanted Engineered Skeletal Muscle Tissue

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The growing deficit in suitable tissues for patients awaiting organ transplants demonstrates the clinical need for engineered tissues as alternative graft sources. Demonstrating safety and efficacy by tracking the migration and fate of implanted cells is a key consideration required for approval of promising engineered tissues. In this study, we hypothesized that fabrication of engineered skeletal muscle using cells isolated from a ubiquitously fluorescent animal would allow tracking of cell migration following implantation. To test this hypothesis, we generated a rat ubiquitously expressing tdTomato (tdT) expression. Following validation of tdT fluorescence expression on the cell and tissue level, we fabricated engineered skeletal muscle units (SMUs) from isolated tdT satellite cells and implanted these SMUs into a volumetric muscle loss defect of the tibialis anterior (TA) muscle in a rat ubiquitously expressing green fluorescent protein (GFP). Following a recovery period of 28 days, the repaired TA muscles were explanted for histological analysis. Explanted tissues demonstrated both tdT and GFP expression in the repair site, indicating involvement of both implanted and host cells in the regeneration process. It is worth noting that tdT fluorescence intensity was consistently greater than GFP intensity. These results demonstrate our successful generation of a novel tdTomato rat while also showing one potential application taking advantage of this tdTomato fluorescence. This unique animal could assist researchers in similar cell tracking experiments and ultimately serve as a valuable preclinical model system for promising tissue engineering therapies.

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Evaluating Cyclic vs. Static Uniaxial Strains for Engineering Functional Skeletal Muscle Grafts

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Tissue engineering of skeletal muscle grafts is a promising strategy for the treatment of volumetric muscle loss. Yet, it remains challenging to engineer grafts with functional properties comparable to those of native tissues. Prior studies have reported enhanced functional outcomes in response to static and cyclic uniaxial tensile strains. However, differences in their experimental designs make it impossible to compare the relative efficacy of applied strain protocols. To address this, we have developed a novel bioreactor platform for the application of various strain regimens to C2C12s grown on aligned, electrospun fibrin scaffolds. We will investigate whether the application of static or cyclic uniaxial strain more effectively increases the structural, morphological, and functional outcomes of tissue engineered skeletal muscle constructs. We seeded 300,000 C2C12s onto each scaffold and cultured under free-swelling conditions for 3 days before beginning the strain application process. The experimental groups included 0% strain (control) or 10% strain amplitude for 6 hours a day using a static or cyclic (triangular waveform; 0.5 Hz) profile. In all groups the C2C12s exhibited multi-nucleation and alignment parallel to the fibrin microfibers. Engineered muscle grafts spontaneously contracted with forces ranging from 5 - 15 mN. Preliminary results have shown that both static and cyclic strain increase the myotube diameter and the number of nuclei/myotube as compared to controls, as well as the appearance of spherical 'myoballs'. Ongoing studies will further investigate the interplay between biophysical cues and cell morphology and explore the molecular mechanisms governing these changes.

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Bioinspired Three Dimensional Construct with Silk Fiber Reinforcement for Regeneration of Load Bearing Soft Tissues

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Fiber-reinforced composite scaffolds have gained ground in tissue engineering applications by integrating mechanical strength with ability to mimic native tissue structures[1]. Advancing this strategy, our work focuses on blending silk fibers (mulberry *Bombyx mori* and non-mulberry *Antheraea assamensis*) with the biocompatible silk matrix material to improve its application for cartilage tissue engineering. The physical characterization studies of the fabricated scaffolds revealed that the scaffolds were porous with interconnected pores, had swelling property for nutrient uptake and biodegradable. The scaffolds supported proliferation of primary chondrocytes and maintained chondrogenic nature of the cells which was evident from alcian blue staining of GAG (sGAG) secreted by the cells. Biochemical analysis showed collagen, sGAG and DNA increased with time on culturing chondrocytes on the scaffold after 28 days. The non-mulberry silk composites showed enhanced growth and proliferation as compared to the mulberry silk. This was further attested by upregulation ($\sim$2 fold) of cartilage-specific ECM gene markers- Collagen-II, SOX9 and aggrecan, post 28 days of culture. The presence of intrinsic integrin binding RGD sequence[2] in *Antheraea assamensis* silk validated its preferable choice over its mulberry counterpart. Easy aqueous based fabrication technique, suitability of fibers as a substratum for greater cell attachment and mechanical robusticity of fabricated silk fiber-reinforced scaffolds establishes them as a sustainable alternative. Such composite scaffolds have tantalizing potential to refill the damaged tissue void by *in vivo* neo-tissue formation post implantation.

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Tissue Engineered Muscle Repair (TERM) for treatment Of A Critically Sized VML Injury in the Rat Latissimus Dorsi

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Volumetric muscle loss (VML) injuries are extremely complex and lead to permanent functional and cosmetic deficits. Improved therapeutics for VML injury largely depend on the development of more germane preclinical animal models. Herein, we tested the effectiveness of the TEMR technology platform (i.e., muscle progenitor cells seeded on an acellular porcine-derived bladder acellular matrix/scaffold) to restore function in a biologically relevant VML injury in the rat latissimus dorsi muscle (LD). The VML injury model entailed resection of 1.8 cm^2 of the LD. TEMR constructs were implanted at the time of injury ($n=6$). Control groups included a no repair group ($n=6$), and implantation of an acellular scaffold ($n=8$). *Ex vivo* muscle contraction force was evaluated 8 weeks post-surgery via electrical field stimulation in an organ bath. Contralateral LD muscles served as positive controls. Samples were submitted to histological analysis. TEMR implantation resulted in generation of 84% of isometric force (TEMR: 2.7 ± 0.2 N *vs* native LD: 3.2 ± 0.07 N), which was statistically significantly greater than the 56% and 65% observed in the untreated NR, and BAM group, respectively (NR: 1.8 ± 0.1 N; BAM: 2.2 ± 0.2 N; $p < 0.05$). TEMR constructs integrated with native tissue, and were well vascularized 8 weeks post-implantation, with new muscle tissue formation evident only in the TEMR-treated group. In summary, TEMR constructs promoted substantial functional recovery in a critically-sized, biologically relevant VML injury in rats. These findings bode well for potential clinical applications to craniofacial repair (e.g., cleft lip) of the TEMR technology platform.

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Carbon Dot Doped SPION Gelatin Freeze Dried Composites for Osteochondral Regeneration

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Mechanotransduction allows MSCs to differentiate into different phenotype. Exploration of magnetic field is one of the major method by which this can be achieved. In current study, carbon dots (CD) doped super paramagnetic nanoparticles (SPIONS) are synthesized and explored for combined effect of chemical induction of CDs and magnetic mechanotransduction of SPIONs. The doped nanoparticles were prepared by hydrothermal method and characterized by TEM, XRD, XPS, FTIR, Raman spectroscopy, SQUID and AES. Further, the nanoparticles were mixed with gelatin to prepare freeze dried scaffold. The scaffold structure and porosity was checked using SEM and *in vitro* cyto-compatibility was performed using fluorescence microscopy, MTT assay and ROS scavenging assay. Osteochondral differentiation was checked by culturing MSCs in to scaffold and actuating them using 0.05 T rare earth bar magnets. Via PCR and ALP assay osteochondral regeneration was observed. The cell cultured scaffolds were implanted on rodent model for ectopic osteochondral differentiation study. On histology, features of both chondrogenesis and osteogenesis were observed. No specific feature related to iron toxicity was observed.

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BMP-2 Indirectly Induces Angiogenesis During Large Bone Defect Regeneration

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Critical-sized bone defects do not heal without intervention and their repair is often limited by insufficient neovascularization. Bone morphogenetic protein-2 (BMP-2) stimulates osteogenesis and has been proposed to induce angiogenesis by directly stimulating endothelial cells. Thrombopoietin (TPO) has been shown to stimulate bone healing through osteoblast activation by megakaryocytes, and may also promote angiogenesis. We hypothesized that BMP-2 or TPO delivery would enhance vascularized regeneration of critical-sized bone defects compared to treatment with scaffold alone. 8mm defects were created in rat femora and stabilized with fixation plates without medullary reaming. Defects were treated with a collagen sponge hydrated with PBS, 5μg BMP-2, 5 μg TPO, or 25 μg TPO. Neovascularization was evaluated by microCT angiography at weeks 3 and 8. Bone formation was measured post-mortem at 3 weeks or *in vivo* at 4 and 8 weeks. *In vitro*, endothelial colony forming cells (ECFC) were treated with 100 or 200 ng/mL of BMP-2 and/or TPO and evaluated for migration, tubulogenesis, and SMAD 1/5/9 nuclear localization. *In vivo*, BMP-2 significantly induced bone formation and neovascularization, but TPO treatment did not significantly affect either. Studies have observed significant effects of TPO delivery on bone regeneration, suggesting limitations of the collagen sponge delivery method or need for medullary reaming to promote megakaryocyte mobilization. BMP-2 and TPO had no significant effect on *in vitro* ECFC migration or tubulogenesis, but BMP-2 supplementation increased SMAD nuclear localization. These data suggest that the effect of BMP-2 on angiogenesis is secondary to bone formation-induced vascular recruitment rather than direct endothelial cell activation.

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A Whole Joint-in-Motion Culture System Reveals a Critical Role of Glucose in Regulating Articular Cartilage and Growth Plate Matrix Production

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Introduction: Currently, there are no suitable *in vitro* systems to evaluate mechanically and biochemically induced osteoarthritis (OA), hampering therapeutic development. We designed a system (JM1) to study cartilage destruction by facilitating joint culture movement, and used this system to evaluate the effects of glucose and mechanical stimuli on cartilage.

Materials and methods: 8-week-old Balb/c mouse knee joints were isolated and mounted into JM1 devices. Joints were actuated through a 0.5 Hz extension-flexion cycle for 8hrs/day (''dynamic stress''). Controls included joints cultured in petri dishes with no movement (''static'') and on rotating platform (''on rotator''). Joints were cultured in varying glucose or mannitol mediums. After 7 days, joints were harvested and histology was assessed via OARSI scoring.

Results: *1. Glucose and mechanical loading synergistically affect joint health.* A) Increasing levels of glucose caused a reduction of matrix in the articular cartilage. B) ''Dynamic stress'' group showed highest cartilage matrix loss at any glucose level. *2. High glucose-induced joint cartilage matrix loss is not due to osmolarity.* Glucose was substituted by mannitol to control for osmolarity effects. Our results showed that high glucose metabolism, rather than osmolarity, induced cartilage matrix loss.

Conclusions: This study demonstrates a uniquely designed dynamic system for culturing whole mouse that recapitulates

mechanically-induced human OA development. It allows for the dissection of biochemical and biomechanical inputs, and serves as a platform for mechanistic and therapeutic testing. Using this device, we found that mechanical stress potentiates cartilage matrix loss under high glucose levels. This study has implications for diabetes-related OA.

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Immune-Dysregulation in a Rat Model of Infected Femoral Segmental Bone Defect

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Open contaminated fractures, particularly in the setting of segmental bone loss, are a clinical challenge. In the setting of trauma and infection, it has been proposed that immune-dysregulation and associated hardware failure. The purpose of this study was to investigate the long-term immune response in a clinically-relevant model of infected segmental bone defect. Thirteen-weeks-old female SD rats underwent unilateral 3 mm mid-diaphyseal femur bone defects surgery, with plate stabilization. Infection group received biomaterial soaked with luciferase expressing *Staphylococcus aureus*. Bacterial growth and bone bridging was monitored using a Bruker Imaging system. Systemic immune response was measured by peripheral serum cytokine array and flow cytometry analysis of splenocytes, bone marrow cells and adjacent soft tissue from defect site. Micro CT scans were performed to quantify bone bridging and periosteal growth. Bioluminescent signal appeared in infection animals at d3 post-surgery and was present up to d7. Serial radiographs showed no bridging from infected animals at any time point. Three of four infected rats had hardware failure and observed gray necrotic soft tissue around the hardware. Luminex cytokine analysis demonstrated infected animal had increased expression of immune suppressive cytokines (IL-10) and reduced concentration of inflammatory cytokines (TNF-α, IL-1) compared to controls. Moreover, infected rats showed increased immune suppressive cell (MDSCs, Tregs) population in spleen, bone marrows and infected tissues. This study would be able to establish a clinically-relevant animal model of infected non-union with hardware failure, and present supportive preliminary evidence of chronic systemic immune dysregulation due to infection.

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3D Printed Biphasic Osteon-like Scaffolds for Bone Tissue Engineering

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Attempting to mimic the physical and chemical architecture of biological tissues is particularly challenging, and often requires advanced fabrication techniques. We are interested in engineering cortical bone tissue, and specifically its component osteons, which consist of organized concentric calcified regions with interpenetrated vasculature. Extrusion-based 3D printing (3DP) offers an efficient tool for accurate fabrication of biomaterial scaffolds with tunable properties. In the present study, we have developed a 3DP biphasic bone-like scaffold, containing two separate osteogenic and vasculogenic cell populations encapsulated in fibrin bioink and co-printed along a supporting polycaprolactone (PCL) carrier scaffold. To this end, fibrin bioink was first optimized to improve the resolution of printed fibers and ensure a reproducible printing process. The influence of printing parameters on cell survival was then investigated. Human umbilical-vein endothelial cells (HUVEC) and human mesenchymal stem cells (hMSC) laden fibrin were printed in concentric rings and cultured *in vitro*. After two weeks, cell viability (Live/Dead) and cell proliferation (Picogreen) were assessed. Immunohistochemistry and quantitative polymerase chain reaction showed an upregulation of osteogenic (alkaline phosphatase, bone morphogenetic protein 2) and vasculogenic (vascular endothelial growth factor) markers. Dynamic mechanical analysis showed an elastic modulus of 0.7MPa ± 0.3MPa under compression. Biphasic scaffolds (fibrin and PCL) showed improved mechanical properties with an average compressive modulus of 103MPa ± 26.8MPa. Finally, biphasic biomimetic scaffolds seeded with rat aortic endothelial cells and MSCs were implanted subcutaneously in rats. Results showcase the capacity of our 3DP biphasic construct to provide accurate physical cues for large-scale vascularized bone.

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Using Ultrasound for Non-Destructive Evaluation of Engineered Cartilage Damage by Sliding Shear

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Overview: Cartilage must function under transient compression and shear. Under these conditions, catastrophic damage to tissue engineered (TE) cartilage occurs, which limits its *in-vivo* utility. Currently, damage is only observed after a test, providing no information as to when the failure occurred. Here, we prototype an ultrasonic (US) system for identifying tissue damage under compression and sliding shear.

Methods: 31 samples of scaffold free MSC-based TE cartilage were glued to stainless steel reflectors, placed in the US testing device, and US signals between the cartilage surface and the reflector were recorded. Samples were then subjected to between 0 and 1000 shear loading cycles, after which US data was recorded again. In some cases, the transducer was scanned across the sample to evaluate roughness. Samples were then processed for histology.

Results and Discussion: US scanning correctly identified several failure modes of TE cartilage under sliding shear. In most samples, damage was observed after tests, despite no visual cues of failure during the tests. Ultrasound easily demonstrated thickness changes in the samples pre- and post-sliding. Samples with eroded or roughened surfaces demonstrated reduced or absent surface reflections. Surface erosion was clearly illustrated by scanning across the sample.

Conclusions: Non-destructive monitoring of sliding shear experiments using an ultrasound transducer built into the test rig identifies early failure, greatly enhancing the evaluation of TE cartilage.

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Modeling and Analysis of Gait Biomechanics after Volumetric Muscle Loss Injury

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Many studies are underway to research methods of regenerating muscle as a treatment for volumetric muscle loss (VML) injury, but few have evaluated VML injury in relation to the biomechanics required for normal function. The ability to quantify changes in rat gait will facilitate identification of relevant functional deficits resulting from injury, as well as the functional effectiveness of regenerated muscle. Four 12-week female Lewis rats were tested on a Panlab Rat Treadmill at 40 cm/s with video recorded at 2, 4, and 8 weeks post-surgery. Baseline testing occurred the week prior to surgery. Surgery

consisted of removal of 20-30% of the right tibialis anterior (TA) muscle and the extensor digitorum longus (EDL) and extensor hallicus longus (EHL) synergists. Prior to all runs, 3mm/5mm reflective markers were placed on the joint centers of the pelvis, spine, tail, hip, knee, ankle, and toe. Rats were recorded using Vicon Nexus motion capture software with a 7-camera setup collecting at 120 Hz. Marker data was exported to a custom OpenSim rat hindlimb model for simulation of gait kinematics. All data was filtered at 30 Hz and post-simulation analyses were performed in Matlab. We saw more hip abduction, hip external rotation, and ankle plantarflexion post-injury of the TA muscle which is indicative of circumduction, a classic compensation for drop foot. These changes were durable over the 8-week time course of study. The addition of force plates to capture ground reaction forces in the future will further inform development of more effective regenerative technologies for VML injuries.

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The Osteogenesis and Degradation of 3D Printed Calcium Silicate/Polydopamine/Polycaprolactone Scaffolds for Bone Regeneration

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The emergence of three-dimensional printing techniques has been a breakthrough in bone tissue engineering. Although there is still a shortage of available biomaterials which exhibit both osteogenic activity and printability could be readily used, incorporation of bioceramics into printable composites which possess good osteogenic activity. Calcium silicate-based materials (CS) have attracted much attention due to their advantages in promoting the proliferation and differentiation of osteogenic cells. Exciting results of the printed CS/polymer scaffolds on the osteogenesis have been reported. Unfortunately, the presence of $Ca(OH)_2$ in CS could accelerate the degradation rate of the thermoplastic polymer, resulting in the premature failure of the implanted scaffolds. Thus, we proposed a facile route to fabricate the CS/polycaprolactone (CS/PCL) composites without affecting the degradation rate through coating polydopamine (PDA) on the surface of CS. The results indicated that the presence of PDA between CS and PCL could be served as an anchor layer to reinforce the compressive strength of the scaffolds. The *in vitro* degradation experiments conducted by monitoring the pH change and weight loss of the scaffolds after soaking in simulated body fluid confirmed that the introducing of PDA into the composite indeed minimized the level of the pH raise and decreased the degradation rate of the composite scaffolds. More importantly, the CS/PDA/PCL scaffolds supported higher levels of proliferation, alkaline phosphatase activity, and mineralization of human mesenchymal stem cells than CS/PCL. Taking together, these results suggest its potential application in bone regeneration.

Neuronal Tissue

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The Effect of Keratin on Spinal Cord Injury Recovery

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Spinal cord injury (SCI) is a devastating neurologic event with complex pathophysiological mechanisms that is difficult to treat: currently no effective therapies are available. Keratin nanomaterials (KN) can induce macrophage polarization and promote the M2 anti-inflammatory phenotype to attenuate inflammatory responses. The purpose of this study was to evaluate the ability of KN to reduce secondary damage after SCI and improve the environment for nerve regeneration and restoration of function. Female Lewis rats were trained on a DigiGait Imaging System treadmill and baseline gait recordings were obtained for two weeks before SCI. SCI was induced at T9 with a blunt force of 150Kdyn, producing moderate injury with partial recovery. Treatment groups were: intrathecal KN, topical KN, and saline dural application controls. Functional recovery was observed at 3 and 6 weeks post-SCI and compared to pre-SCI recordings, followed by tissue harvest for histology. Gait metrics of Stance phase, %Swing/Stride, %Brake/Stride, Stride frequency, Stride length, Paw area variability, and Paw angle were compared by two-way repeated measures ANOVA followed by Bonferroni post-hoc comparisons. Topical KN rats demonstrated increased fine motor control, normal stride length and frequency, and normal paw angles compared to controls or intrathecal KN and demonstrated values similar to baseline. Quantification of lesion area and glial scar protein yielded no significant differences between groups. KN-treatment may limit secondary damage to improve motor function but future experiments are required for understanding tissue healing and mechanism of action.

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Soluble Fractions Of ECM Decrease Viability Of Primary Glioma Cells

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Gliomas are the most lethal and common primary tumor type in the central nervous system (CNS) across all age groups; affected adults have a life expectancy of just 14 months. As glioma cells invade the surrounding normal parenchyma they remodel the surrounding extracellular matrix (ECM), suggesting that the native normal microenvironment is not ideal for their progression. Recent reports describe suppressive and/or lethal effects of mammalian ECM hydrogels derived from normal (non-neoplastic) sources upon various cancer types, though the ECM components responsible for these effects are unknown.

We have shown that soluble fractions of ECM derived from porcine dermis, small intestinal submucosa (SIS), and urinary bladder (UBM) all decrease the viability of human primary glioma cells, and that UBM-ECM soluble fraction showed the most potency. In a cell viability assay (MTT) normalized to the media treatment, non-neoplastic CHME5 and N1E-115 cells scored between 103% and 114% when treated with a 3X concentrated soluble fraction solution of dermis-ECM, SIS-ECM, or UBM-ECM. Two primary high grade glioma cell types scored 78.5% and 90.5% with dermis-ECM, 48.5% and 64% with SIS-ECM, and 17% and 30.5% with UBM-ECM (average of $N = 2$). We further observed that glioma cells have decreased migration, and markedly decreased proliferation in the presence of these matrix-embedded cues, indicating that non-neoplastic ECM contains potent homeostatic regulators that are capable of abrogating malignancy. Delivering soluble fractions of ECM to a tumor site may represent a novel approach to cancer therapy, sidestepping traditional cytotoxic therapies in favor of utilizing putative endogenous anti-tumor pathways.

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Peptide Amphiphile Scaffolds For Spinal Cord Regeneration

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Spinal cord injury (SCI) is a debilitating condition affecting an estimated 1,275,000 Americans at a cost of over 40 billion dollars each year. SCI causes immediate damage of nervous tissue accompanied by loss of local blood vessels, release of inflammatory mediators and the cellular immune response at the site of injury. These

processes lead to further axonal injury and cell death, which impede endogenous tissue repair and limit prospective repair approaches. To date, there is no single biological intervention that can address all of the events that damage the spinal cord. Previously, our group demonstrated that peptide amphiphile (PA) molecules self-assemble into nanofibers that can be used as bioactive scaffolds, which were beneficial upon injection in an experimental mouse model of SCI. Here, I integrated orthogonal bioactive moieties that addressed different aspects of the injury response in order to increase the potency of the bioactive biomaterials that have already been shown to bridge the lesion site effectively. This was accomplished by designing and testing new fibroblast growth factor 2 (FGF-2) mimetic PA molecules that self-assembled into nanostructures as an angiogenic and neurogenic biomaterial platform for treating SCI. The FGF-2 mimetic sequence incorporated in a PA was co-assembled with a known PA that presents the neuroactive pentapeptide epitope from laminin, IKVAV-PA, to promote vascularization, nerve regeneration, functional recovery, and to limit the damage. This project evaluated the synergies between the different PA sequences and defines the most effective combinations, and determine the best stoichiometry *in vitro*.

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The Development of a Human Cortex Model for High Throughput Neurotoxicity and Drug Screening

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Increased cerebrovascular permeability due to blood brain barrier (BBB) disruption is known for destabilizing brain homeostasis, neuronal function and nutritional distribution in brain tissue. The BBB controls these functions through a dynamic structure of tight junctions and adherent junctions formed mainly between endothelial cells. The integral selectivity characteristic of the BBB limits therapeutic options for many neurologic diseases and disorders. Currently, very little is known about the mechanisms that govern the dynamic nature of BBB. To date, most *in vitro* models only utilize endothelial cells, pericytes and astrocytes. These models neglect the role of other cell types in the brain cortex such as the neurons, microglia and oligodendrocytes. Thus, we seek to create a 3D spheroid model of the blood brain barrier consisting of all major cell types that closely recapitulate normal human brain tissue. Spheroids containing 6 cell types were maintained in static culture with growth media exchange every other day and were fixed in 4% formaldehyde and Immunohistochemistry was performed for TJ, AJ and cell specific markers. Our data demonstrates expression of TJs and AJs. Furthermore, our data on BBB functionality assessment using MPTP, MPP+ and Mercury chloride in our spheroids indicate charge selectivity through the barrier. Our spheroid model would have applications in drug discovery and neurotoxicity and cytotoxicity testing. This model can serve as a tool for individualized, patient-specific blood brain barrier disease models through the use of representative cell types derived from induced pluripotent stem cells (iPSCs).

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3D Bioengineered Model of Human Enteric Nervous System

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Bidirectional interactions between the central nervous system and the gastrointestinal tract (GI) via the enteric nervous system surrounding the GI tract are unmapped. Assortments of neurodevelopmental disorders are associated with disturbances in the gastrointestinal system. There is a need for 3D intestinal systems *in vitro* that permit the study of complex human cell interactions, including the enteric nervous system. Tissue engineered systems offer the potential to encompass the properties of endogenous human systems in a modular, tunable format as *in vitro* models. Therefore, we focus on the development of *in vitro* 3D human innervated intestinal tissues that encompasses both human induced neural stem cells (hiNSCs) differentiated into pertinent enteric nervous system neural cell types, as well as enterocyte-like (Caco-2) and goblet-like (HT29-MTX) cells that create the intestinal epithelial layer, building off of our earlier systems. Cultures of *in vitro* ENS (ivENS) scaffolds indicate the capability to function for at least 27 days, indicating the long term viability of experiments. Seeded hiNSCs migrate from seeding locations toward the lumen of the scaffold to the epithelial cells. Additionally, nNOS-expressing neurons were found within the ivENS system. These tissue models elevate experimental options in technology to understand neural circuits controlling the intestine and communicating with the microbiome.

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Fabrication of Anisotropically Organized 3d Neuronal Bundles Using 3d Bioprinting

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Nerve tissues have several structural features such as bundle of axons and linearly ordered cellular components. Despite many efforts for engineering of artificial nerve tissue, the introduced methods still have limitations in producing 3D biomimetic architectures. Herein, a method to construct linearly organized, cable-like bundles of 3D neuronal tissues has been proposed by using contractile force of cells. A cellular bundle structure was fabricated by applying neural cells, gelatin based bio-ink and synthetic polymer into 3D bioprinting. Boundary conditions to induce contractile force were obtained by printing fixed pillars on both side. The printing results were evaluated by imaging and cytocompatibility tests. Staining results successfully showed that bioprinted artificial tissues were anisotropically organized. Overall structures were well maintained for than week. Moreover, cellular activities such as cell proliferation and differentiation to neuronal tissue were well maintained after printing. These results indicated that the proposed method is quite suitable to produce 3D biomimetic circuit for nerve tissue engineering.

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Nerve Growth Factor incorporated Coaxial Electrospun Cellulose Acetate/Polyvinylpyrollidone(CA-PVP-CA) Nanofibers for Neural Cell Differentiation

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Efficient repair and regeneration of nerve gaps requires scaffold to biomimic topographical structure of the native tissue along with sustained supply of neurotrophic factors[1]. Electrospun nanofibers mimicking extracellular matrix (ECM) have been extensively used as nerve grafts because they could modulate diverse cellular functions like differentiation[2]. Here, we present the synthesis of core-shell nanofibers with Cellulose acetate (CA) shell and Polyvinylpyrrolidone (PVP) core material with nerve growth factor (NGF) encapsulated in the core using coaxial electrospinning

technique. After extensive optimization, (CA-PVP-CA) nanofibers with average diameter of 493 nm were successfully obtained and characterized by SEM, TEM, XPS and FTIR techniques. Next, enzyme-linked immunosorbent assay (ELISA) was used to evaluate the release of NGF from the coaxial nanofibers over period of 10 days. The effect of this coaxial scaffolds on the differentiation of PC12 cells were evaluated over a period of 14 days and results show that coaxial nanofibers could effectively promote differentiation of PC12 cells.

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Polymeric Microparticles and Extracellular Matrix Hydrogels Facilitate Stem Cell Delivery for Spinal Cord Repair

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Traumatic spinal cord injuries (SCI) cause devastating neurological deficits and disabilities. Initial trauma leads to immediate disruption of neural tissue by axon shearing, blood vessel rupture and cell death. Subsequently a cascade of secondary events occurs composed of ischemic injury, inflammation, cell death, demyelination of axonal tracts and the creation of a glial scar. Cell transplantation to replace damaged cells, provide trophic support and promote functional recovery is a favourable strategy for SCI. Stem cells derived from dental tissues are an attractive source due to accessible supply, high proliferation rates and the potential for autologous transplantation. The objective of the present study was to evaluate the suitability of different biomaterials, including surface modified PLGA microparticles and extracellular matrix hydrogels, to act as cell delivery vehicles for spinal cord repair. Polymeric microparticles coated with spinal cord ECM hydrogels promoted dental stem cell viability. Furthermore, release of glial derived neurotrophic factor (GDNF) from microparticles stimulated neural differentiation of the dental stem cells. Viability of dental stem cells was similar in hydrogels derived from bone and spinal cord extracellular matrix (ECM). Spinal cord ECM hydrogels did not gel *in vivo* whereas bone ECM hydrogels had higher storage moduli, providing a stiff and fast gelling material to overcome the flow of blood and cerebrospinal fluid *in vivo*. The combination of bone and spinal cord ECM hydrogels facilitated gelation, enhanced gel properties and provided a homologous ECM hydrogel. These results will inform further studies utilizing combinations of ECM hydrogels and microparticles for spinal cord repair.

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3D Printing for Spinal Cord Injury

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There exists a great unmet medical need to develop novel therapies that promote axonal regeneration after spinal cord injury. While bioengineered scaffolds have been reported to support axon regeneration into spinal cord lesion sites, these technologies have been limited by foreign body responses at implantation sites, cumbersome production requirements, limitations in scaling to human size injuries and lack of biomimicry of the natural spinal cord. We now report the first use of advanced microscale 3D bioprinting to fabricate a scaffold mimicking the complex fascicular architecture of the spinal cord. The printed scaffold is simply and rapidly produced, reduces foreign body responses, and supports linear, aligned host axonal regeneration in the most challenging model of spinal cord inure - complete transection. Moreover, loading neural stem cells into the scaffold supports host axon regeneration and functional recovery in long-term *in vivo* studies. This technology advances efforts to promote neural repair.

Production Assistance for Cellular Therapies

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Role of Myelin Topography And Alignment on the Activation of Astrocytes

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Introduction: Myelin sheath is thick layers of neuronal plasma membrane that serves as a capacitor during neuronal signal transmission. Decay in myelin sheath is associated with several neurodegenerative diseases such as multiple sclerosis and Alzheimer disease. Although its primary purpose is to insulate and accelerate the neuronal impulse, very few studies focused on the geometrical aspect of myelination in neuronal function. Here, we developed a versatile platform to study the effect of myelin topography and alignment on the neuronal signal transmission.

Materials and Methods: Electrospinning of Poly-epsilon-caprolactone (PCL) was performed to achieve fibers with 0.5 μm diameter. After electrospinning, the fiber surface was treated with oxygen plasma and activated surfaces finally exposed to myelin solution in 100% EtOH for surface coating. For astrocyte activation 10,000 astrocytes/10 mm disc shaped scaffolds were seeded onto sterilized scaffolds and cultured for 5 days and tested for astrocyte activation markers. For neuronal signal transmission, myelinated scaffolds were conjugated with motor proteins and further microtubule movements were recorded using fluorescent microscope.

Results: Astrocyte spreading and *in vivo* like morphology was observed on biomimetic fibrous scaffolds. Astrocyte activation markers (GFAP and nestin) were checked using immunostaining. The topographies and alignment on neuronal signal transmission results show a direct correlation between myelin topography and microtubule gliding speed.

Discussion and Conclusions: This study demonstrates of an experimental platform to investigate the effect of myelin topography and astrocyte function as well as neuronal signal transmission using a novel microtubule gliding assay.

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Therapeutic Outcomes of Insulin-like growth factor-1 Released from Alginate-gelatin Microbeads on Stress Urinary Incontinence in Rats with Simulated Childbirth Injury

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Introduction and Objectives: Insulin-like growth factor-1 (IGF-1) treatment can accelerate recovery from stress urinary incontinence (SUI) induced by simulated childbirth injury in rats. A local sustained delivery method is ideal for further clinical applications to avoid side effects of IGF-1. Our goal was to determine the effects of controlled release of IGF1 from alginate-gelatin microbeads (IGF1-A-G-beads) on sphincter tissue repair in a rat SUI.

Methods: Female SD rats (n = 44) were divided into 4 equal groups: A) sham vaginal distension (VD) + saline; B) VD + saline; C) VD + empty A-G-beads; D) VD + IGF1-A-G-beads. All rats received periurethral injections of A-G-beads immediately after VD. Leak point pressure (LPP) testing and external urethral sphincter (EUS) electromyography (EMG) were performed 1 week after VD. Urethral tissue and anterior vagina were dissected for further analysis.

Results: LPP was decreased in B or C group compared to A group. In contrast, LPP of D group was greater than C group. The increase in EUS EMG amplitude was reduced in B or C group compared to A. Rats in D group had EUS EMG amplitude response to LPP testing partway between and not significantly different from either the A or B group. Histological analysis demonstrated well-developed and organized skeletal muscle fibers in D group. In contrast, substantial muscle fiber attenuation and disorganization was observed in B or C group.

Conclusions: IGF1-A-G-beads improved recovery in rat model of SUI, suggesting that these microspheres could provide a local sustained delivery method for IGF-1

Regenerative Pharmacology

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Micropatterned Substrates To Promote and Dissect Reprogramming of Human Somatic Cells

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Reprogramming of human somatic cells to generate induced pluripotent stem cells (iPSCs) produces valuable precursors to disease modeling and regenerative medicine. However, the reprogramming process can be inefficient and noisy, creating many partially reprogrammed cells in addition to fully reprogrammed cells. Understanding and managing this population heterogeneity arising from reprogramming could result in significant gains in creating robust cell therapies and disease models. To address these shortcomings, we have developed a novel platform that enables dynamic live-cell microscopy of thousands of cell subpopulations undergoing reprogramming. This platform also allows multiplexed and independent control of the biophysical environment. Micropatterning at island diameters below 450 microns facilitated a change in shape, size and clustering of nuclei to promote somatic identity erasure. Increased proliferation, cell density and decreased intercellular YAP signaling accompanied these nuclear changes. A combination of eight nuclear characteristics could be used to track reprogramming progression and distinguish partially reprogrammed cells from those that were fully reprogrammed. We could use these characteristics to develop a regression model to aid in the purification of high-quality iPSCs. Thus, micropatterned substrates constitute a new tool for facile iPSC production and can be used in high-throughput to probe and understand the subcellular changes that accompany human cell fate transitions. Enhanced capabilities to produce iPSCs on these micropatterned substrates can move both allogeneic and autologous PSC-based regenerative medicine closer to the clinic and use in precision medicine.

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Regenerative Pharmacology for Post-Prostatectomy Sexual and Urinary Dysfunction in a Nonhuman Primate Model

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The objective of this study is to characterize a nonhuman primate model of post-prostatectomy erectile and urinary dysfunction and test local chemokine (CXCL12) therapy for these chronic conditions. Eleven adult male cynomolgus monkeys were used; 2 for the study of normal pelvic anatomy. Nine animals were evaluated for sexual behaviour and abdominal leak point pressures (ALPP) pre- and 6 months post-prostatectomy**.** Five animals were necropsied for tissue evaluation 6 months post-surgery. The other four animals received urethral injections of the chemokine CXCL12 (125 ng) 6 weeks following the prostatectomy and were evaluated for up to 4 months thereafter. Erectile function was assessed by measuring sexual behaviour in mating situations. The anatomy, innervation and vascular supply to the prostate and surrounding tissues of these monkeys are substantially similar to those of human beings. After prostatectomy, there was sustained and marked decrease in mating success (mounting attempts, lack of intromission, ability to achieve and to maintain an erection) and in ALPP. Histologically there was marked reduction in nerve supply to the urethra and penis. In the 4 animals receiving CXCL12, mating success and ALPP improved within one month post injection and urethral and penile innervation were restored by 4 months post injection. We conclude that radical prostatectomy in this model produces persistent erectile and urinary dysfunction. Local injection of the cell mobilizing chemokine CXCL12 restored urinary and erectile structure and function and may represent a treatment for these conditions.

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Adiponectin Improves The Osteointegration of Titanium Implant Under Diabetic Conditions by Reversing Mitochondrial Dysfunction and Oxidative Stress via the AMPK Pathway

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Diabetes-induced reactive oxygen species (ROS) overproduction would result in compromised osteointegration of titanium implant (TI) and high rate of implant failure, yet the underlying mechanisms remain elusive. Adiponectin (APN) is a fat-derived adipocytokine with strong antioxidant, mitochondrial-protective and anti-diabetic efficacies. We hypothesized that mitochondrial dysfunction under diabetes may account for the oxidative stress in osteoblasts and titanium-bone interface instability, which could be ameliorated by APN. To test this hypothesis, we incubated primary rat osteoblasts on TI and tested the cellular behaviors when subjected to normal milieu, diabetic milieu (DM), DM + APN, DM + AICAR (AMPK activator) and DM + APN + Compound C (AMPK inhibitor). *In vivo*, APN or APN + Compound C were administered to diabetic *db/db* mice with TI implanted in their femurs. Results showed that diabetes induced structural damage, dysfunction and content decrease of mitochondria in osteoblasts, which led to ROS overproduction, dysfunction and apoptosis of osteoblasts accompanied by the inhibition of AMPK signaling. APN alleviated the mitochondrial damage by activating AMPK, thus reversing osteoblast impairment and improving the osteointegration of TI evidenced by Micro-CT and histological analysis. Furthermore, AICAR showed beneficial effects similar to APN treatment, while the protective effects of APN were abolished when AMPK activation was blocked by Compound C. This study clarifies mitochondrial dysfunction as a crucial mechanism in the impaired bone healing and implant loosening in diabetes, and provides APN as a novel promising active component for biomaterial-engineering to improve clinical performance of TI in diabetic patients.

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Novel Target in Medical Devices

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Introduction: Calcium channel blockers are widely used in the treatment of cardiovascular diseases. Calcium homeostasis is critical for multiple functions including motility and biofilm formation in bacteria. Biofilm formation are leading causes of infectious complications in patients with indwelling catheters, artificial cardiac valves. We report here that swimming motility and biofilm formation in pathogenic *Escherichia coli* and *Proteus mirabilis* is inhibited by verapamil, a calcium channel blocker. The central hypothesis for this study is that Calcium channel blockers will prevent bacterial motility and aggregation and biofilm formation.

Methods: First, we tested the effect of various concentrations of verapamil on growth of common bacterial pathogens including *E. coli*, *P. mirabilis* and *Klebsiella pneumoniae*. At high doses (≥12 mM) verapamil was able to inhibit growth of these pathogens. Next, we tested the effect of verapamil, at levels that does not affect

bacterial growth (3 and 6 mM), on swimming motility *in vitro*. Lastly using crystal violet staining and optical density meter we evaluated biofilm formation in plastic catheter.

Results: Our results revealed that verapamil inhibits flagella-mediated motility in *E. coli* also in *P. mirabilis*. Importantly it was able to decrease biofilm formation on plastic catheter dose dependently.

Conclusion: We identified for the first time that verapamil decreases bacterial aggregation and biofilm formation of tested bacterial pathogens in a dose-dependently in a plastic catheter. Further *ex vivo* and *in vivo* experiments are necessary to determine if Calcium channel blockers could be repurposed as novel therapeutics or prophylactic targets in medical devices.

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Inhibition of Nf-kb and Inos Inflammation Signaling Pathway Through Nipep-1 Peptide on Raw 264.7 Cells

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Development of anti-inflammatory peptide NIPEP-1 that suppresses *NF-kB* and *iNOS* expressions in lipopolysaccharide-stimulated RAW 264.7 cells Da hyeon Choi[1)], Gookjin Yoon[2)], Yeonsu kim[3)], Dong Woo Lee[3)], Buem Soo Jo[3)], Hyun Jung Lee[3)], Jue-Yeon Lee[3)], Chong-Pyoung Chung[3)], Sangmee Ahn Jo[4)], Inho Jo[5)], Yoon Jeong Park[2)], Yoon Shin Park[1),* 1)] School of Biological Sciences, College of Natural Sciences, Chungbuk National University, Cheongju, Korea 2) Dental Regenerative Biotechnology Major, Seoul National University, Seoul, Korea 3) Central Research Institute, Nano Intelligent Biomedical Engineering Corporation (NIBEC), Seoul, Korea 4) Department of Pharmacology, College of Pharmacy, Dankook University, Cheonan, Korea 5) Department of Molecular Medicine, School of Medicine, Ewha Womans University, Seoul, Korea Current inflammation therapeutic agents have several limitations such as non-specific bio-distribution and induction of cytotoxicity. Peptide drugs have been proposed as promising agents that can overcome these drawbacks, we designed NIPEP-1 peptide that has strong anti-inflammatory functions. We treated lipopolysaccharide (LPS: 1 μg/ml) to RAW 264.7 cells to induce the inflammatory response for 0.5 hr. Thereafter, RAW 264.7 cells were co-treated with NIPEP-1 with different concentrations (10, 50, and 100 μg/ml) and time points (0.5, 1, 4, and 24hr). Western Blot analysis was adopted to check the protein expression related to inflammation such as p-IKK-a/b, iNOS and p-NF-kB-p65. Here, we confirmed successful induction of inflammation stimulated by LPS treatment to RAW 264.7 cells and checked inhibition of NF-kB and iNOS expression. Therefore, we suggest that NIPEP-1 can be a promising agent for a variety of inflammatory diseases treatments including rheumatoid arthritis and dermatitis.

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Manipulation of Regeneration-Related Genes in the Adult Zebrafish Heart: Gene Silencing Effect and Cell-Level Biodistribution of siRNA Nanoparticles

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With the damaged cardiomyocytes losing their contractile and regeneration capacity, the heart would be dysfunctional, resulting in the cardiovascular diseases which are the leading cause of mortality and morbidity worldwide. SiRNA technology opened a new avenue for effective manipulation of regenerative pathways in cardiac tissues. To facilitate the siRNA delivery into the cells, numerous nonviral nanomaterials were developed while the delivery mechanisms have not been elucidated due to the difficulty in tracking and imaging the nanoparticles *in vivo*. Herein, taking advantage of zebrafish, the delivery efficiency of a polyamidoamine (PAMAM)-based siRNA delivery system was demonstrated. Moreover, the delivery mechanisms, especially the cell-level biodistribution of siRNA nanoparticles in the heart were investigated. The nanoparticles were detectable in different specific GFP-expressing cells in the transgenic zebrafish heart and allowed the precise quantification of the biodistribution at the cellular level. It was shown that in the injured heart, the 40 % uptake of siRNA was mediated by the leukocytes, accompanied by 19% by cardiac and 19% by endothelial cells. The profiling of the distribution of nanoparticles in the heart with the cell-level precision provide important insights into how the nanomaterial-based delivery systems can be designed as regenerative nanomedicine for the treatment of cardiovascular diseases.

Skin & Wound Healing

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Poloxamer-Based Hydrogel for Vaginal Tissue Healing

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Vaginal reconstructive surgery (VRS) requires extensive postoperative care. A common complication after VRS is vaginal scar tissue (VST) occurring in 73% of patients. There is no standard of care to reduce VST, and reduction requires improving vaginal angiogenesis while preventing vaginal wall fusion. One approach involves vaginal placement of estrogen cream (EC) daily to promote tissue healing and reduce VST. This does not allow for assessments of delivered estrogen due to cream egress from the vagina. Hydrogels are prominent platforms for wound healing applications, but are not used extensively in the vagina.

We have targeted a unique hydrogel-based hyaluronic acid (HA) mechanism to promote angiogenesis and wound healing in the vagina. The combination of HA/Interleukin 10 (IL10) delivery reduces kidney fibrosis following injury in a murine model. We have quantified differences between hydrogel-based EC and HA/IL10 induced wound healing in a murine vaginal injury model. Furthermore, we explored synergies between hydrogel-based CEE and HA/IL10 in a temporally staggered release system. An effective vaginal wound-healing system will reduce health care costs and improve clinical outcomes.

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Low-Level Laser Therapy (LLLT) 670 nm and Carboxyethyl Gamma Aminobutyric Acid (CEGABA) Topical Treatment in the Time Course on Wound Healing in Rats

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Introduction: The laser therapy is indicated in wound healing by stimulating fibroblasts proliferation and angiogenesis[1]. The CEGABA has a positive growth effect and induces cell proliferation[2]. The purpose was evaluate the effect topical CEGABA and laser GaAlAs in the repair of cutaneous tissue.

Methodology: Forty Wistar male rats separated in 4 groups: Control(CG); Laser Group(LG) GaAlAs, 670 nm, 39 mW of power output, energy per point of 1,17 J, radiating 1 point in 30 s; CEGABA Group(CEGG) with 1% cegaba/volume-based and Laser with CEGABA Group(L+CEGG). Was used punch with 10 mm of diameter in the back of the animals. The treatment took 16 consecutive days. Biopsy time points were made on the days 4, 11, 16. H&E analysis were performed in addition to immunohistocchemistry (IHC) for VEGF-a, and analyzed of variance the test two-away ANOVA with post hoc Tukey (p&lt 0,05).

Results: Inflammatory infiltrate decreased significantly to LG on 11Th day (p&lt 0,001), on the 16Th day LG, CEGG and L+CEGG (p&lt 0,01; p&lt 0,01 and p&lt 0,05 respectively) compared to CG. In

the analysis of VEGF-A expression factor had increased from the 11^{Th} to CEGG and L+CEGG (p&lt 0,01; p&lt 0,05 respectively) and 16^{Th} day to LG (p&lt 0,05).

Discussion: The proposed treatments were effective in the healing process and the granulation tissue was improved, shown that Laser associated to CEGABA potentiates enhanced healing and may have potencial aplication for cutaneous wounds.

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Amnion Membrane Biomaterials for Wound Healing

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Problem: There is a dire need for more effective products to accelerate wound closure and healing. Here we describe the development of several amnion-derived biomaterials for wound healing applications, including a lyophilized wound covering, and a cross-linkable hydrogel composed of hyaluronic acid (HA) and solubilized amniotic membrane (SAM).

Objective: Evaluate whether the application of amnion-derived biomaterials will promote faster wound healing and reduced scarring compared to her commercially available wound healing products in a porcine model of wound healing.

Methodology: Porcine skin wound model: A total of 72, 4.0 × 4.0 cm full thickness wounds were created on the dorsal skin (n = 9). Amnion membrane-derived biomaterials were evaluated for their potential to accelerate wound healing, and compared to several other commercially available and amnion membrane-derived wound healing products.

Results: Analysis of wound closure, contraction and epithelialization demonstrated that amnion-derived biomaterials were superior in wound closure and epithelialization and in preventing contraction. They achieved this through the significant acceleration of wound re-epithelialization. The epidermis from Amnion hydrogel and Amnion powder-treated wounds looked similar to healthy skin in regards to the coverage, thickness and presence of rete peg protrusions into the dermal area. Histochemical stains demonstrated that amnion-derived biomaterials had a much more similar ECM composition to healthy skin, with a dermis that consisted of thick, organized mature collagen fibers and mucins/GAGs. Treatment of wound with amnion-derived biomaterials did not result in any signs of immune cell infiltration.

Significance: This study demonstrates that amnion-derived biomaterials accelerate the re-epithelialization of full thickness wounds.

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Antibacterial Selenium Nanoparticle Coatings for Field Hospitals

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While advancing antibacterial resistance has recently come to demonstrate a looming threat to the health-care infrastructure of developed nations, many areas throughout the developing world still suffer persistently high mortality rates from commonly treatable bacterial strains. Humanitarian efforts in these regions, primarily seen today in the form of non-governmental organizational (NGO) outreach as well as peace-keeping military operations, are significantly strained by an incapability to reproducibly produce and maintain sterile conditions in the field. Injured personnel are often required to be rapidly transported out of the region in order to receive safe medical treatment, incurring large financial costs while demonstrating severe risks to the individuals in the process. In order to address this issue, antibacterial selenium nanoparticulate coatings were prepared for application in rapidly constructed field hospitals operating in diverse environments. These coatings have demonstrated antibacterial properties when applied to wall paneling suspended in various bacterial cultures. Ongoing work includes determining the effects of local weather (principally, heat and humidity) and transportation (principally, vibrational and shock disturbances) conditions on the persistence of applied coatings. New means of rapid, on-site administration of antibacterial nanoparticulate coatings, onto both medical instrumentation and housekeeping materials, are also currently under development.

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Small Intestinal Submucosa-Chitosan Hydrogel as a Deep Wound Healing Material

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Skin wounds are a major public health problem, representing an annual approximate cost of 25 billion dollars in the USA. This is rapidly growing problem due to risk factors such as diabetes, obesity and aging population. Current solutions cannot satisfactorily solve this problem so we propose a Small Intestinal Submucosa-Chitosan (SIS/Ch) hydrogel as a deep wound healing cost/effective material with hemostatic, angiogenic and reepithelization capacities. The objective of this study was to biologically evaluate the effect of SIS/Ch hydrogels in deep wound re-epithelialization.

SIS and SIS/Ch hydrogels *in-vitro* tests included a MTT assay (Vero cells) to test cytotoxicity, and an erythrocyte lysis test to assess hemolytic effects. The SIS/Ch hydrogels were also evaluated *in-vivo* in a murine model with excisional full thickness wound, including the panniculus carnosus (n = 6). Carboxymethylcellulose hydrofiber and open wound were used as positive and negative controls, respectively. Wound contraction, histology and inmunohistochemical analysis were performed.

SIS/Ch hydrogels showed a cell viability percentage greater than 80 %. Hemolysis index less than 5 % confirmed absence of hemolytic effects. SIS and SIS/Ch hydrogels showed a significantly higher reduction in wound area after 21 days compared to both controls. Histological and inmunohistochemical results demonstrated a major epithelial regeneration with myofibroblast presence, angiogenesis and adequate collagen arrangement. Controls showed greater granulation tissue area with a non-full epithelial regeneration at 21 days.

These results demonstrate that the SIS/Ch hydrogels possess enough biocompatibility and viability to reepithelialize deep wounds and are a new promising material for clinical use.

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In vivo Deep Wound re-epithelialization through Small Intestinal Submucosa/Chitosan Tridimensional Scaffolds

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Chronic wounds usually do not enter a normal reparative phase and need external signals to promote extracellular matrix restoration and adequate healing. We have previously demonstrated that new Small Intestinal Submucosa-Chitosan (SIS-Ch) tridimensional scaffolds have appropriate physicochemical properties as wound dressings. We aimed to evaluate SIS-Ch scaffolds' biological capability to induce skin wound re-epithelialization.

In-vitro biological evaluation (n = 3) included: a MTT assay (Vero cells) to test cytotoxicity; an erythrocyte lysis test to assess hemolytic

effects; and exposure to *P. aeruginosa* and *S. aureus,* to evaluate scaffolds' antibacterial effects by inhibition zone and turbidity measurements. Also, scaffolds' regeneration ability was studied through a full thickness skin wound in a murine model (n = 6). A commercial hydrofiber and absence of dressing were used as positive and negative controls, respectively. Wound area reduction was calculated; Hematoxylin-Eosin, Masson's trichrome staining and immunohistochemical analysis were performed.

Vero cells viability greater than 90% was obtained, indicating cytotoxicity absence. Hemolysis results confirmed a biologically accepted hemolytic index (less than 2 %). SIS-Ch scaffolds' antibacterial properties were demonstrated through the presence of an inhibition growth zone around the scaffolds and a lower bacterial optical density. Wound area was significantly lower in SIS-Ch groups after 14 days, compared to both controls. Reduced inflammation, complete epithelium regeneration, collagen deposition, hair follicle presence and angiogenesis with the SIS-Ch scaffolds demonstrated their ability to induce deep wound healing in rats.

In conclusion, SIS-Ch scaffolds could be used as wound dressings that induce a greater healing and regeneration effect compared to commercial hydrofibers.

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Bioactivation of Nanofibrous Scaffold through Collagen Protrusion for Partial Thickness Wound Healing

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Dermal ECM has collagen I as the major structural and functional protein, which possesses cell binding sequences such as Gly-Phe-Hyp-Gly-Glu-Arg (GFOGER) and Arg-Gly-Asp (RGD), making it the preferred choice of support matrix for skin tissue engineering. Collagen type I built scaffold overwhelmingly controls and mediates fibroblast and keratinocyte functions and its breakdown products are chemotactic for different players of wound healing cascade. Nanofibrous scaffold promotes better cell attachment due to its high surface area to volume ratio. Integra® made of collagen-GAG freeze-dried structure is considered as ''gold standard'' skin substitute. However, owing to presence of silicon layer, Integra® needs a double surgical innervation to replace synthetic silicon layer with thin autograft. To address this issue, we have developed a hierarchical porous bioactive scaffold with overlying porous 3D nanofibrous layer and an underlying thin freeze-dried collagen fibrillary layer. Collagen would provide faster cellular adhesion, enhancing cellular infiltration within scaffold, while nanofibrous scaffold would further boost cellular migration, proliferation, and differentiation leading to faster re-epithelialization and wound closure. The collagen layer upon fibrillogenesis resulted in fibers of diameter 12.23 ± 4.49 μm. The water contact angle was 60 °, while its water uptake capacity was 132%, degrading 38% in two months. The architecture and composition of the scaffold promoted efficient cell attachment, migration and proliferation from initial days of cell seeding, eventually covering the entire fiber structure. Scaffold implantation on full thickness wound in rat model proved beneficial with faster healing and re-epithelialization in comparison to Tegaderm™ standard dressing material.

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Vascular Network Formation is Regulated by Tensile Forces in 3D Bioprinted Tissue

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Vascular Network Formation is Regulated by Tensile Forces in 3D Bioprinted Tissue Guangliang Zhang[1,2#], Mathew Varkey[1#], Zhan Wang[1], Anthony Atala[1] 1. Wake Forest Institute for Regenerative Medicine, Winston-Salem, 2. Ruihua affiliated hospital of Soochow University, Suzhou, China, 25104; mvarkey@wakehealth.edu, #Equal contribution The purpose of this study is to further understand the role of tensile forces in vascular network formation using 3D bioprinted tissue. Briefly, print code was generated; polycaprolactone (PCL) support scaffold frames with gelatin spacer layer were printed, on top of this a layer of PCL into which fibrin-gelatin hydrogel containing HUVECs and fibroblasts was printed as vessel lines. Post-printing the constructs were cultured and assessed. Angle, length and branches of the vessel-like structures were measured. Formation of vessel-like structures was assessed 7 days post-printing. In the test group the vessels mostly grew in one direction, while in the control group they grew in chaos. Fibrinogen concentration affected vascular network formation; 5mg/ml fibrinogen scaffolds showed better vessel formation, with vessels growth parallel to the direction of force. Y27632 reduced cell-generated forces resulting in damaged networks and less-elongated vessels, while blebbistatin reduced network quality. This suggests that tensile forces due to the interaction between PCL scaffolds and printed cell-laden hydrogels regulate vascular network formation. Further understanding of underlying mechanisms will enable better control of vascular network formation in bioprinted tissue. 1. Kang HW, Lee SJ, Ko IK, *et al*. Nat Biotechnol. 34, 312, 2016. 2. Rosenfeld D, Landau S, Shandalov Y, *et al*. Proc Natl Acad Sci. 113, 3215, 2016.

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Single Stage Procedure to Repair Full Thickness Wounds in Porcine Model

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Introduction: Clinical application of skin substitutes is often a two-step procedure: application of the scaffold to the wound followed by engraftment of split thickness autograft. This two-step procedure requires multiple interventions due to wound contracture and autograft morbidity. In this study, we hypothesized that a single stage procedure can be achieved by combining autologous cells in acellular fetal bovine dermal matrix (ADM) for the treatment of full thickness wounds (FTWs).

Methods: A split-thickness biopsy was harvested from pig and processed using a cell harvesting device for preparing cell suspension. Cells at density of 80,000 cells/cm^2 were seeded onto ADM. A total of 12, 4×4 cm FTWs were created on the dorsum of Yorkshire pigs (n = 12) and treated with: cells; cells+ADM; ADM or empty. Wound measurements and biopsies were taken on days 9, 14, 28 and 42. Excised tissue was processed for histopathology. Mechanical testing was performed on excised tissue via indentation mapping, a novel application in skin, and tensile extension to failure.

Results: Empty and cells wounds contracted the most between days 0 and 14 compared to cells + ADM and ADM groups. By day 14, re-epithelialization (%) was significantly greater in cells + ADM (77%) compared to ADM (28%), cells (30%) and empty (29%) groups. From indentation mapping, differences in healing wound and contractile tissue was noted. Groups containing cells exhibited lower mean load at rupture (1.61 kg) in tensile testing than those without (2.39 kg).

Conclusions: The combination of autologous cells with ADM can be promising single stage procedure for dermal reconstruction.

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The Effect of Amniotic Membrane on Adipose Derived Stem Cells for Chronic Wound Repair

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Introduction: Chronic wounds have impaired healing due to growth factor deficiency and inadequate vascularization. Amniotic membranes are rich in growth factors, which have the potential to improve tissue regeneration by recruiting stem cells at wound site and enhance neovascularization in chronic wounds. In this study, effect of amniotic membrane (AM) on human adipose derived stem

cells (ADSCs) pre-seeded on collagen-GAG matrix (CG) was evaluated for its ability to alter stem cell activity *in vitro*.

Methods: ADSCs were seeded at 200,000 cells/cm^2 on CG, CG+AM (HydraTek^{TM} Amniotic membrane), and tissue culture polystyrene (TCP) in serum or serum-free growth media. Samples were measured for DNA, release of VEGF and KGF and production of collagen type I. Confocal imaging was performed to visualize cell morphology and skin extracellular matrix proteins.

Results: Cells on CG+AM had significantly greater release of VEGF over CG and TCP as early as day 4. Cell-free controls confirmed that the factors released were from the cells. By day 14, cells on CG + AM produced over ten times more collagen type I than cells on CG and TCP, which was supported by immunostaining results.

Conclusions: These findings suggest that the Amniotic membrane can stimulate ADSCs to secrete endogenous growth factors and produce more extracellular matrix. This combination of CG with an amniotic membrane would be an attractive treatment for chronic wounds.

Stem Cells

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Fibroblast Looks Like a Mesenchymal Stem Cell and Talks Like a Mesenchymal Stem Cell: is It a Mesenchymal Stem Cell in Disguise?

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Induced Pluripotent Stem Cells (iPSCs) are adult somatic cells genetically reprogrammed to an embryonic stem cell-like state. Fibroblast (FB) is the adult somatic cell most commonly used for reprogramming and is defined by spindle-shape morphology, plastic-adherence and expression of several markers. Nonetheless, such properties also define Mesenchymal Stem Cells (MSCs), that also share with dermal FB the ability to differentiate into osteoblasts, adipocytes and chondroblasts.

Moving from the hypothesis that the most striking difference between FBs and MSCs is in the name, we isolated and cultured human dermal and visceral FBs from tissue waste fragments of patients undergoing surgical procedures, and evaluated their morphology and expression of markers typical of MSCs, at gene and protein level. Additionally, we analyzed synthesis and release of specific growth factors in culture medium and, finally, we tested FB ability to differentiate into osteoblasts, adipocytes and chondroblasts.

Interestingly, all FBs in culture adhered to plastic culture dishes, expressed markers typical of MSCs, like CD90, CD105, CD146, SSEA-4, ECM2, ID-1, and were morphologically undistinguishable. As reported for MSCs, FBs released EGF, FGF-4, GDNF, HGF, IGF, TGF-β and VEGF, and were capable of differentiating into cells of mesodermal origin.

Our findings raise the question of whether FBs and MSCs are not admittedly the same population. We might infer that previously described differences are due to different stages of differentiation, which also account for reports on high variability in both expression of mesenchymal markers by MSCs and efficiency of FB reprogramming to iPSCs.

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In Vivo Chondrogenesis In 3D Bioprinted Human Cell-laden Hydrogel Constructs

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Cartilage is an avascular tissue that once degenerated or wounded has limited ability to heal. Untreated cartilage injuries can lead to osteoarthritis(OA) which is a leading cause of disability among older adults, and affects upwards of one in eight adults [1]. This highly widespread disease and associated disability have a huge effect on individuals and on society. The demand for cartilage tissue restoration is therefore high.

3D bioprinting technology allows creation of 3D constructs using biologically relevant materials such as biopolymers and cells. The aim of this study is to investigate the use of 3D bioprinting in a clinically relevant setting to evaluate the potential of this technique for *in vivo* chondrogenesis and thus repair of human cartilage.

Bioprinted cell-laden constructs were implanted subcutaneously in mice. Human nasal chondrocytes (hNCs) were cultured alone or co-cultured together with human bone marrow derived mesenchymal stem cells (hBMSCs), human adipose derived stem cells (hASC) or human stromal vascular fraction (SVF). After 30 and 60 days, the scaffolds were harvested for histological and immunohistochemical analysis.

Preliminary data show an increase in both proliferation of chondrocytes and glycosaminoglycan production and co-culture with stem cells are enhancing these effects. We also show that nanocellulose/alginate bioinks demonstrate good long-term mechanical and structural stability *in vivo*.

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Effect Of Ecm Proteins And Their Elasticity On iPS Cell Differentiation To Self-beating Cardiomyocytes

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The purpose of this study is to clarify the effect of culture substrates with different ECM proteins and different elasticities on the cardiac differentiation of induced pluripotent stem cells (iPSs). We investigated the differentiation in three steps: (1) cardiac marker gene expression, (2) contraction-relating gene expression, and (3) beating and its duration. To this end, in addition to iPS cells, neonatal cardiomyocytes (NCMs) were selected and used. These cells were cultured on hydrogel (HG) substrates with the young modulus of 9, 20, and 180 kPa and on hard tissue culture polystyrene dishes (TCPS). These substrates were immobilized with collagen (Col), gelatin (Gel), or fibronectin (FN). The most effective niches for the above three stages differed very much. The cardiac marker gene expression of iPSs was improved on TCPS/FN and TCPS/Gel. In contrast, on HG/FN the cardiac differentiation was greatly suppressed and the undifferentiated marker gene was still highly maintained. The contractile gene expression and self-beating high on TCPS/FN and HG/Col, which suggests that the combination of ECM and matrix elasticity is more important for inducing the cardiac differentiation. In addition, the beating behavior lasted longer period of time on HGs irrespective of the ECM type. These results suggested that the culture substrates might be changed and controlled stepwise along with the cell differentiated stages in order to prepare the beating cardiomyocytes from stem cells at a high efficiency.

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Dexamethasone Released from PEUUR Promoted Cell Viability of Cardiac Stem Cells

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Stem cell-based therapy holds great potential to treat myocardial infarction (MI). Unfortunately, the MI-induced inflammation results in a poor engraftment and survival rate of the transplanted cells. To address this, we propose co-delivering stem cells with an anti-inflammatory dexamethasone (Dex)[1]-releasing elastomeric scaffold.

Our previous work has shown that electrospun poly(ester-urethane urea) (PEUUR) scaffolds support c-kit$^+$ human cardiac stem cells (hCSCs) viability and differentiation into cardiomyocytes[2]. The present study examines the impact of Dex released from PEUUR scaffolds on hCSCs under normal and induced-cell death. Dex-PEUUR scaffolds were fabricated by electrospinning blends of PEUUR and Dex in hexafluoroisopropanol, and hCSCs were cultured on Dex-PEUUR scaffolds, ordinary PEUUR scaffolds, and tissue-culture polystyrene (TCPS) substrates. Cell viability, proliferation, cellular morphology, and cardiac differentiation were analyzed before and 7, 14, 21, 28 days after 5-azacytidine-differentiation using Live/Dead assay, PCR, and immune antibody analysis. The present data indicated that there were similar cell viabilities between Dex-PEUUR and TCPS, while the cell morphology and proliferation seemed significant different between PEUUR and TCPS. Ongoing studies are expected to demonstrate increased hCSCs viability on Dex-PEUUR scaffolds under induced-cell death using interleukin or Doxorubicin.

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In Vitro Biomimetic Mineralization of GAG/Chitosan Modules by Osteoinduced MSCs

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Introduction: Biomaterials that facilitate mineralization may improve strategies for generation of tissue engineered bone. We examined the *in vitro* mineralization of glycosaminoglycan (GAG)/chitosan microcapsules containing osteoinduced mesenchymal stem cells (MSCs), to evaluate these microcapsules as a platform for modular bone tissue engineering.

Methodology: MSCs were encapsulated in GAG/chitosan microcapsules with or without hydroxyapatite (HAP) microgranules, and cultured in expansion (Exp) or osteogenic (Osteo) induction media. After 4 weeks of culture, capsules were sectioned, and imaged via SEM to characterize the cell-deposited matrix.

Results: Osteoinduced MSCs deposited significant bone-like mineral in microcapsule interiors, while no significant mineral was deposited under Exp media conditions. New mineral was mainly deposited on the pre-existing HAP in the Osteo+HAP condition, while the interior surface of the microcapsule membrane was well mineralized in the absence of HAP microgranules. Furthermore, mineral morphology differed depending on HAP presence. New mineral presented as 1-5 μm plate-like crystals in the absence of HAP, while mineral deposited on pre-existing HAP formed 1-5 μm diameter spherulites. Spherulites were composed of smaller 30 nm diameter spherulite. Compressive stress/strain analysis of fused capsule constructs showed that mineralization increased construct mechanical properties by $\sim$3 orders of magnitude; however, the HAP and HAP-free capsules possessed similar yield strengths (10.4$\pm$4.4 MPA). These results demonstrate the ability to alter deposited mineral architecture based on presence or absence of templating mineral. Additionally, incorporation of HAP mineral was not necessary to achieve high increases in compressive yield strengths *in vitro*.

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Culturing and Expanding Human Mesenchymal Stem Cells in Three-dimensions

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Introduction: Tissue-engineering strategies often demand a large number of stem cells and cell-passaging, which is normally achieved by two-dimensional (2D) cell expansion on Petri dishes or culturing flasks. Stem cells on these 2D systems become confluence in about two weeks, reaching 3 to 10 folds maximum proliferation, and must be trypsinized, detached and re-plated to continue expansion. Trypsinization, however, exposes stem cells to biochemical and biophysical stresses known to accelerate senescence, phenotype changes, or lost of stemness. A cell-expanding strategy that maximize proliferation per passage, while minimizing the need for trypsinization, can promote the quality of stem cells post expansion and benefit the research and applications of cells based therapy.

Method: In contrast to traditional cell expansion, we created a three-dimensional (3D) cell-expansion system using Micro-Ribbons made of polystyrene, which is the same material for culture flasks. These microribbons (500 micron wide and 50 micron tick) produced a large surface area per weight to support high cell proliferation, leading to a maximum cell number per passage that is dramatically higher than the traditional system (by more than 10 folds). Cells reaching confluence in 3D were re-suspended by trypsin following the similar protocol for 2D cell expansion. In this presentation, we will demonstrate the results of our study on the expansion of human mesenchymal stem cells (MSC). Results on MSC proliferation rate per passage, change of gene expression post passaging, and the differences between the results from our 3D platform and traditional 2D system will be explained in detail.

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Vimentin Intermediate Filaments May Influence Chondrogenic Extracellular Matrices Over Time

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Vimentin intermediate filaments have been shown to be involved in short term chondrogenesis. However, vimentin's role in human mesenchymal stem cell (hMSC) long term chondrogenic extracellular matrix (ECM) deposition remains to be clarified. Better understanding of vimentin's role in chondrogenesis may help in understanding therapeutic outcomes of MSC therapies. Here, hMSCs expressing lentiviral shRNA targeting vimentin or LacZ (Control) for 14 days were formed into pellets. Chondrogenesis was induced via chondrogenic media supplemented with 10 ng/ml TGFβ3. ECM deposition and gene expression were analyzed on days 14 and 21 using immunohistochemistry/histology for Type II collagen and sulfated glycosaminoglycans (sGAGs) and RT-PCR for *18s, sox9, mmp13, adamts4,* and *adamts5*, respectively. Statistical differences were determined using Student's T-test. A potential, but not definitive, disruption of Type II collagen and sGAG deposition was observed. Gene expression revealed few significant differences between shVim- and shLacZ-hMSCs. However, shVim-hMSCs demonstrated gene expression changes from Day 14 to Day 21 (Day21 relative to Day14 - $\uparrow$*sox9*: $p=0.025$, $\uparrow$*mmp13*: $p=0.042$, $\uparrow$*adamts4*: 0.267, $\downarrow$*adamts5*: 0.012) while shLacZ-hMSCs did not (Day21 relative to Day14 *sox9*: $p=0.103$, *mmp13*: $p=0.101$, *adamts4*: 0.982, *adamts5*: 0.221). While prior evidence suggests vimentin influences chondrogenesis in the short term, it may have a lesser influence in long term cultures. However, a decrease in vimentin does appear to increase the dynamism of gene expression over time, which could tune changes to the ECM. Ongoing studies will delve more deeply into the relationship between vimentin and chondrogenic, degradative, and ECM adhesive protein gene expression.

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Influence of Vimentin Intermediate Filaments on Mesenchymal Stem Cell Spreading in Response to Environmental Cues

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Vimentin intermediate filaments have been shown to be involved in cellular mechanotransduction and adhesion. However, the role of vimentin in mesenchymal stem cell (MSC) response to environmental cues such as adhesion to different extracellular matrix (ECM) proteins is still uncertain. Investigating how vimentin is involved with MSCs' response to such changing environmental cues will improve understanding of how MSCs interact with differing environments such as microcarriers for manufacturing or tissue engineered scaffolds. Here, hMSCs expressing lentiviral shRNA vectors targeting vimentin or LacZ(Control) for 14 days were seeded onto glass coverslips coated with 10 or 50 μg/ml of either fibronectin(Fn) or type I collagen(ColI). Cell areas were measured after 2 hrs using ImageJ to determine initial response to different ECM proteins. Statistical differences were determined using Mann-Whitney U tests (alpha = 0.01). Cellular area measurements revealed that shVim-hMSCs had smaller cell areas than shLacZ-hMSCs on fibronectin (10 μg/ml Fn: $p < 0.0005$, 50 μg/ml Fn: $p < 0.0005$), but not on collagen regardless of concentration (10 μg/ml ColI: $p = 0.051$, 50 μg/ml ColI: $p = 0.186$). Further, shLacZ-hMSCs appeared to be responsive to changes in collagen concentration (ColI 10 μg/ml vs. 50 μg/ml - shLacZ: $p = 0.005$, shVim: $p = 0.015$), while shVim-hMSCs appeared to be responsive to changes in fibronectin concentration (Fn 10 μg/ml vs. 50 μg/ml - shLacZ: $p = 0.02$, shVim: $p = 0.006$). These results demonstrate that vimentin may be involved in MSC early sensing of fibronectin. Cues like ECM proteins can impact downstream MSC activity and investigating these can lead to improved understanding of mechanisms that drive MSC behavior. Investigation of the expression of cell-surface adhesion proteins will elucidate this response further.

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Mesenchymal Stem Cell Immunomodulatory Effects depend on the Degree of Existing Inflammation

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Human mesenchymal stems cells (hMSCs) have been investigated for the treatment of numerous diseases due to their immunomodulatory capacity. Recently, their ability to not only decrease inflammation but also be polarized to a pro-inflammatory phenotype has been hypothesized, a factor which may underlie the high variability in hMSC clinical outcomes. The present study evaluates the impact of existing macrophage-mediated inflammation on the immunomodulatory capacity of hMSCs.

Bone marrow-derived hMSCs and pro-inflammatory, LPS-activated macrophages (AMs) were used to establish different inflammatory environments by modulating AM:hMSC ratios. Poly(ethylene glycol) hydrogels containing various levels of AMs or hMSCs were combined using a Transwell system to produce mono-culture or co-culture groups. After 72 hours of culture, cell protein expression was assessed by western blot and multiplex protein analysis.

As expected, hMSC expression of COX-2, a key anti-inflammatory factor in hMSCs, increased in a dose-dependent manner following exposure to AMs. However, the increase in hMSC COX-2 did not always correlate with reduced pro-inflammatory factor secretion by co-cultured AMs. Specifically, at low AM:hMSC ratios, AM secretion of TNF-α and IL-6 decreased substantially. However, high AM:hMSC ratios were associated with no change in AM pro-inflammatory cytokine production. The AM results may be due to the associated increase in hMSC pro-inflammatory/pleiotropic cytokine production seen with increased AM levels concomitantly with increased COX-2. These results increase the knowledge of the paracrine effects of hMSCs and indicate that their successful use must account for the existing inflammatory environment.

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Alginate Encapsulated Multipotent Adult Progenitor Cells Enhance Bone Defect Repair

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A current challenge for many cell-based therapies is the long-term retention or initial homing to the target region in order for the stem cells to elicit their therapeutic effects [1]. The use of alginate for the encapsulation of cells or delivery of drugs has been extensively investigated, but to date the encapsulation of multipotent adult progenitor cells (MAPC) in alginate remains uninvestigated. This study examined the use of alginate encapsulated MAPC to enhance the repair of bone defects. We have shown that MAPC in alginate can remain viable for at least 28 days *in vitro*, and significantly upregulate their expression of key angiogenic cytokines GRO-α and VEGF during encapsulation, when compared to MAPC cultured on normal tissue culture plates. Given that revascularization is a key step in the healing of bone defects, an *in vivo* pilot study was performed to determine if the angiogenic cytokine upregulation observed *in vitro* would translate into improved healing in a rat fibular defect model. The preliminary histopathological evaluation has shown that encapsulated MAPC have increasing bone healing and neovascularization over a 4 week time course. The results of these studies have shown that alginate encapsulated MAPC have promise to improve current treatment options for poorly vascularized bone defects.

Reference:

1. Hocking AM. The Role of Chemokines in Mesenchymal Stem Cell Homing to Wounds. Adv. Wound Care 4, 623, 2015.

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Role of Nanoelectrodes and Electrical Stimulation to Modulate Cellular Functions

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The application of external stimuli to influence cell proliferation and differentiation is an important aspect of tissue engineering. Physical (2D/3D structures, material properties, topography) and chemical (growth factors, hormones, drugs) stimulus have improved structure and functionality of tissue engineered products. Another type of external stimuli is electrical potential and current, which remains fairly unexplored despite its huge value[1]. In this project, we explore 3D penetrating nanoelectrodes (of various aspect ratios) to deliver electrical stimuli and its potential to differentiate human Adipose Derived Stem Cells (hADSCs) to neuronal lineage. The degree of differentiation of hADSC to neural lineage varied based on the strength of the electric field and the aspect ratio of the nanoelectrodes. The extent of differentiation was evaluated by immunostaining for markers of PAX6, NESTIN and MAP2. The functional ion channels in hADSCs and neural lineage were further analyzed using whole-cell patch-clamp recording. This study demonstrates that electrical stimulation can play a vital role in modulating cellular functions, particularly in the differentiation of hADSCs and thereby aid in the development of a growth factor-free differentiation protocol.

Reference: 1. Balint, R.; Cassidy, N. J.; Cartmell, S. H., Electrical stimulation: a novel tool for tissue engineering. Tissue engineering. Part B, Reviews 2013, 19(1), 48-57.

Acknowledgments: This work was performed at Joint School of Nanoscience and Nanoengineering, a member of the National Nanotechnology Coordinated Infrastructure supported by the National Science Foundation (Grant ECCS-1542174).

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Adipose-Derived Stem Cells Delay Muscle Atrophy after Peripheral Nerve Injury in Rats

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Peripheral nerve damage contributes to atrophy of its innervated muscles; even if immediate primary nerve intervention is performed, the successful reinnervation of the nerve does not ensure the return of muscle function, which then requires additional therapies [1]. This study investigates the ability of adipose-derived stem cells (ASCs) to delay muscle atrophy after peripheral nerve injury in a rat model. Twenty Lewis rats underwent excision of the right sciatic nerve; left hindlimbs were used as controls throughout. Investigation conditions included: empty defect, autograft, autograft with injection of ASCs into the gastrocnemius, or autograft with injections of ASCs on Days 0 and again on 21. All rats were sacrificed on Day 42 and bilateral gastrocnemius muscles were harvested and weighed. Weights were reported as a percentage of the contralateral, unaffected muscle. Specimens were evaluated histologically to evaluate muscle fibers, fatty infiltration, and fibrosis. DiR imaging confirmed presence of ASCs at sacrifice. Statistical analysis of the muscle weights by ANOVA revealed significantly larger masses in muscle with the single ASC injection compared to controls, suggesting that injection of ASCs delayed progression of muscle atrophy.

Reference: 1. Grinsell D, Keating CP. BioMed Research International. 2014.

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Tissue Chips & Tissue Organoid Models

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Development of a Human Tissue-Engineered Model of Duchenne Muscular Dystrophy

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Current animal models of Duchenne muscular dystrophy (DMD) fail to fully recapitulate the severity of the disease seen in humans. Given the wide range of mutations underlying DMD, the ability to study DMD from patient biopsies would allow patient-specific drug screening. Here we sought to determine if a 3D tissue-engineered skeletal muscle system[1] (myobundle) can replicate the contractile and morphological phenotype of DMD muscle. Myobundles were generated from primary myoblasts derived from 2 DMD and multiple healthy (H) patients. Functionally, DMD myobundles generated significantly lower tetanic force (H: 1.64 ± 0.21 mN, DMD: 0.24 ± 0.05 mN) and exhibited greater force loss (H: $30.1 \pm 2.3\%$, DMD: $50.4 \pm 2.91\%$) following repeated eccentric contractions than healthy bundles. The decrease in force may be partially due to a decrease in differentiation as shown by a decrease in muscle cross-sectional area and mean myotube diameter. Additionally, by quantifying fiber size distribution, we found an increased abundance of fibers over 20 μm in DMD bundles due to the presence of hypercontracted fibers that could be directly visualized in longitudinal myobundle sections. The human myobundle system recapitulated contractile (i.e. muscle weakness and increased susceptibility to eccentric contraction) and morphological (i.e. hypercontracted fibers and increased variation in myofiber size) seen in DMD patients. This system provides a novel platform for the study of DMD pathogenesis and drug development in a patient-specific manner.

Reference: 1. Madden L *et al*. eLife 4: 2015.

Acknowledgments: NIH grant UH3-TR000505

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Adhesive Film-based Microfluidic Device For Motility Assessment Of Cancer Cells

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Recent studies have shown that cell motility plays a central role in metastasis and tumor invasion [1]. Studying motility in cancer models that mimic the *in vivo* tumor microenvironment and testing drugs on this model can aid in developing treatments for aggressive

cancers. 3D cell culture recapitulates the tumor microenvironment more accurately than in 2D models and translates to humans better than animal models [2]. We have developed a photopatterning technique through which 3D cell cultures of arbitrary size and shape can be formed *in situ* in the chambers of an active adhesive film-based microfluidic device, enabling complex structures to be produced and allowing independent chemical delivery to parallel models [3]. Here, we expand this system through a novel architecture that enables direct, quantitative imaging of cell motility throughout an experiment. In this study, multiple replicates of 3D cell culture motility structures containing human colon carcinoma cells (HCT116) are produced *in situ* in a microfluidic device and challenged in parallel with various concentrations of the chemotherapeutic drug 5-Fluorouracil. Cancer cell motility and viability are probed as a function of drug exposure over 7 days. We envision this system as a valuable tool for assessing the inhibition of motility, and therefore as an aid in the development of drugs for the prevention of metastasis.

References:
1. Wells A. Adv. Cancer Res. 2000, 78: 31-101.
2. Imamura, *et al*. Oncol Rep 2015 33(4):1837-43.
3. Skardal, *et al* Biofabrication 2015, 7(3):031001.

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Adhesive Film Based Multi-organoid Body-on-a-chip Platform For Assessing Drug Efficiency And Toxicity

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Current drug development techniques are expensive and inefficient, partially due to the use of preclinical models such as 2D cell cultures and animal models that often do not accurately recapitulate *in vivo* drug efficacy and cytotoxicity. For example, understanding the cytotoxic side effects in non-target organs in animal models may not accurately translate to humans. To address this challenge, we demonstrate a double-sided adhesive film-based body-on-a-chip microfluidic device that enables the assessment of drug efficiency and toxicity on multiple 3D tissue organoids in parallel. These miniaturized structures require less than 200 μL fluid volume and are amenable to both 3D cell culture and spheroid integration. Here, we describe a demonstration of this technology by presenting a multi-chamber device consisting of (1) liver organoids, (2) cardiac organoids, (3) lung cell constructs, (4) brain organoids, and (5) testes organoids in tissue volumes roughly proportional to *in vivo* organ sizes. We first show that these multiple tissue types can be kept in common circulation with high viability for 14 days. We then validate the platform for accurate drug response by introducing the prodrug capecitabine, and demonstrating that its metabolism by liver into 5-fluorouracil (5-FU) induces downstream toxicity in lung and cardiac organoid. Importantly, removal of the liver from a subset of devices prior to drug administration resulted in no downstream toxicity, illustrating the importance of the metabolically active liver organoids. In summation, we present a multi-organoid body-on-a-chip system that can be used for the accurate characterization of drug interactions *in vitro*.

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An *in vitro* Model of Fibrosis-Induced Abnormal Development of the Biliary Tract

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Purpose: 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 (aHSC), which produce chemokines, ECM proteins and restructuring of the ECM. Our lab has developed a unique *in vitro* model that simulates a fibrotic environment. We propose to use to model the effects of the fibrotic environment during development and if aHSC cause abnormal development.

Methods: Fibrosis models were produced by culturing the HepaRG cell line with aHSC or LX-2 (immortalized HSC cell line) in a collagen gel. Several experiments were conducted: HSC's or LX-2 only and (HSC's or LX-2 + HepaRG within collagen gel. Cells were culture for 1 and 3 weeks. Cells were analyzed for changes in developmental pathways using qPCR and changes in the ECM were quantified by image analysis.

Results: Co-cultures of liver progenitors and HSC resulted in a variation of gene expression in progenitor cells compared to progenitors alone. aHSC's modification of the collagen gel resulted in larger collagen I fibrils as compared to LX-2 quantified by picrosirius red. HepaRG's formed primitive biliary structures, which were larger within the aHSC co-culture as compared to LX-2 or HepaRG alone.

Discussion and Conclusions: aHSC's interrupt normal developmental gene expression and growth of liver progenitor cells in this model system. A larger analysis of additional developmental pathways such as OPN, TBX3, Notch etc. will be conducted and compared to tissue samples from patients who suffer from these liver disorders.

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Enhancing the Stability of Self-Assembled Toroid-Shaped Tissue Constructs via Media Composition

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During cellular self-assembly, monodispersed cells are seeded into a non-adhesive mold, adhere and exert forces on one another resulting in compaction into a highly dense tissue construct. By modifying the geometry of the non-adhesive agarose gel in which cells are seeded, it is possible to control the alignment of the cells as they assemble around precisely placed peg features.[1] However, as highly contractile cell types such as fibroblasts coalesce into a self-assembled tissue and continue to exert forces on one another, tension in the tissue builds and ultimately results in necking and failure.[2] In order to maximize the long-term stability of these tissues, media composition was optimized with matrix promoting supplements ascorbic acid and l-proline. Similarly, fetal-bovine serum (FBS) was removed due to its implication in cellular contractility. Toroids from the FBS medium group rapidly self-assembled, thinned and $71\% \pm 8\%$ ($n = 12$) broke by day 4. Conversely, only $8\% \pm 17\%$ ($n = 12$) of serum-free medium (SFM) with supplementation tissues broke within the same time span. This has the potential to become a platform for the production of precisely aligned ECM of tunable composition, for harvest or designer biomaterial.

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1. Schell, J. Y. *et al.* Harnessing cellular-derived forces in self-assembled microtissues to control the synthesis and alignment of ECM. *Biomaterials* **77,** 120-129 (2016).
2. Wang, H. *et al.* Necking and failure of constrained 3D microtissues induced by cellular tension. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 20923-8 (2013).

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Embryoid Body Culture of Mouse iPS Cells using an Oxygen-permeable Microwell Chip

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Embryoid body (EB; iPS cell aggregate) can promote the initial differentiation of iPS cells. As a new platform of EB culture, we have developed a microwell chip, in which microwells of several hundred micrometers were regularly fabricated on a culture substratum. In this study, we investigated the effects of oxygen environment in microwell chip culture on the EB properties. We fabricated the chips which contained 139 microwells (500 μm in diameter) on poly-methylmethacrylate plate (PMMA chip; oxygen non-permeable material) or polydimethylsiloxane plate (PDMS chip; oxygen permeable material), and evaluated the EB growth and cell differentiation properties. Mouse iPS cells formed a single EB in each microwell within 1 d of culture. Although the EB size in both chips increased with the increase of culture period, the EB growth in PDMS chip was higher than that in PMMA chip. The expression of hepatic markers (TTR and AFP) in the PDMS chip was lower than that in the PMMA chip, while the expression of vascular markers (Flk1 and PDGFRβ) in the PDMS chip was higher than that in the PMMA chip. These results indicate that the oxygen environment is important factor to control cell proliferation and differentiation fate of the EBs, and the microwell chip culture is a promising platform to control the EB properties.

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In Situ Monitoring of Engineering Muscle to Assess Myopathy

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Tissue engineered human skeletal muscle provides a new platform to model muscle diseases and to study the muscle function in response to drugs and toxins *in vitro* [*Stem Cell Research & Therapy*, 4(1): S10 (2013)]. In this work, we developed a novel in situ system to monitor the muscle force with higher throughput and low cost. Myobundles were fabricated from human myoblasts using a hydrogel molding technique. PDMS molds with four seeding chamber were designed to fabricate multi-myobundles array. Each myobundle was independently bonded in series with an ecoflex (platinum-catalyzed silicone) film containing beads (53-63μm). Then force generation of myobundles in the frame was obtained from mapping the displacement of the beads and the measured elastic modulus of ecoflex membrane. Myobundle contractile forces measured with the microbeads embedded in ecoflex agreed well with simultaneous measurements with a force transducer. This novel system was tested through monitoring the force change of myobundles treated with doxorubicin and cerivastatin. For doxorubicin (1 μg/ml), the contractile force generation of myobundles in all groups were maintained or increased in first three days after shifting to differentiation media (then doxorubicin was added) and this trend was maintained for another 5 days in control groups. In contrast, the tetanus force for myobundles treated with doxorubicin continuously dropped from 5 day onward. The force declines after day4 of continuous exposure to 100 nM cerivastatin and the inhibitory effect of cerivastatin could be eliminated by incubation with 0.3 mM mevalonate. This method opens new possibilities for investigating the mechanical stresses of muscle.

Poster Session 3

Tuesday, December 5, 2017, 4:30 PM - 6:30 PM

Biomaterial Scaffolds

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Synthesis and Evaluation of Dual Crosslinked Alginate Microbeads

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Alginate hydrogels have been investigated for a broad variety of medical applications. The ability to assemble hydrogels at neutral pH and mild temperatures makes alginate a popular choice for the encapsulation and delivery of cells and proteins. Alginate has been studied extensively for the delivery of islets as a treatment for type 1 diabetes. However, poor stability of the encapsulation systems after implantation remains a challenge. In this paper, alginate was modified with 2-aminoethyl methacrylate hydrochloride (AEMA) to introduce groups that can be photoactivated to generate covalent bonds. This enabled formation of dual crosslinked structure upon exposure to ultraviolet light following initial ionic crosslinking into bead structures. The degree of methacrylation was varied and *in vitro* stability, long term swelling, and cell viability examined. At low levels of the methacrylation, the beads could be formed by first ionic crosslinks followed by exposure to ultraviolet light to generate covalent bonds. The methacrylated alginate resulted in more stable beads and cells were viable following encapsulation. Alginate microbeads, ionic (unmodified) and dual crosslinked, were implanted into a rat omentum pouch model. Implantation was performed with a local injection of 100 μl of 50 μg/ml of LPS to stimulate a robust inflammatory challenge *in vivo.* Implants were retrieved at 1 and 3 weeks for analysis. The unmodified alginate microbeads had all failed by week 1, whereas the dual-crosslinked alginate microbeads remained stable up through 3 weeks. The modified alginate microbeads may provide a more stable alternative to current alginate-based systems for cell encapsulation.

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Self-foldable 2-D Micro-patterns for Creating 3-D Cell Niches with Tunable Micro-topography

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2-D cell-culture platforms are well established and easy for cell seeding and culturing, whereas 3-D scaffolds are promising for tissue

regeneration study, as they better resemble the dimensionality of extracellular matrix (ECM) *in vivo*. Despite the progress in 3D technology, bio-fabrication of 3-D scaffolds often demands complicated and time-consuming procedures. 3-D scaffolds are often difficult to cellularize due to inefficient cell penetration or diffusion. Here we present an easy-to-use platform that consists of 2-D, self-foldable, hydrogel-based micropatterns, which can turn into biomimetic 3-D structures. In brief, biomaterials with distinct swelling ratios were inkjet-printed by layers into cell-sized patterns on glass substrates pre-coated with a releasing layer. The patterns were seeded with human mesenchymal stem cells (MSC), incubated for 1 day for cell attachment, and released to activate self-folding. Due to heterogeneous swelling, rectangular micropatterns folded into microtubes, which are promising for engineering linear tissues including muscles, blood vessels and nerves. Fluorescent imaging on day 1 showed extensive cell spreading and uniform cell distribution inside the self-folded scaffolds. On day 12, the cytoskeleton of MSC spread in parallel to each other along the tubular scaffold, due to the unidirectional ECM shape. Our platform enables the formation of uniformly-cellularized 3-D micro-scaffolds with customized ECM topography, while supporting conventional 2-D cell-seeding procedure. The inkjet-printing approach is efficient and enables the rapid production of 3-D scaffolds at large quantity, which benefits high throughput studies and is promising for clinical applications.

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PC-12 Cell Proliferation On Coaxial Electrospun Microfibers Containing FGF-2

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Fibroblast growth factor (FGF-2) is an important bioactive agent involved in tissue repair that can be encapsulated through coaxial electrospinning for controlled release. The aim of this study has been to analyze the biological potential of these electrospun fibers on rat pheochromocytoma PC-12 cell line. The core of the fibers consisted of 100 μg/ml FGF-2, 10% polyethylene glycol and 2% bovine serum albumin in water; the shell solution consisted of 18% poly(lactic-*co*-glycolic acid) (PLGA 75:25) in 1-1-1-3-3-3-hexafluoro-2-propanol. The morphology of the PLGA/FGF-2 microfibers was analyzed by scanning electron microscopy (SEM) and their diameter was calculated using ImageJ software. Verification of the core-shell structure was performed by transmission electron microscopy (TEM). The scaffolds were seeded with PC-12 cells. Cell viability was evaluated by MTT assay; cell proliferation and morphology was analyzed using images from SEM and CLSM. Coaxial electrospinning resulted in fibers with a uniform morphology and without any beads. Through TEM analysis, it was possible to visualize the core-shell structure of the microfibers. Additionally, by using fluorescein in the core, it was possible to confirm the presence of fluorescence inside the fibers by CLSM. The average diameter of the fibers was 2.18 ± 0.74 μm. *In vitro*, the coaxial fiber scaffold supported the attachment of PC-12 cells, as shown by the SEM images. The MTT assay demonstrated that the cells proliferated and maintained viability. These results indicate the cytocompatibility of the FGF-2 loaded core-shell microfibers and their great potential in tissue engineering.

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3D Macro-porosity Regulates Stem Cell Fate in Hand-spun Micro and Nanofibers

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Introduction: 3D topographic cues, including shape and macro-porosity of extracellular matrix, regulate mechanosensing and subsequent cell bioactivities including survival, proliferation, and differentiation. Research on how 3D topographic cues influence cell behaviors is limited by a lack of proper ECM model of customizable, cell-size features. Here we present Fiber-Gel, a new biomaterial platform to help study the biological effects of matrix porosity. Fiber-Gel is made of photocrosslinkable gelatin microfibers of a widely customizable diameter, ranged from 200 nanometers to 100 microns. The microfibers form highly tunable macro-pores, ranging from 500 nm to 500 μm in pore size, providing an easy-to-used platform for studying how cells or stem cells respond to the porous topographic cues. The elasticity of individual fibers is customizable. We invented a 'hand-spinning' technique, which repeatedly stretches and folds a ring of gelatin core into micro/nano fibers with diameter ranged from 200 nm to 100 μm. This hand-spinning method will be introduced in detail.

Cell study and Results: Cultured in mixed adipogenic/osteogenic media and encapsulated in Fiber-Gel of varied pore size and fiber elasticity, human mesenchymal stem cells (MSC) in Fiber-Gel of macropores (>> cell size) and high elasticity (~50 kPa) presented elevated osteogenic gene expression. MSC in macropores and low elasticity (~1 kPa) presented elevated adipogenic gene expression. In contrast, regardless of the elasticity of fiber, MSC in Fiber-Gel of subcellular pores (<cell size) resented elevated adipogenic gene expression. Above results demonstrated the great potential of our Fiber-Gel as a novel platform for the fundamental study on stem cell mechanosensing.

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The Impact of Sterilization Upon the Biologic and Viscoelastic Properties of Extracellular Matrix Hydrogels

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Prior to clinical use, ECM hydrogels undergo processing, sterilization and storage. Each of these treatments has the potential to alter the structure of the hydrogel and compromise *in vivo* performance. Evaluation of stability and function of ECM hydrogels after sterilization and storage is critical in creating an off-the shelf biomaterial with retained desired properties. Yet, no studies to date have addressed how sterilization and storage effect ECM hydrogel mechanical properties and bioactivity. In the present study, a new form of material, lyophilized digest, was introduced to mitigate potential storage issues with ECM hydrogels. Well-accepted terminal sterilization methods currently used for ECM sheets and powders (i.e., gamma irradiation, electron beam irradiation, ethylene oxide (EO) exposure) and supercritical CO2 (scCO2) sterilization, a relatively new method, were used to investigate sterilization effects upon the biologic and viscoelastic properties of ECM hydrogels. 30 kGy irradiation and scCO2 treatment were the only methods to completely inhibit bacterial growth in ECM hydrogels. However, exposure to 30-kGy E-beam or gamma irradiation prevented hydrogel formation. Rheological properties of the hydrogels were not affected by scCO2 sterilization with stiffness equivalent to non-sterilized control samples. Macrophages exposed to LPS prior to treatment with terminally sterilized ECM hydrogels did not show any statistically significant change in TNFα secretion compared to the non-sterilized control. These results will inform future clinical translation of ECM hydrogels.

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Controlled Release of IGF-1 from IGFBP-3 Conjugated Scaffolds for Volumetric Muscle Injury

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Current treatment options to repair volumetric muscle loss injury involve the use of existing host tissue like muscular flaps or grafts.

In many instances, this approach is challenged by the host muscle tissue availability and donor site morbidity such as functional loss and volume deficiency. In this study, we aimed to develop a biofunctionalized scaffolding system to utilize endogenous stem/progenitor cells for *in situ* tissue regeneration [1]. To this end, we fabricated an insulin-like growth factor-binding protein 3 (IGFBP-3)-conjugated scaffold for the controlled delivery of IGF-1. *In vitro* evaluations, including release kinetics and cellular infiltration, proliferation, and migration, were performed. The results revealed that the IGFBP-3 conjugated scaffolds provided the sustained release and increased biological activity of IGF-1 via the IGF-1 binding domain. Also, higher cell infiltration, proliferation, and migration were determined in the IGFBP-3 conjugated scaffolds when compared with control scaffold (no conjugation). Our current data strongly suggest that IGF-1 delivery from IGFBP-3 conjugated scaffolds may accelerate host stem cell recruitment that can improve the muscle regeneration *in situ*.

Acknowledgments: This study was supported by the Musculoskeletal Transplant Foundation.

Reference: 1. Ju YM, Yoo JJ, Atala A, and Lee SJ, Biomaterial induced host stem cell recruitment for in situ muscle regeneration, Acta Biomater., 2014;10(10):4332-9.

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The Biomimetic Meniscus Scaffold Can Enhance Meniscus Regeneration in Rabbit Model

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The inner region of meniscus cannot be self-healing due to the lack of blood supply. The aligned structure of the native meniscus can make it easy to resist body load-bearing. Meniscus native microenvironment can be beneficial to the tissue regeneration. Thus, one of the major solution that may regenerate the meniscus injuries is designing a biomimetic scaffold that not only can own a good biomechanical capacity but also mimic the native microenvironment. A biomimetic meniscus scaffold that consisted of the poly(ε-caprolactone) (PCL) and the decellularized meniscal extracellular matrix (MECM), was constructed via combination of 3D printing and decellularization approach. The biomimetic scaffolds exhibited an excellent biomechanical characteristics and biocompatibility. We constructed the passaged meniscal fibrochondrocytes (MFC)-scaffolds composites and culture *in vitro* for 7 and 14 days, the results showed that those constructs not only can enhance cell proliferation but also can improve the glycosaminoglycan and collagen secretion. We also implanted the GFP rat MFC-scaffolds composites into the nude rat subcutaneous. The results show that those composites can form a meniscus-like tissue by GFP rat MFC compared with the native meniscus after 4 weeks implantation. The repair effect was evaluated by implanting the biomimetic scaffolds into meniscus defects of a rabbit model. Our findings demonstrated that the biomimetic scaffolds were able to induce meniscus regeneration, and the newly formed tissues were similar to the native tissues. Consequently, the current study not only achieves meniscus repair, but also suggests a promising strategy for the fabrication of the biomimetic scaffolds.

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Mimicking Cartilage Zonal Organization by Spatial Patterning of Microribbon-Based Stem Cell Niches

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Introduction: Articular cartilage is characterized by zonal organization, with gradient transitions of biochemical and mechanical properties. Regenerating cartilage with biomimetic zonal organization remains a great challenge. Our research group recently developed a method to fabricate hydrogels from microribbon (μRB)-like building blocks, which can be mixed with cells and intercrosslinked to form cell-laden macroporous scaffolds. The objective of this study was to create a spatially-patterned, multi-compositional extracellular matrix (ECM)-based μRB scaffold and evaluate its potential for mimicking cartilage zonal organization.

Methods: Microribbons were fabricated by wet-spinning as we previously reported. To mimic zonal transitions, five layers of MSC-loaded μRBs were patterned in a laminar manner. The layers consisted of mixed compositions of chondroitin sulfate and gelatin, with decreasing percentages of CS from 100% to 50% to mimic the superficial to deep zones of articular cartilage, respectively. All polymers were methacrylated to enable intercrosslinking of μRBs between adjacent layers. Scaffolds were cultured in chondrogenic media for three weeks. Outcome analyses include viability, biochemical assays (DNA, sGAG, collagen), mechanical testing, and histology.

Results: μRBs enabled one-step crosslinking to form an integrated macroporous scaffold with a gradient cue of ECM composition. Tuning μRB compositions led to MSC zonal-specific responses. From superficial to deep, the resulting cartilage demonstrated an increase in compressive moduli (9 to 456 kPa), as well as a 3-fold increase in sGAG content.

Significance: Spatial patterning of μRBs with tunable compositions facilitated MSC-based cartilage regeneration with zonal organization. This method may be broadly applied for recreating tissue organization of various tissue interfaces.

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In Vitro Raw 264.7 Monocyte Biocompitibility Of Casting Chitosan Film And Electrospun Chitosan Membranes Treated By Two Methods

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Guided Bone regeneration (GBR) membranes are widely used in clinical surgery as a barrier to direct the formation of bone in the graft space by protecting it from soft tissue intruding during healing. Chitosan is explored for making GBR membrane because of its biocompatibility and degradability. In the previous study, trimethylamine (TEA)/acetone and di-tert-butyl dicarbonate (tBOC) treatment was investigated for acidic salt removal from chitosan electrospun instead of the common Na2CO3 treatment to keep the nano-fibrous structure.1 In this study, electrospun chitosan membranes treated by TEA/tBOC method and Na2CO3 method, and casting film were evaluated in cytocompatibility with TIB 71TM RAW 264.7 monocyte cells. All the TEA/tBOC treated and Na2CO3 treated membranes and casting film were cytocompatible and supported cell proliferation for 3 days. TEA/tBOC treated membrane did not active monocytes to produce nitric oxide (NO) *in vitro* in the absence of lipopolysaccharide (LPS). In the presence of 2 μg/ml LPS, TEA/tBOC treated membrane and casting film inhibited LPS-induced NO production of RAW 264.7 cells by 50% - 75% as compared to tissue culture plastic and Na2CO3 treated membrane. Further evaluation are needed in the fibroblasts and osteoblasts compatibility and *in vivo* study.

Reference: 1. Su H, Liu KY, Karydis A, Abebe DG, Wu C,... & Bumgardner JD. Biomedical Materials. 12, 015003, 2016.

Acknowledgments: Work supported by the Biomaterials Applications of Memphis (BAM) labs, UM-UTHSC, Memphis, TN.

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Conductive Scaffold Made From Micro-scale Wet-spun PCL Fibres

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Normal 0 false false false EN-AU X-NONE AR-SA /* Style Definitions */ table.MsoNormalTable {mso-style-name:''Table Normal''; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:''''; mso-padding-alt:0 cm 5.4 pt 0 cm 5.4 pt; mso-para-margin-top:0 cm; mso-para-margin-right:0 cm; mso-para-margin-bottom:8.0 pt; mso-para-margin-left:0 cm; line-height: 107%; mso-pagination:widow-orphan; font-size:11.0 pt; font-family:'' Calibri'', sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-fareast-language:EN-US;} Fibrous scaffolds are excellent for tissue engineering due to their similarities to the tissue extracellular matrix (ECM). Conductive PCL fibres were wet-spun and coated by polypyrrole (PPy) and scaffolds were developed using a salt leaching process applied on wet-spun fibres. The Young's modulus of the PPy coated fibres was higher than that of the uncoated fibres. Neurite outgrowth of PC12 cells was observed on both coated and uncoated samples and fibroblast cells had a suitable growth and infiltration into the structure of the scaffolds. The promising results of the cell growth, infiltration and differentiation on these scaffolds show the benefits of this fabrication procedure derived from salt leaching and wet-spinning where fibres form spongy structures with large pores.

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Evaluation of Two Distinct Biomaterials in Critical Bone Defects in Calvaria of Rats

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Bone defects are referred to as clinical problems[1]. Thus, new biomaterials based on hydroxyapatite and PLGA have been extensively studied[2,3]. The objective was to evaluate the action of two different biomaterials on critical bone defects. 48 rats (*Wistar*), divided into CG, BG1-(HA/PLGA/Bleed) and BG2-(HA/PLGA) were used, subdivided into experimental periods of 15, 30, 60 days. The bone defect was induced in the medial region of the calvaria with a trephine drill 8 mm in diameter. Histologically, BG1 presented the best evolution of the repair process with greater presence of newly formed bone when compared to the other groups studied. Morphometric analysis confirms the histological findings. BG1-15 presented 9.49% of newly formed bone compared to 5.3% presented by CG-15 and 1.5% presented by BG2-15. BG1-30 presented 18.4% of newly formed bone compared to 17.5% presented by CG-30 and 3.73% by BG2-30. BG1-60 presented the largest area of newly formed bone 50.9% compared to 18.06% presented by CG-60 and 7.06% by BG2-60. The difference found between the treated groups may be associated with both chemical properties of each biomaterial as well as the porosity and rigidity. Thus, it is suggested that BG1 has interesting properties that induce the necessary stimuli that contribute to the evolution kinetics of bone repair. 1 Petrids, X.; Diamanti, E.; Trigas, G.C.; Kalyvas, D.; Kitraki, E. Bone regeneration in critical-size calvarial defects using human dental pulp cells in an extracellular matriz-based scaffold. **Journal of cranial-maxillo-facial surgery**, 43, 483-490, 2015. 2 Torquato, S., Random Heterogeneous Materials: Microstructure and Macroscopic Properties. **Springer-Verlag**, New-York, 2002.

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A Novel 3-D Micro-fibrous scaffold fabricating method for Engineering Linear Tissues

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Tissue-engineering scaffolds mimicking the microenvironmental topography in our body are promising for desirable stem cell responses and tissue-formation. Microfibers with linear alignment and customized diameters are particularly useful for forming linear tissues, as aligned microfibers form channel-like lumen spaces to induce unidirectional cell spreading and mechanosensing, which are known to promote linear-tissue cell phenotypes. However, most methods to prepare microfibers are extremely slow and have limited diameter tunability. Cellularization of fibrous scaffolds is also challenging, due to inefficient cell penetration or diffusion in 3-D. Here we present a novel, ''stretch-and-fold'' method to mass-produce aligned microfibers of customizable diameter. In brief, a ring of photo-crosslinkable porcine gelatin were repeatedly stretched and folded for N cycles. Within minutes, the cycles increased the length of gelatin ring by 2^N folds, decreased the diameter of the gelatin ring by $2^{N/2}$ folds, turning the ring into a bundle of microfibers. This method leads to a widely tunable fiber diameter, from micron- to nanometer scale, while maintaining diameter uniformity and fiber alignment. To verify the efficacy for tissue engineering, human mesenchymal stem cells (hMSCs) were mixed among the aligned microfibers, and the microfibers were photo-crosslinked into a 3-D scaffold. Fluorescent images on day 7 showed uniform cell distribution and unidirectional cell spreading. The stress-fibers of hMSCs, which plays a central role in mechanosensing and mechanotransduction, were found to organized along the fibers. Above results demonstrated the great potential of our stretched and folded, multi-scaled microfibers for tissue-engineering and regenerative medicine.

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Evaluation of Biocompatibility of Silk Fibroin and Application to Tissue Engineered Materials for Cardiocvascular System

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Introduction: Current commercial material as a cardiovascular medical device is expanded polytetrafluoroethylene (ePTFE) and in use a broad treatment spectrum. However, there are still physical and functional limitations of this material. In order to make ePTFE a more useful material, modification of the material itself is required because of current problems such as biodegrability, self-organized dysplasia, thrombosis and infectivity. We therefore developed on a hybrid material prepared by blending a silk fibroin (SF) and Polyurethane (PU) for the cardiovascular system, and evaluate the biocompatibility and tissue response with comparison of current materials.

Materials and Methods: Composite of SF/PU sheets prepared by electrospinning with several ratio (ww) and insolubilized in proportion to previous report. Structural analysis and compatibility of material observed by Solid state NMR measurements. SF/PU patches implanted to rat abdominal aorta and dog descending aorta from 3 to 6 monthes.

Results: SF and PU composite sheets showed cell infiltration that result will be anticipated the tissue remodeling in long term. These results indicated that the SF/PU composite sheet have some good functions supports to use as a cardiac repairing patch. Moreover, the SF/PU = 7/3 sheet demonstrated significantly higher tissue infiltration, elastogenesis, endothelialization, and no neointimal hyperplasia compared with SF/PU = 4/6 sheet. In conclusion, an increase of SF concentration in the SF/PU patch had effects on the vascular remodeling and physical properties.

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Biomimetic Surface Modification of Polymeric Scaffolds for the Development of *in vitro* tumor Models

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Traditional chemotherapy regimens put a high degree of emphasis on the use of historical data to predict a cancer patient's response to a proposed therapy. Unfortunately for the patients, this often leads to continuous rounds of trial-and-error in the search for a compatible treatment, decreasing their chances for survival. Tumor engineering seeks to alleviate this issue by growing patient tumors outside of the body, providing a high throughput avenue for treatment discovery. Utilizing a variety of techniques for 3D culture, researchers have created models that more closely resemble and predict *in vivo* tumor drug responses; however, there is still more room for improvement. In particular, these *in vitro* models consistently exhibit poor cell proliferation and distribution, which severely limits their predictive capabilities. To combat this major issue, we have leveraged our patented biomimetic surface modification platform for tumor engineering applications. In particular, we have identified various moieties specific to certain tumors that are integral to cellular adhesion, and have used these to modify our scaffolds and trick the cancer cells into exhibiting higher rates of adhesion. For instance, in terms of prostate cancer, poly(L-lactic acid) (PLLA) scaffolds were modified to express n-cadherin, which is a highly upregulated protein used for cellular adhesion. After cell seeding, we were able to significantly increase PC3 seeding efficiency and potentially improve cell physiology without compromising the mechanical and degradation properties of the underlying PLLA.

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Novel Heterogeneous Polycaprolactone/human Serum Albumin Electrospun Membrane for Tissue Engineering with Improved Properties

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Development of electrospun membrane with improved mechanical properties, proper biodegradation, and excellent biocompatibility simultaneously is the great challenging task. In this background, we have prepared the heterogeneous polycaprolactone/human serum albumin (PCL/HSA) membrane by blending the solution of the different mass weight ratio of PCL to HSA using electrospinning technique. Physio-chemical and biological properties of the as-fabricated membranes were evaluated. FE-SEM images revealed that all PCL/HSA mats were composed of interlinked nano-nets along with conventional electrospun fibers while nano-nets were not found for pristine PCL mat. PCL-HSA membranes showed excellent mechanical properties (3 fold tensile stress compared to pristine PCL), improved biodegradation, enhanced mineralization and extraordinarily supported the cells early adhesion and proliferation compared to pure PCL membrane. These results indicate that the nano-nets supported PCL/HSA membranes with the ability to induce progressive cell growth could be promising in tissue engineering applications.

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Evaluating the Degradation of Alginate/Chitosan Mixtures in Growth Plate Injuries

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Alginate and chitosan are promising yet unexplored biomaterials for regenerating growth plate cartilage. Growth plate injures can cause bone bridge formation that can result in traumatic bone growth abnormalities for children, yet no treatment exists that can restore growth plate cartilage. The goal of this study was to develop a material that would prevent bone bridge formation, and degrade in a timely manner. We evaluated the ability of Alginate:Chitosan 90:10 with calcium (90:10+C), Alginate:Chitosan 50:50 with calcium (50:50+C), Alginate:Chitosan 50:50 without calcium (50:50-C), and Irradiated Alginate (IA) to inhibit bone bridge formation, and degrade in a rat growth plate injury model. All animal procedures were approved according to the University of Colorado IACUC. Alginate/chitosan mixtures were prepared as above, and injected into proximal tibial growth plate drill-hole injuries of 6-week Sprague-Dawley rats. Animals were sacrificed at 28 days, and tibiae were processed for Alcian-Blue Hematoxylin staining. Groups 90:10+C, 50:50+C, and 50:50-C remained present in the drill track with variable levels of degradation. Further, repair tissue in these groups showed a mix of chondrogenic, bony, and fibrous tissue. IA was mostly degraded, and resulted in fibrous/chondrogenic repair tissue without bony repair. Results from this study suggest that of these groups, IA degrades quickly and may enhance growth plate cartilage restoration.

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3D Electrospun PLGA-Cardiac ECM Hybrid Scaffold for Cardiac Tissue Engineering

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Electrospinning is a widely used technique to fabricate polymeric fibers, which mimics the structural organization of native extracellular matrix (ECM). Despite these mimicking structural properties, conventional electrospinning only results in 2 dimensional mats rather than 3 dimensional structures and require further biochemical modification to improve their bioactivity. Polymeric scaffolds are generally conferred bioactivity using biomolecules such as collagen, fibronectin, and laminin. But, more specific organotypic molecules are required to modify the polymer scaffolds to obtain a native ECM mimicking structure, as the complex ECM provides a tissue specific biochemical makeup, and 3D structural organization that governs eventual cellular functionality. In this study, we combined a PLGA scaffold and liquid porcine cardiac ECM (L-pcECM), to form a hybrid scaffold. Wet electrospinning was used to obtain 3D scaffolds of PLGA. L-pcECM was obtained by the process of decellularization of porcine cardiac ECM. The 3D PLGA scaffolds were modified with L-pcECM using EDC-NHS chemistry. The resulting 3D scaffolds showed similar morphology, fiber diameter, and pore size as characterized using SEM in comparison to a native ECM. FTIR analysis presented successful modification of the scaffold with the L-pcECM. Preliminary cell interaction between the unmodified PLGA scaffold and human mesenchymal stem cells show improved cell interaction, viability, proliferation and phenotypic expression. Taken together, these results indicate that this natural-synthetic composite scaffold provides a native mimicking cardiac microenvironment to facilitate stem cell survival and proliferation and may be a potential platform for cardiac tissue engineering applications.

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3D Microprinting of Poly(propylene fumarate) for Drug Delivery

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Photocrosslinkable polymers have great applicability in tissue engineering as resins for 3D printing applications. Photocurable materials are particularly good candidates for 3D microprinting (3DMP), a rapidly emerging technology in which a two-photon laser source is used to fabricate scaffolds with nanoscale resolution[1]. Yet, of the extensive library of photocrosslinkable biomaterials currently developed, only a limited fraction is currently used in 3DMP. In this work we investigated the possibility to scale down the photochemistry of poly(propylene fumarate) (PPF), a biomaterial extensively used in tissue engineering, for microscale scaffold fabrication via 3DMP. PPF was synthesized using standard techniques[2] and crosslinked

in the presence of bis-acylphosphine oxide photoinitiator at different 3DMP conditions, resulting in scaffold with microscopic features, as shown via electron microscopy. Laser power/speed affected PPF crosslinking and mechanical properties, as measured by infrared spectroscopy and atomic-force microscopy. Furthermore, delivery kinetics from drug-loaded PPF scaffolds was tuned by 3DMP conditions, resulting in highly controlled drug release in an animal model. Together, this work highlights the use of established photocrosslinkable polymers in 3DMP and its high-throughout characterization of polymer properties (including degradation and delivery kinetics) on a microscopic level, avoiding resource-consuming experiments with larger scaffolds.

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Preparation of Chondroitin Sulfate Crosslinked, Thermally Responsive Hydrogels for Osteochondral Tissue Regeneration

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In situ gelling hydrogel constructs have shown great promise for the repair of bone, cartilage, and other tissue types. Our research group has developed in situ thermally gelling hydrogels for bone repair, as well as bi-layered hydrogels that affect the repair of osteochondral tissue containing bone and cartilage. Because thermogelling hydrogels have inherent issues with syneresis, or shrinking, chemical crosslinkers have been utilized to prevent such behavior. In this study, we aimed to study the feasibility of using chondroitin sulfate (CS), a sulfated glycosaminoglycan, as the biological crosslinking agent. Once modified with adipic acid dihydrazide (ADH), it undergoes crosslinking reaction with (N-isopropylacrylamide) (PNiPAAm)-based thermogelling macromer. Pendant epoxy group was chosen as the site of crosslinking, since amine-epoxy reaction does not require exogenous catalyst, and can occur in mild conditions without generating harmful by-products.

Carboxylate groups on glucuronic acid repeat units of CS were modified with ADH using carbodiimide crosslinking chemistry, and the degree of modification was quantified via ^{1}H-NMR. The results show that CS can be modified with ADH, and its modification rate can be easily modulated by changing the amount of carbodiimide in the reaction. The chemical crosslinking between modified CS and PNiPAAm-based hydrogel was also verified via simple inverted tube method, in which the hydrogel underwent, along with thermal gelation at 37 °C, irreversible chemical crosslinking. These results show that CS can indeed be utilized as the chemical crosslinker for PNiPAAm-based hydrogel.

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Localized Release of Corticosteroid from Macroporous Organosilicone Beads Scaffolds

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The local delivery of anti-inflammatory agents provides a promising approach to improve the efficacy of islets transplantation for treatment of Type 1 Diabetes. The delivery profile, however, needs to be carefully designed to ensure the stable release of the agent in a range that mitigates inflammatory responses while avoiding deleterious effect to the graft. In our previous study, Dexamethasone (Dex) was loaded within PDMS directly to fabricate macroporous scaffolds (regular scaffolds) for controlled release of the drug. This local release was found to acceleration islet engraftment via promotion of anti-inflammatory M2 macrophages [1]. In an effort to provide a more controlled and extended drug release, porous scaffolds were fabricated using Dex-microbeads. First, Dex was loaded within PDMS and microbeads were fabricated by emulsion (size range $75.39 \pm 50.51\mu m$). Dex-beads were then mixed within PDMS and NaCl to obtain a 85% macroporous scaffold. The kinetic release of Dex from these new PDMS beads scaffolds was found to be significantly modulated, resulting in the stable release for over 30 days (within the range from 50 to 150 ng/mL for the 0.5% loaded scaffold). This was in stark contrast to standard Dex scaffolds, where the majority of the drug was released within the first 10 days (burst followed by plateau). A scaffold that provides a more sustained and controlled Dex release should lead to mitigation of different transplant-associated inflammatory responses while minimizing deleterious effects associated with a burst and expiration drug release profile.

Reference: 1. Jiang, Kaiyuan, *et al*, Biomaterials 114 (2017) 71-81.

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Fabrication of Functional Biomimetic Vascular Scaffolds for Use in Renal Tissue Constructs

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One challenge common to large tissue engineered constructs is the need for vascularization *in vivo* and *in vitro* to facilitate oxygen delivery. Pre-vascularization is particularly challenging in the kidney due to the organ's size and unique vascular architecture. Biofabrication and microfluidics techniques can produce simple branching vessel-like structures, however they do not replicate the vascular organization seen in native tissues. In addition, these methods are often complex and expensive. We propose a simple, cost-effective and novel method for the fabrication of biomimetic microvascular scaffolds for pre-vascularization of constructs based on vascular corrosion castings of kidneys. Vascular corrosion casts are capable of capturing the entire vascular network of an organ, including glomerular capillaries. Starting with these corrosion casts as a template, we are able to mold microvascular scaffolds that reflect the native anatomy. The microvascular scaffolds can support endothelialization and incorporation into hydrogel-based renal constructs. Human renal cells in 3D culture around the vascular scaffold self-assemble into tubule-like structures, which anatomically and functionally resemble segments of the nephron. We believe that these pre-vascularized renal constructs will be capable of integration after implantation and may be used to augment the function of diseased or damaged kidneys.

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Osteogenic Differentiation of Human Mesenchymal Stem Cells on Osteopontin Mimetic Peptides Modified Electrospun Nanofibers

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Osteopontin is one of the major extracellular matrix protein that plays vital role on mineralization. Osteopontin structure contains 40% of glutamic acid (GLU) and aspartic acid (ASP) sequences. Although there have been studies that show the individual effect of GLU and ASP sequences on osteogenic differentiation when those used to modify the surface of PLGA nanofibers (NF), to best of our knowledge comparison the effect of GLU and ASP on human mesenchymal stem cells (hMSCs) osteogenic differentiation has not been studied. The objective of this study is to determine the major effective sequence on adhesion, proliferation, osteogenic differentiation and mineralization of hMSCs. EEEEE and DDDDD were synthesized manually on Rink Amide resin and conjugated to Ac-PLA by

Michael addition reaction to produce the PLA-GLU and PLA-ASP conjugate[1]. Peptide conjugation was characterized via AFM, FTIR and XPS. Next, hMSCs are seeded on the surface modified NF and cultured in osteogenic media for 28 days. Differentiation of MSCs was evaluated by measuring the alkaline phosphatase activity, alizarin red staining, total calcium content, gene expression levels and immunofluorescence staining of osteogenic markers of collagen type I, osteocalcin and osteopontin. Surface characterization of PLA-GLU and PLA-ASP successfully confirms peptide conjugation on NF and osteogenic differentiation of hMSC on surface modified NF are underway. The outcomes of this study would help to determine the most effective sequence on hMSCs on osteogenic differentiation for further modification of the synthetic scaffolds.

1. Karaman O. *et al*, J Tissue Eng Regen Med, **10**(2): p. E132-46(2016).

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Mechanically Tuned Aligned Nanofibers and GDNF to Improve Nerve Growth Conduits

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Treatments for peripheral nerve injury includes autografts and nerve growth conduits (NGC). This project enhances existing NGCs by incorporating aligned nanofibers (topographical) made from a compliant substrate (mechanical) with growth factor (GF) releasing microspheres (chemical). We hypothesize that these cues along with physical therapy (PT) will result in enhanced function recovery. Polycaprolactone conduits with longitudinally aligned methacrylated hyaluronic acid fibers, with or without microspheres containing glial cell line-derived neurotrophic factor were tested in rat sciatic nerves. The animals were divided into five groups: fibers, fibers + PT, fibers + GF, fibers + GF + PT, and autograft control. All animals received behavior and functional testing prior to surgery and weekly post-surgery for 60 days. Prior to sacrifice the Compound Muscle Action Potentials (CMAP) of the gastrocnemius muscles were measured. Gastrocnemius muscles and sciatic nerves were harvested for histological analysis. All surgical procedures and animal testing was approved by the Wayne State University Institutional Animal Care and Use Committee. Weekly testing showed that fibers with GF enhanced or accelerated functional recovery. By 4 weeks both the GF groups were performing similarly to the autograft in the footfall test. Interestingly, starting at week 5, the fibers + PT group was also near autograft. Von Frey fibers were used to test the sensory perception. This study indicates that GFs combined with aligned fibers can improve functional recovery following peripheral nerve injury and that physical therapy can have a positive effect, though it may not have an additive effect when combined with GFs.

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Development of an Elastic and Adhesive Sealant for Surgical Applications

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Conventional surgical sealants have been used for sealing or repairing defects often suffer from low adhesion strength, insufficient mechanical stability and strength, cytotoxic degradation products, and weak performance in biological environments. Therefore, in this study we aimed to engineer a photocrosslinked and highly biocompatible sealant with tunable mechanical and adhesion properties using tropoelastin, as a genetically modified human protein. We tuned the degree of methacrylation of tropoelastin and prepolymer concentration to optimize the physical properties and adhesion strength of the methacryloyl-substituted tropoelastin (MeTro) hydrogel for sealing of elastic and soft tissues. Following ASTM standard tests, the MeTro hydrogels revealed superior adhesive strength and burst pressure values compared to the commercially available sealants. The subcutaneous implantation of the engineered MeTro hydrogels in rats exhibited minimal inflammatory host responses and slow biodegradation of sealant. The *in vivo* and *ex vivo* burst pressure resistance of bioengineered MeTro sealants was tested on lungs and arteries in small as well as translational large animal models. Our results proved MeTro sealant to effectively seal lung and artery leakages without the need for sutures or staples, presenting a significant improvement compared to the commercially available clinical sealants (Evicel® and Progel™) and sutures only. Combining these results, we envision that the engineered MeTro sealant has the potential to be commercialized due to its remarkable mechanical strength, biocompatibility, biodegradability and strong adhesive interaction between the sealant and the wound tissue without the need for suturing.

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Seeding and Recellularization of Porcine Acellular Muscle Matrix Biomaterials with Adipose-Derived Mesenchymal Stem Cells and C_2C_{12} Myoblasts

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The ability of skeletal muscle to repair itself via regenerative mechanisms is limited to instances where tissue damage is relatively small. When volumetric muscle loss occurs, the regenerative capacity of skeletal muscle is exceeded. This results in a permanent loss of muscle volume and function. Current strategies to replace or repair such damage are inadequate. The goal of this project is to develop natural biomaterials that facilitate the engineering and/or regeneration of skeletal muscle tissue by providing a myoinductive environment to seeded and/or infiltrating cells. We hypothesized that scaffolds and hydrogels composed of porcine acellular muscle matrix (PAMM) could be efficiently recellularized and support myogenic differentiation. Here, we describe the production and characterization of PAMM scaffolds and gels. Histological analyses, DNA content measurement, and scanning electron microscopy show that porcine skeletal muscle tissue can be effectively decellularized and processed into both a sheet-like scaffold and a hydrogel. We also demonstrate that PAMM biomaterials can be recellularized with adipose-derived mesenchymal stem cells and support the differentiation of C_2C_{12} myoblasts into myotubes. These results demonstrate the potential for PAMM biomaterials to be employed in tissue engineering and regenerative medicine-based strategies for repairing volumetric muscle loss.

Cardiovascular

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Bioprinting of Engineered Heart Tissue

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For cardiac tissue repair, 3D bioprinting holds great potential enabling to create multicellular constructs with defined shape and cell composition. However, materials selection and effective structures that can be reliably used to bioprint cardiac tissue constructs remain elusive. Here we present a concerted effort to fine tune

existing materials and protocols to generate customizable 3D printed designs which can support cardiomyocyte or fibroblast live cell printing. To achieve this, variable concentrations of gelatin methacrylate were used to print freeform 3D cell-containing structures using a BioBots bioprinter. To increase precision, the concentration of UV cured gelatin methacrylate inks was changed from the standard 10% to 15%. Printing parameters were monitored and adjusted to achieve free standing structures before UV crosslinking. Primary rat neonatal cardiac myocytes and cardiac fibroblasts were used as main cell sources. To address possible adverse effects of bioprinting-induced shear stress, bioprinted 10 mm wide ring-shaped constructs were then analyzed using CytoscanTM LDH assays, immunocytochemistry and bioluminescence imaging. Results from these experiments demonstrate that 3D bioprinting can be effectively tuned to create on demand cardiac constructs with different scaffold porosity, cell composition, and cell concentration.

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M1/M2 Macrophage Secretome Differentially Alters Valve Interstitial Cell Remodeling Behavior within Mechanically Constrained Microenvironments

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There is an increased appreciation for the contributions of macrophages as regulators of tissue and matrix remodeling through their ability to produce cytokines and chemokines. Their role in altering valve remodeling and driving disease has not yet been elucidated. While others have implicated classically activated macrophage (M1) microvesicles in driving valve interstitial cell (VIC) calcification, they do not address bulk 3D remodeling behavior of VIC populations in mechanically constrained environments as well as the contributions of the M2 secretome. Previously, we showed that boundary constraints can promote hyperactive remodeling within VIC populations. Here, we show that VIC remodeling differs greatly in the presence of secreted factors as a function of macrophage polarization (M1, M2a, M2c). Porcine VICs were encapsulated within isotropically mechanically anchored type I collagen hydrogels, and cultured with conditioned media collected from THP-1 derived M0, M1 (LPS, INFγ), M2a (IL4, IL13), or M2c (IL10) macrophages. Extracellular matrix remodeling and cellular differentiation varied significantly between conditioned media states. The M2a and c conditioned media showed increased compaction relative to M0 and M1 conditioned samples. Additionally, these samples showed increased osteogenic differentiation potential via increases in Runx2 and BMP2/4 expression. Using this novel platform, we can begin to understand how macrophages can alter VIC homeostasis by driving fibrosis and calcific remodeling.

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Tissue Engineering Based 3D Modelling of Cardiomyogenesis

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We report the creation of a reproducible and prototypical *in vitro* 3D prevascularized cardiac tissue, which encompasses all the indispensable characteristics of a tissue-engineered cardiac graft, such as consistent and synchronized spontaneous contractility, stable and responsive electrophysiological properties, vascularization, and a somatic stem cell source, to address the most chronic problem that is facing cardiac therapy as of today, i.e., the repair and/or regeneration of damaged myocardium. Additionally, we have demonstrated that it is feasible to recreate *in vitro* a 3D model of mammalian cardiomyogenesis that has been long-awaited. This 3D vascularized cardiac tissue can be utilized to address various outstanding questions that linger in the highly controversial field of adult stem cell-based cardiac regeneration, and allows us to dissect various molecular mechanisms that are underpinning the orderly cellular differentiation and tissue morphogenesis, which are probably difficult to be examined and/or accomplished in the clinical scenario. The mosaic of myocardial tissue recreated by means of modular and bottom-up tissue engineering approaches reiterated not only several crucial aspects of *in vivo* neo-vasculogenesis and neo-cardiomyogenesis, but it enabled us to critically evaluate the controversial aspects of the role of somatic stem cells (hMSCs) on the induction/differentiation of embryonic cardiomyocytes, and most significantly, whether hMSCs had multilineage differentiation potential in this milieu, undoubtedly. Eventually, the outcome from this study may translate to human personalized medicine by rendering a targeted approach to regulate the maintenance (physiological) or reparative (pathological) process of myocardium, and in turn can lead to functional enhancement of a failing heart.

Matrix & Extracellular Matrix Biology in Regenerative Medicine

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Integrated Hydrogel Culture System for Co-Culture of Muscle & Tendon Cells for *In vitro* Modeling of Myotendinous Junction

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Muscle tissue disorders can result from chronic conditions, including Duchenne's Muscular Dystrophy, or acute conditions, such as volumetric muscle loss resulting from trauma. In many cases, the myotendinous junction is also affected and is a key target for tissue engineering therapies. *In vitro* co-culture systems that allow for the characterization of the cross-talk between myoblasts and tenocytes could reveal mechanisms that could be targeted to guide current tissue engineering strategies towards tissue healing. C2C12 myoblasts (ATCC) and tenocytes were seeded in decellularized extracellular matrix hydrogels[1] which were self-assembled adjacent to each other with a controlled gradient transition between the two tissue specific gels. This platform allows for the characterization of both myoblasts and tenocytes separately and in co-culture on a controllable scale to analyze disorders at the myotendinous junction. This platform is the first step towards the development of new therapeutic techniques targeted at creating a more favorable environment for the regeneration of functional muscle tissue.1. Freytes, D. O.; Martin, J.; Velankar, S. S.; Lee, A. S.; Badylak, S. F., Preparation and rheological characterization of a gel form of the porcine urinary bladder matrix. Biomaterials 2008, 29 (11), 1630-7.

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Tissue-Engineered Submillimeter Diameter Vascular Grafts for Free Flap Survival in Rat Model

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The technological innovation of microsurgery has allowed to anastomose smaller diameter vessels, but synthetic vascular grafts with that small diameter, which could replace the autologous vein grafting, have not been developed. In this study, we studied acellular tail arteries of rats with 0.6-mm inner diameter and 5-cm length for free flap transfers. Their inner surfaces were modified with the peptide '$(POG)_7G_3REDV$', which we previously reported to catch circulating endothelial progenitor cells. Tail arteries of ACI rats were treated with the high-hydrostatic pressure and then incubated in peptide solution at each condition; two arteries were at 60°C for 1 h (group A), three were at 37°C for 1 h (group B), and three were at 4°C for 24 h (group C). Each graft was transplanted to connect the epigastric flap of Lewis rat to the contralateral femoral artery while the

flap venous drainage remained intact. All flaps survived at 3 weeks, whereas three flaps with the axial artery ligated showed total necrosis. All grafts demonstrated immediate patency, but each patency was 0/2 in group A, 3/3 in group B, and 2/3 in group C at 3 weeks. Although partial pseudoaneurysms were seen in patent grafts, the endothelialization was observed in grafts of group B. Since flap survival does not need long-term patency of the axial artery, our tissue-engineered vascular grafts would be useful for replacing flap arterial deficits temporarily.

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A Bone Ecm-derived Porous Scaffold As Biomaterial For Bone Tissue Engineering.

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The current approach to the treatment of bone defects involves the use of scaffolds that provide a biological and mechanically stable niche to favor tissue repair. Despite the significant progress in the field of bone tissue engineering, several main problems associated are attributed to giving a low biodegradation degree, does not promote osseointegration and regeneration, if the bone is not healing as well as expected or fails to heal, will not be given a proper ossification or new bone formation. The actual approaches of bone tissue regeneration are directed to the use of decellularized native extracellular matrices, wich are able of retain their own architecture, mechanic properties, biodegradability and promote new bone formation because they are capable of conserving proteins and other factors that are founded in physiological concentrations. Therefore, we propose an extracellular matrix-based bioscaffolds derived from bovine cancellous bone, which is processed by decellularization, demineralization, and hydrolysis of the collagen protein, these protocols have been successfully carried out in other organs and tissues; the effectiveness of its biosafety has also been previously evaluated *in vivo* and FDA approved. In the specific case of bone a more complex treatment is needed in comparison with other organs and tissues, because is necessary demineralization and collagen denaturalization. The present work was made in order to obtain a temporal scaffold that succeed in degradation in an inversely proportional way to the synthesis of extracellular matrix and the maturation of the bone by the cells of the host.

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The Use of Intrarticular Acellular ECM in Knee OA: A Clinical Study

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The Intrarticular Use of Acellular UBM in Knee OA: A Clinical Study

Purpose: To evaluate the utility of UBM in human knee osteoarthritis (OA)

Methods and Materials A retrospective random assessment of 14 patients with symptomatic knee OA were treated with Matristem Micro Matrix. Patients were confirmed to have knee OA using Kellgren and Lawrence (KL) assessment grades 2-3. Patients were monitored at 1 week, 1 month 6 months and 1 year.

Results: All patients received one intra-articular of Matristem. No severe adverse events or side effects were noted in any patient. In terms of efficacy, all but one patient showed a period of sustained pain relief (92.8). At one year 8 of 14 patients (62.5) reported no return of pain. Patients that did experience pain relief of up to one year tended to have lower VAS scores prior to injection than patients that experienced pain relief for less than 6 months.

Conclusion: This clinical study of the use of Matristem MicroMatrix injection in the treatment of human knee OA reflects a favorable response in patients, correlating with the animal literature. Exclusion of known factors impacting the progressive nature of knee OA such as obesity or more advanced KL score resulted in 100 efficacy. In this study, the small sample size and non-randomized non-clinical trial warrant further investigation. The use of knee Matristem for symptomatic human knee osteoarthritis provides a therapeutic regenerative medicine and tissue engineering application where prior strategies have been ineffective in altering the progressive degenerative cascade of knee OA.

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Behavior of Fibroblastic Cells under the Influence of Variable Biaxial Cyclic Stress Regimes

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Mammalian cells such as adipocytes and myocytes are constantly subjected to varying mechanical stimuli during both normal physical and exercise regimes. Even though mechanical stimuli are a major factor in determining cell function, the role of complex mechanical environments have remained unexplored. In this study, we report on our understanding of applying variable stress on NIH/3T3 fibroblastic cells cultured on flexible membrane in a custom-built biaxial cell stretching system[1]. In order to understand the cell behavior in the above-mentioned environment, we applied both regular (5%) and variable (up to 75% variability with mean stress amplitude maintained at 5%) biaxial cyclic stress. The normal cellular functions namely viability, proliferation, adhesion, migration and directionality of actin fibers were studied. It was found that even though viability was not affected by stress, proliferation increased significantly under regular and decreased marginally under variable stretch regimes. Lastly, the spreading factor, which is an indicator for cell adhesion and fibronectin distribution increased significantly following variable stretching. These results indicate that variable stretching, in particular may affect normal cellular functions such as proliferation and adhesion significantly, which in turn may affect cell-matrix interactions. Control of such cell-matrix interactions has wide applicability in tissue engineering and wound healing processes.

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The Effect of Regular and Diet Coca-Cola™ on Cell Adhesion, Proliferation and Integrin Expression of Human Foreskin Fibroblasts

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Objective: Observe trends of Human Foreskin Fibroblasts-1 cells in different concentrations of Coca-Cola and Diet Coca-Cola initially coated with fibronectin that may increase proliferation, migration and modify integrin expression.

Methodology: HFF-1 cells were cultured in DMEM, 10% fetal bovine serum and grown in 2% Coca-Cola, 4% Coca-Cola, and 4% Diet Coca-Cola media. HFF-1 cells were seeded on 5µl/ml fibronectin, 10µl/ml fibronectin, 5µl/ml collagen, and 10µl/collagen initially coated wells (10,000 cells/well) and incubated at 37°C, 5% CO2. Samples were quantified via Calcein-AM staining using FilterMax F5 MultiMode microplate reader. Images were taken using Olympus IX71 inverted microscope using Cy3 filter at 10x magnification. 24-well plates with Boyden Chamber inserts were coated with 10µl/ml collagen and 10µl/ml fibronectin. Cells were counted using Countess II FL from Life Technologies. FACS analysis

performed using Millipore Guava easyCyte. Statistical significance by $p < 0.05$.

Results: Proliferation rates and initial adhesion of HFF-1 cells were examined on varying concentration of collagen and fibronectin, revealing that cells in a fibronectin environment had an increased rate of initial adhesion. Proliferation increased in the presence of 2% Coca-Cola. HFF-1 cells migrate at a higher rate in the presence of ECM proteins such as collagen and fibronectin. β1 integrin expression was slightly increased in the presence of 2% Coca-Cola and 4% Coca-Cola, suggesting the increase in focal adhesion and HFF-1 cell spreading due to many ingredients such as phosphoric acid blocking mineral absorption and therefore allowing cellular proliferation.

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3-D Physiomimetic Porcine ECM Hydrogels Preserves the Function and 3-D Morphology of Rodent and Human Islets

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Clinical islet transplantation is a promising cell-based therapy for type 1 diabetes. Following islet isolation, the disruption of islet-to-matrix interaction results in ''anoikis''-induced apoptosis. Recent studies have shown that anoikis can be reduced if islets are co-cultured with an extracellular matrix (ECM). Decellularized tissues are abundant in cell adhesion motifs and provide an environment that can recapitulate the peri-islet niche. In this study, we sought to develop 3-D porcine ECM hydrogels using optimized tissue-specific decellularization protocols to enhance islet survival. Decellularized porcine bladder and pancreas were generated, with resulting matrices exhibiting no visible nucleic residue and minimal detectable DNA. IHC and HPLC-MS-based proteomic analysis found a high presence of peri-islet proteins and structural collagens in decellularized bladder and pancreas, respectively. Stable hydrogels reconstituted from solubilized decellularized tissue (1 mg/mL) were formed and used to encapsulate pancreatic rodent or human islets within this 3-D fiber network structure. After 3-7 days of culture, islet viability, function, and morphology were examined. Islets embedded in bladder and pancreas hydrogel demonstrated an increase of glucose-stimulated insulin production after culture, when compared to unencapsulated controls. Notably, ECM hydrogels also supported the outgrowth of $CD31^+$ endothelium and F-actin$^+$ fibroblast-like cells from islets, as well as recapitulated islet adhesion with increased expression of β1-integrin. This unique morphology could be beneficial during transplantation, to accelerate engraftment. Overall, these results demonstrate the benefits of ECM hydrogels in preserving islet function *in vitro*, while also supporting a 3-D morphology.

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Effect of Soluble RGD Peptide on Vasculogenesis of Scaffold-Free Micro-Tissues

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3-dimensional (3D) tissue formation characteristics of mainly vascularized tissues requires extensive exploration to investigate the characteristics of such tissue formation in micro scale. RGD peptide sequence is the primary integrin binding sequence to effectively modify biomaterials surface to enhance cellular adhesion and migration on synthetic surfaces. Immobilized RGD peptide on substrate promotes cellular adhesion and soluble form of RGD peptides enhances cell-to-cell communications. In this study, the effect of soluble RGD peptide on vascularization of Human Umbilical Cord Stem Cells (HUVECs) in scaffold-free micro-tissues was investigated. Scaffold-free micro-tissues were formed by 3D petri dish[1] by seeding 10^5 HUVECs in 100 µl with control (no RGD), 1 mM and 2 mM RGD peptide experimental groups containing serum-free media. Micrographs were taken every day for 7 days and the diameter of developed micro-tissues was determined by ImageJ. After culturing for 7 days, the cell viability of micro-tissue was assessed using a live/dead assay. Vascularization of HUVECs in micro-tissues were assessed by evaluating gene expression levels and immunofluorescence staining of key vasculogenic markers of VEGF, Tie-1, Tie-2, vWf, and PECAM-1. Micro-tissues were formed successfully for both experimental groups. 2 mM RGD peptide significantly enhanced the size of the micro-tissues. Vasculogenic differentiation of HUVECs with different concentrations of RGD peptide is underway. The findings of these study will enlighten the effect of integrin binding peptide on vasculogenesis of HUVECs. 1. Dissanayaka WL *et al*. Journal of endodontics. 2015;41(5):663-70.

Musculoskeletal

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Engineered *In Vitro* Models for the Guidance of Rehabilitation Regimens that Promote Cartilage Regeneration after Repair Surgery

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Microfracture (MFX) to repair focal lesions of articular cartilage injury is the most common cartilage repair procedure. MFX consists in the creation of small holes through the subchondral bone, the defect site is then filled with a clot rich in bone marrow-mesenchymal stem cells (BM-MSCs) that form of new fibrocartilaginous tissue. In general, early passive motion and limited weight bearing are adopted for rehabilitation after surgery, but it is unclear how these mechanical regimes affect BM-MSCs differentiation at the defect site and cartilage repair. To study this, we devised an *in vitro* model of cartilage defect filled with fibrin and BM-MSCs to mimic MFX repair. Composite constructs were then subjected to loading regimens simulating different rehabilitation exercise sessions. Weight bearing (compressive loading) was simulated using the Mechano-Active Tissue Engineering (MATE) system (MATEsystems, Clackamas, OR) with dynamic compression at 9 N load force, 1.5 Hz for 2 minutes followed by 2 minutes pause, repeated for 1h a day total, over 7 consecutive days. Passive motion (shear loading) was simulated using a rotatory cell culture system (RCCS TM-4;Synthecon Inc., Houston, TX). Samples were assayed by qRT-PCR, histology, GAG/DNA content, push-out test (integration of the construct). Statistical comparisons were done by paired t-test with significance of $p < 0.05$. Our results suggest that early passive motion exercise may be the most effective promoter of chondrogenesis in the early stages of MFX repair. Ultimately, we aim at identifying the long-term effects on regeneration of each exercise regime to guide the optimization of post-op rehabilitation.

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Control of Fiber Size and Tortuosity in Direct-Write Electrospun Structures for Tissue Engineering

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Abstract: Direct-write electrospinning (DWE) combines the advantages of electrospun[1] and 3D printed[2] scaffolds for fibrous soft tissue engineering by creating polymeric scaffolds with both submicron diameter fibers[3] and macroscopic structure. However, the influence of process parameters on fiber size and direction is not well understood. Our objective was to investigate the feasibility of controlling poly-caprolactone fiber formation via DWE by systematically

varying polymer solution concentration (14, 17.5, 21, 24.5, and 28wt%) and stage translation speed (10-90 mm/sec). Preliminary scanning electron microscopy showed that fiber diameter decreased with increasing concentration. Tortuosity also appeared to decrease with increasing concentration. No clear effects for stage speed were observed. These initial results show the promise of controlling fiber size and tortuosity for *in vitro* tissue engineering studies evaluating the effect of fibrous scaffolds on cellular morphology and collagenous tissue formation.

Acknowledgments: We thank the North Carolina State University Game-changing Research Incentive Program (GRIP) for its support.

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Cell-based Enhancement of the Tissue Engineered Muscle Repair Technology Platform

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Skeletal muscle has a remarkable ability to undergo regeneration post-injury. However, there is a limit to which endogenous mechanisms can recover bulk loss of tissue. One example is volumetric muscle loss (VML), which can result from trauma, as well as other congenital and acquired conditions. VML injury is associated with permanent functional and cosmetic deficits. There are currently no treatments that can adequately address VML injury. We have developed a tissue engineered muscle repair (TEMR) technology platform to enhance endogenous repair by creating a more favorable microenvironment for regeneration. TEMR consists of muscle progenitor cells seeded on a porcine bladder acellular matrix (BAM) and conditioned in a bioreactor prior to implantation. TEMR constructs have been evaluated in two distinct, surgically-created and biologically relevant rodent VML injury models in the tibialis anterior and lattisimus dorsi muscles, respectively. In both instances, TEMR-treated animals exhibited significant, durable, functional recovery (60-70%), as well as observable muscle regeneration. In pursuit of more rapid and complete functional recovery from VML injury, we have begun to incorporate additional cell types into the TEMR construct to more fully recapitulate the endogenous regenerative microenvironment. Specifically, satellite cells or human pluripotent stem cell-derived neurons have been added to the TEMR construct. Initial investigations have documented that both cell types can adhere to and survive on the TEMR construct during our bioreactor preconditioning process. Incorporation of these cell types into our existing technology platform should further improve functional recovery from VML injuries.

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Antibacterial Effects of Silver Nanoparticle-loaded 3D Bioprinted Construct for Infected Bone Repair

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Osteomyelitis is a common manifestation of invasive *Staphylococcus aureus* infection, often resulting in extensive bone loss and destruction. Reconstruction of large bone defects secondary to Osteomyelitis is still a major challenge, mainly due to chances of re-infection of bone grafts and rise of Methicillin-resistant *Staphylococcus aureus* (MRSA). The objective of this study is to use 3D bioprinting for custom fabrication of bone constructs containing silver nanoparticles (AgNPs); where a sustained release of AgNPs from the bone constructs would provide antibacterial activity, and the construct architecture would allow new bone ingrowth. This bone construct was designed and 3D printed using a combination of poly(ε-caprolactone) (PCL) and poly(lactide-*co*-glycolide) (PLGA) containing AgNPs (PCL-AgNP and PLGA-AgNP, respectively). AgNP release kinetic studies showed that almost 100% of AgNPs was released from PLGA-AgNP in 6 weeks; while for only 4% of the AgNPs release from PCL-AgNP. A NaOH pre-treatment method was used to accelerate the degradation of PCL, which resulted in about 30-35% AgNPs release in 2 weeks. Additionally, an *in situ* hydroxyapatite (HA) plus AgNP deposition method to surface-coat the bone constructs resulted in an enhanced antimicrobial activity *in vitro* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The combination of differentially degrading PCL and PLGA polymers containing AgNPs along with surface coating would enable the sustained release of AgNPs for treating Osteomyelitis infection during the bone regeneration.

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Curcumin-loaded PLGA Particles for the Treatment of Osteoarthritis

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Osteoarthritis (OA) affects 27 million adults in the U.S and is the leading cause of disability in the country. It consists of a painful progressive articular cartilage degeneration and synovium inflammation that could lead to total joint replacement. Current treatments of OA are limited to pain management and there is no approved therapy to prevent OA progression. The anti-inflammatory and chondroprotective properties of curcumin make it a good candidate for OA treatment, but its poor oral bioavailability has limited its use. Therefore, this work aims to develop poly-lactic-co-glycolic acid (PLGA) curcumin-loaded microparticles (PLGA-Cur) for the intra-articular treatment of OA in a Medial Meniscus Transection (MMT) rat model. Particles of 0.26, 1 and 8 μm in diameter were synthesized using oil-in-water emulsion evaporation method and evaluated for their *in vitro* curcumin release profile and residence time in *naïve* rat knee joints. Particles with longer residence time in the knee joint and higher amount of curcumin cumulative release (1 and 8 μm) were evaluated for their effectiveness at reducing OA progression in a pilot study (n = 3) using the MMT model. Preliminary results suggest that 1 μm PLGA-Cur particles qualitatively reduced lesion volume compared to empty particles (control) as shown by Equilibrium Partitioning of an Ionic Contrast agent microCT (EPIC-μCT). Future work includes fully powered studies to confirm the effectiveness of 1 μm PLGA-Cur particles to reduce OA progression and dosing studies. This preliminary work suggests that intra-articular curcumin delivery could be an effective disease-modifying therapy able to reduce the progression of OA.

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Combination of Silk Fibroin and Fiber Alignment Cues in Nanofibers for Tendon Tissue Engineering to Repair the Achilles Tendon Defects

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In this study, we used silk fibroin/polycaprolactone to produce random and aligned nanofibrous membranes through electrospinning while silk microfibrous membranes were purchased. SF could facilitate cell attachment and proliferation while PCL could impart favorable mechanical properties and biocompatibility. We embedded a silk microfibrous membrane at the bottom of the electrospung nanofibers to enhance the mechanical strength of the scaffold. The MTS test revealed the effect of SF in proliferation of rabbit dermal fibroblast (RDFB) while fluorescent cytoskeleton staining and SEM analysis showed that the aligned nanofiber membranes (APSF)

facilitated the allied proliferation of cells with extracellular matrix (ECM) deposition. The quantitative real-time polymerase chain reaction studies indicated that the APSF facilitated expression of type I collagen, fibronectin, and biglycan. Tendon reconstruction was confirmed by the presence of type III collagen on APSF. Subsequently, an *in vivo* animal experiment on New Zealand white rabbits with Achilles tendon deficiencies were performed to examine tendon reconstruction. Histological and immunohistochemical staining, mechanical testing, tissue section staining for tenascin-cytotactin, collagen type I and type III staining were performed to evaluate the tendon regeneration potential of the scaffolds. Effects of fiber alignment in ECM production was verified by histological and immunohistochemical staining. *In vivo* mechanical testing of tendon tissues grown on APSFm+RDFB indicated the similar Young's modulus values to original tendons. In short, the aligned nanofiber composite membrane scaffold enabled a faster tendon reconstruction by utilizing the aligned morphology and good mechanical properties.

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3D Displacement Fields in Cartilage under Compression using Two-Photon Microscopy

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Introduction: The structure and mechanical properties of articular cartilage are known to vary with depth. Depth dependent mechanical behavior has been evaluated using planar (2D) methods based on optical imaging to track tissue features. It has generally been assumed that tissue displacements were limited to the imaging plane. In this investigation, we used two photon microscopy techniques to track the 3D spatial location of labeled chondrocytes under compression.

Materials and Methods: A biaxial loading apparatus for applying compressive and shear displacements to a sample was designed and built. Servo motors applied specified compressive and shear displacements to a cartilage sample while a load cell measured the corresponding shear and compressive forces. Osteochondral samples were cored from a calf femoral condyle. Before imaging, cell nuclei were labeled with ethidium bromide. Three-dimensional images of the tissue in uncompressed and compressed states were then obtained using two-photon microscopy. 3D displacement fields were obtained from volume images using Imaris software.

Results and Discussion: Cell displacements under compression did not remain in the imaging plane; they had displacement components perpendicular to the imaging plane. We observed that cell displacements vary spatially suggesting that it should not be assumed that displacements in this type of commonly used loading device are planar. Cells close to the articular surface displaced more compared to those near bone, suggesting that surface and middle zones are more compliant.

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Contractile hPSC derived Skeletal Muscle Tissues for Human Disease Modeling

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Despite established protocols for differentiation of human pluripotent stem cells (hPSCs) into myogenic cells, 3D hPSC-derived skeletal muscle tissues that are able to respond to electrical and biochemical stimuli have not been reported. Here we describe engineering of contractile human skeletal muscle tissues using induced myogenic progenitor cells (iMPCs) generated via paraxial-mesoderm induction of hPSCs followed by transient overexpression of Pax7. Under optimized 3D culture conditions, iMPCs form skeletal muscle tissues (iSKM bundles) contain aligned multi-nucleated myotubes that display functional properties akin to native muscle, including positive force-frequency relationship in response to electrical stimulation as well as forceful contractions in response to acetylcholine. During 4-week culture, iSKM bundles undergo increased structural and molecular maturation, hypertrophy, and force generation. When constructed with iMPCs derived from patients with various muscular dystrophies, the iSKM bundles allowed quantitative comparisons of histology, contractile forces, and calcium handling between control and diseased cells. The iSKM bundles hold the promise as a platform for personalized modeling of human muscle disease and screening of candidate therapeutics.

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Engineering of Volumetric Skeletal Muscle Tissue for Accelerated Restoration of Pelvic Floor Muscle Function

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Damages in the pelvic floor muscles often cause dysfunction of the entire pelvic urogenital system. Current treatments for the injury include physical therapy, autologous muscle flap transfer, and surgical interventions using synthetic and biological materials. However, none of them entirely address the problems associated with long-term restoration of normal anatomy and function in the injured pelvic floor muscle system. Engineering of functional muscle tissue constructs may provide a solution to this unmet medical need. However, the current muscle engineering techniques are limited by the ability to build sizable constructs with timely innervation for successful graft survival. To this end, this study aims to fabricate and optimize volumetric 3-D bioprinted skeletal muscle constructs with innervation capability for repairing pelvic floor muscle injuries. Bioprinted skeletal muscle constructs that mimic native skeletal muscle organization were fabricated by using a 'bioink' formulation consisting of fibrin-based hydrogel containing human muscle progenitor cells, and muscle tissue formation capacity was investigated in a pelvic floor muscle injury in rats. Our results demonstrate that the bioprinted cells within the engineered skeletal muscle constructs are able to maintain their viability and formed muscle fibers *in vivo*, suggesting that the engineered muscle constructs may contribute to the restoration of pelvic floor muscle function anatomically and functionally.

Respiratory, Urologic and Gastrointestinal

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Anastomosis of Tissue Engineered Intestine in Line with Host Intestine in Rats

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Objectives: The purpose of this study was to anastomose tissue engineered intestine (TEI) in line with host intestine to examine the patency and peristaltic function of the TEI.

Methods: Intestine harvested from 2-5 day old transgenic GFP rat pups was digested to obtain organoids which were seeded onto tubular polyglycolic acid (PGA) scaffolds and implanted into the peritoneal cavity of six nude rats. After 4 weeks of *in vivo* incubation, the TEI was located and anastomosed with host intestine. Four weeks after anastomosis, the patency and peristaltic function of TEI was assessed by barium contrast study, and the TEI with adjacent host intestine was harvested for morphological evaluation.

Results: Four animals survived the anastomosis surgery. One animal died from intestinal obstruction and another from leakage at the anastomosis. In surviving animals, the average time for passage of barium through TEI was 9 ± 3 minutes, whereas it was 5 ± 2

minutes for passage through the same length of native intestine ($p < 0.05$). Histology revealed TEI lined with neomucosa. GFP staining demonstrated the presence of positively stained cells at the site of TEI, which confirmed that the cell source of TEI was from implanted organoids.

Conclusion: Anastomosis of TEI in line with host intestine maintained patency and displayed intact but slower intestinal transit compared to native intestine.

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An Autonomous Bronchoscope for Drug Delivery into Target Regions in the Lungs

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Locally administered therapeutics into injured or diseased lung regions can increase treatment efficacy and reduce systemic toxicity by concentrating therapeutic action within a specific region [1, 2]. With existing methods, however, localized drug delivery within the structurally complex airway tree is technically challenging, time consuming, and spatially inaccurate because of manual navigation-path determination and tedious device operation required. To address these challenges, we developed an autonomous robotic bronchoscope that can enter predetermined target pathologic sites and release drugs in the lungs with enhanced spatial accuracy. This device is integrated with a fiber-optic imaging probe that visualizes the inside of the lung airways. Using machine learning algorithms, the visual information obtained is processed to determine a navigational direction in real time. During navigation, the device is steered using computer-controlled servos attached to the distal tip of the device through pulling wires. We present the first application of machine-learning guided technology that could improve the treatment outcomes of lung disease by enabling precisely controlled and spatially focused drug delivery into target pathologic sites in the lungs.

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Re-Epithelialization of Whole Porcine Kidneys with Renal Epithelial Cells

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Re-epithelialization of whole porcine kidneys with renal epithelial cells could result in improved regeneration of human tissues. Preservation of vasculature and uniform cell perfusion are critical criteria for achieving regeneration. Decellularized porcine kidneys were recellularized with renal epithelial cells by 3 methods: perfusion through the vasculature under high pressure (VHP), perfusion through the ureter under high pressure (UHP), or perfusion through the ureter under moderate vacuum (UMV). Vasculature preservation and cell perfusion throughout the kidneys were analyzed in this study by histology, scanning electron microscopy (SEM), confocal microscopy, and magnetic resonance imaging (MRI). Renal epithelial cells were detected in the MRI by labeling them with iron oxide. It became apparent that the cell solution concentration was optimal at 4-5 million cells/ml of culture medium. Higher cell concentrations resulted in clot formation and reduced homogeneous distribution of cells throughout the kidney. High pressure perfusion of cells through the vasculature (VHP) resulted in only a few cells settling in the capillaries of the glomerulus in the cortex region. Perfusion of cells through the ureter under high pressure (UHP) resulted in significant damage to the cell membranes. Perfusion of the cells through the ureter under moderate vacuum (UMV at 40 mmHg) produced the most uniform distribution of cells throughout the kidneys. We suggest 4-5 million cells/ml of culture medium perfusion through the ureter under moderate vacuum as the best procedure for complete and uniform recellularization of porcine kidneys.

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Engineered Trachea with Mucosal Epithelium

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Goal: The overall goal is to engineer a viable, functional trachea replacement tissue (''neotrachea'') for large segmental tracheal defects. The current goal is to develop a method to line neotracheas with functional mucosal epithelium.

Methods: Epithelial cells were obtained from rabbit tracheas incubated with 0.1% pronase and 20 units DNAse in Joklik's medium and expanded on collagen I-coated plates. For air/liquid differentiation, cells were cultured on collagen type IV-coated 12 mm Millicell inserts in BEGM medium. Sheets were fixed in 2% glutaraldehyde and o1% smium for transmission (TEM) and scanning electron microscopy (SEM); the SEM samples were critically point dried. Sheets were fixed in formalin and either flash frozen or embedded in paraffin for immunostaining. Sections were incubated in antibodies to polyglutamate, pan-cytokeratin, cytokeratin-14, or mucin, followed by biotinylated secondary antibody and peroxidase-streptavidin, and visualized with the Vector VIP Peroxidase Substrate Kit.

Results: SEM data showed that cultured mucosal cells had formed ciliated cells by 14 days in culture. TEM images confirmed the presence of ciliated cells, showed mucosal-like cells and a pseudostratified epithelium that included a basal cell-like morphology. Pan-cytokeratin staining confirmed the epithelial nature of the sheets, while the polyglutamate localization showed the presence cilia-specific tubulin. The cytokeratin-14 results confirmed the presence of basal cells observed with TEM and, similarly, the mucin staining confirmed the presence of mucosal cells.

Conclusions: The results demonstrate a methodology for isolating, expanding and differentiating rabbit tracheal epithelial cells, *in vitro*, into sheets containing ciliated, mucosal and basal cells.

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Modular Bioreactor with a Simple and Intuitive Touch-Screen Supervisory with Flow/Temperature/pH/Stirring Capabilities

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Bioreactors are a broad category of devices used in the areas of bioprocessing and tissue engineering/regenerative medicine, to improve, refine or optimize a biological process.

We have developed a modular bioreactor system capable of simultaneous control, monitoring and data logging of different parameters, through the central Supervisory software (Cairo™, Eva Scientific, São Paulo, Brazil), useful for tissue bioreactor purposes. Scalability occurs in multiples of four, with no limit on the number of tissue/organ chambers. Flow is controllable at a range of 0.36-2900 mL/min, with a simple configuration of steady state, ramp-based or pulsatile flow patterns, e.g. for cardiovascular tissue engineering. Stirring is controllable at a range of 100-1500 RPM, with steady state, ramp-based or pulsatile programs, with different impeller designs available for different flow requirements. Temperature is controllable by ramps or at a steady state at a range of 4 °C above ambient up to 50 °C. Flow, stirring and temperature all benefit from Monitoring and Data logging functionalities, within the same Supervisory responsible for the Control functionalities. The fourth parameter, pH, is not controllable in the present embodiment, but allows Monitoring and Data logging, like the other parameters, across a pH range of 0-14.

The system is suitable for a wide variety of applications in tissue engineering. So far, we have tested the system with whole kidney,

liver and trachea decellularization and recellularization setups, in addition to cardiac and lung physiological flow setups.

Kaasi & Jardini (2016) Bioreactors.

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In vitro culture of Spermatogonial Stem Cells in Klinefelter syndrome: a step forward to generate fertility

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Klinefelter Syndrome (KS) is characterized by masculine phenotype, supernumerary sex chromosomes (47 XXY) and impaired fertility due to loss of spermatogonial stem cells (SSC) at puberty. To understand this process and find new therapies it is critically important to develop a culture system for KS testicular cells.

Our current method of SSC isolation and propagation have been adapted to neonatal mouse using 40 XY 3-day old mice. Propagated cells were characterized using qPCR, digitalPCR and Flow Cytometry. The same optimized system was used to isolate and propagate testicular cells from UCLA 41XXY (KS) mouse model and from human KS cryopreserved testicular tissue.

Preliminary data culturing KS mouse testicular cells showed viable testicular cells with 10 folds increase up to 116 days. Characterization of propagated KS testicular cells by qPCR confirmed the presence of undifferentiated SSC. Preliminary data culturing KS human testicular cells showed viable testicular cells with 17 folds increase up to 80 days in culture and presence of spermatogonial cell at least in first 40 days of culture.

Ongoing work is focusing on full characterization of the cell populations present in KS testicular cells culture and ultimately testing their potential to restore fertility.

With this study we were able to overcome the quiescent stage of neonatal testicular germ cells from both XY and KS mouse to successfully expand them in culture while conserving their characteristic gene expression. Preliminary data proofed the reproducibility of this method in human KS tissue, which may lead to new therapeutic options for KS patients.

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Controlled Delivery of Stem Cell-derived Trophic Factors To Treat Acute Kidney Injury

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Renal disease is a worldwide health issue. Besides transplantation, current therapies revolve around dialysis, which is limited to delaying disease progression through filtering metabolic wastes in blood. However, dialysis is unable to replace other renal functions, such as synthesizing erythropoietin. To address these limitations, cell-based approaches have been proposed to restore damaged kidneys as an alternative to current therapies. Particularly, recent studies have shown that stem cell-derived secretomes could enhance tissue regeneration. This study explores a gel-based delivery system for controlled delivery of trophic factors secreted from human placental stem cells (hPSC) (conditioned medium: CM) and evaluates the effect of trophic factors on renal regeneration. *In vitro* cell viability and proliferation assays demonstrated that CM treatment significantly enhanced cell proliferation when compared with the control without CM. Platelet-rich plasma (PRP) was used as a delivery vehicle for CM. To test the feasibility of controlled delivery, CM was encapsulated within the PRP, followed by assessing the release kinetics of CM from the gel. The release profiles show that CM can be released from PRP in a controlled manner by altering gel stiffness. An *In vivo* study using a rat acute kidney injury model showed that CM delivery using the gel system into the injured kidney tissue facilitated less renal tissue damage, leading to rapid functional recovery than that of saline, CM or vehicle only group. These results suggest that the delivery of hPSC-derived trophic factors in a controlled manner may contribute to efficient kidney repair from renal tissue injury.

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Mesenchymal Stem Cells versus Steroids in Inflammatory Bowel Disease

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Introduction: Inflammatory Bowel Disease (IBD) is a chronic disease without a medical cure, requires a lifetime care. IBD includes range of intestinal pathologies as Crohn's disease (CD) and ulcerative colitis (UC). The idea of treatment of IBD patients with MSCs came into mind accidentally after reporting remission of IBD in a woman with 20 years CD who was treated with chemotherapy and autologous bone marrow stem cells for non-Hodgkin's lymphoma, 6 months later she was free of CD.

Materials and Methods: 72 female albino rats were divided into 4 groups; control, Dextran Sulphate Sodium (DSS) induced IBD, DSS rats treated with MSCs and DSS rats treated with steroids. Bone marrow was harvested by flushing the tibias and femurs of 10 healthy male albino rats. During the duration of the experiment, Living animals were assisted for weight loss and occult blood in stool. Biochemical tests for IBD markers, colon length measuring beside histological and immunohistochemical examinations were done. Flowcytometry and PCR were done to insure homing of right cells vehicle.

Results: IBD markers were highest at DSS untreated group and steroid treated group. PCNA immunoreaction marked significant colonic cell proliferation in MSCs treated group. MSCs succeeded to restore normal colon length and avoid strictures. Notable avoidance of weight loss and stool occult blood were recorded.

Conclusion: - Steroids have limited effects on complicated IBD. - MSCs are capable of homing at sites of bowel inflammations. - MSCs carry hope for resistant complicated cases of IBD.

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Modeling Early Granuloma Formation In Engineered Human Lung Tissue Using Mycobacterium Smegmatis

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The granuloma of human Tuberculosis (TB) infection is an organized structure of infected and non-infected macrophages, leukocytes and fibroblasts. Virulent Mycobacterium tuberculosis (Mtb) containing RD1 and ESAT-6 loci produce human lung granulomas. *In vitro* granulomas have been produced using pulmonary blood mononuclear cell (PBMC) systems and virulent Mtb or avirulent

mycobacteria. Whether *in vitro* PBMC granulomas produced using avirulent bacteria are merely cellular aggregates or whether they recapitulate key features of Mtb granulomas is unknown. Early granuloma formation has been studied by adding Mtb infected PBMCs to an *in vitro* Transwell model of human lung mucosa containing epithelial and fibroblast cells. Although this model has several advantages, it does not accurately mimic the distal lung microenvironment, where dynamic strain and blood flow are found *in vivo*. We hypothesize that mechanical strain and perfusion forces could significantly affect granuloma formation and maintenance. Towards elucidating these effects, we have performed preliminary experiments utilizing THP-1 monocytic cells and avirulent Mycobacterium smegmatis (M. smegmatis). THP-1 cells and M. smegmatis were encapsulated within silk-collagen-type I hydrogels and viability was assessed using CellTracker dyes and confocal microscopy. At a lower multiplicity of infection (MOI) of 1:1, THP-1 derived macrophages killed most bacteria and at a higher MOI of 10:1, fast multiplying M. smegmatis resulted in a higher bacterial burden. Future work will include conducting infections using Mtb within an established *in vitro* lung tissue model that recapitulates the distal human lung microenvironment with strain and perfusion forces.

Skin Regeneration and Immunomodulation

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The Superiority Of The Autografts Inactivated By High Hydrostatic Pressure To Decellularized Allografts For Dermal Reconstruction In A Porcine Model

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In Japan, the JACE® cultured epidermal autograft (CEA) was approved and covered by public healthcare insurance for use in the treatment of giant congenital melanocytic nevus (GCMN) in 2016. We are developing a novel skin regeneration therapy in which the inactivation of nevus tissue via high hydrostatic pressure (HHP) is used in the dermal reconstruction in combination with CEA. In this study, we used a porcine skin graft model to explore whether autologous skin including cellular debris inactivated by HHP or allogeneic skin decellularized by HHP is better for dermal reconstruction. Grafts ($n=6$) were prepared for 5 groups each: autologous skin without pressurization group (control group), autologous skin inactivated by 200 MPa group, autologous skin inactivated by 1000 MPa group, allogeneic skin decellularized by 200 MPa group, and allogeneic skin decellularized by 1000 MPa group. All of the grafts at 1, 4, and 12 weeks showed complete engraftment macroscopically. The mean areas of the grafts of the control group and autologous 200 MPa group were larger than that of the allogeneic 1000 MPa group at four weeks after implantation. The thickness of the control group and autologous 200 MPa group was comparable, and that of the autologous 200 MPa group was significantly thicker than that of the allogeneic 200 MPa group. Our result suggests that the autologous dermis was superior to the allogeneic decellularized dermis as a skin graft, and that HHP at 200 MPa provided a better outcome than HHP at 1000 MPa.

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Poly(Diol Fumarate) Microparticles Restore Wound Healing in a Neutropenic Murine Model of Cutaneous Fungal Infection

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Introduction: Cutaneous fungal infection is a challenge to treat in immunocompromised patients. We have recently described poly(decanediol fumarate) (PDF), a biodegradable aliphatic polyester whose degradation products have potential antifungal (decanediol) and immunomodulatory (fumarate ester) properties. In this work, PDF microparticles were loaded with a traditional antifungal therapeutic (voriconazole), characterized, and injected into a cutaneous wound infected with *Aspergillus fumigatus* in a neutropenic mouse model to measure subsequent wound healing and fungal burden.

Materials and Methods: Briefly, microparticles were fabricated by oil/water emulsion and *in vitro* drug release was assayed. A 6 cm diameter wound inoculated with *A. fumigatus* (or saline in control animals) was treated with loaded microparticles, non-loaded microparticles, or saline 72 hours after inoculation ($n=10$ mice/group). Six days after treatment, animals were euthanized. Wound surface area size, fungal CFU count, and histological presence of fungi were measured.

Results: Mice treated with loaded microparticles had significantly greater rates of wound healing than non-treated mice ($p=0.002$) and no significant differences were observed between blank and loaded microparticles compared to non-infected controls ($p=0.497$ and $p=0.947$). Although wounds treated with microparticles had less fungal burden, these results were not significant ($p>0.05$).

Discussion and Conclusion: PDF microparticles can restore normal rates of wound healing in a neutropenic murine model of cutaneous fungal infection. As fungal burden was not significantly reduced, the mechanism of this healing may be due to reduced inflammation due to immunomodulatory effects of released fumarate esters on the Nrf2 stress response pathway rather than direct fungal toxicity.

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Identification of Immunosuppressive (IFNgamma-Stimulated) MSC Morphological Subpopulations Using viSNE, A Tool for Visualizing Cellular Heterogeneity

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Introduction: In many mesenchymal stromal cell (MSC)-based clinical trials, common markers of MSCs do not adequately predict *in vitro* or *in vivo* responses. Therefore, improved methods to identify MSC quality attributes are needed. We previously developed techniques to identify population-based morphological features predictive of MSC osteogenic and immunosuppressive capacity and sought to expand upon these methods by quantitatively assessing heterogeneity based on unique morphological subpopulations (SPs).

Methods: Morphological data from images of $\sim 3\times10^5$ single cells was uploaded to Cytobank and viSNE (visual stochastic neighbor embedding) was performed. SPs were identified for each IFNγ concentration and both SP frequency and total cell numbers were determined for each MSC sample and correlated with their overall immunosuppressive capacity.

Results and Discussion: viSNE permitted visualization of high dimensional morphological data, which allowed for identification of distinct SPs using a contour density plot. Representative cells from different SPs illustrate the ability of viSNE to identify distinct morphological phenotypes. Using viSNE, we were able to identify morphologically-distinct SPs following IFNγ stimulation, which demonstrated donor and passage-dependent differences in SP frequency and number. The immunosuppressive capacity of MSCs was significantly correlated with one SP identified used viSNE (SP9) and MSC samples with greater immunosuppressive capacity possessed greater numbers of SP9 cells ($p<10^{-10}$). This approach could be used as a predictive measure of MSC immunosuppression and could also be used as a tool for both identifying molecular markers to enrich for desired functional SPs, as well as optimizing manufacturing conditions to improve MSC functional characteristics.

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Estimation Of Cell Death Related To Skin By High Hydrostatic Pressure

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High hydrostatic pressure (HHP) technology is a physical method for inactivating cells and tissue. Previously, we have reported that skin and nevus specimens were inactivated after HHP of 200 MPa for 10 minutes. The inactivated nevus could then be used as autologous dermis for covering skin defects. However, the estimated cell death by HHP of 180-200 MPa or the duration of time needed for skin cell inactivation remains unclear. In the present study, we evaluated the time and pressure necessary for complete skin cell inactivation as well as cell-death pathways. We prepared the following four human cell types: dermal fibroblasts, adipose tissue-derived stem cells, malignant melanoma cells, and squamous cell carcinoma cells. We applied HHP of 150, 160, 170, 180, and 190 MPa over three different exposure durations (1 second, 2 minutes, or 10 minutes). To determine cell viability, we performed LIVE/DEAD assay immediately after re-pressurization and WST-8 assay after culturing for 3 hours, 1 day, 3 days, and 7 days. In addition, we performed apoptosis assay using Annexin V. Results revealed that at HHP >170 MPa, cells were not inactivated after an exposure of 1 second or 2 minutes but were completely inactivated after an exposure of 10 minutes. Cell-death pathways were predominantly necrosis. Therefore, HHP >170 MPa for 10 minutes completely inactivated four kinds of human cells.

Stem Cells

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A Computational Model of the Hematopoietic Stem Cell Niche Microenvironment

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The bone marrow microenvironment provides a complex set of extrinsic signals that regulate hematopoietic stem cell (HSC) biology and consequently the production of the body's full complement of blood and immune cells. An artificial marrow would have significant value for expanding HSCs or to study the onset and treatment of hematologic diseases. The challenge consists of identifying key niche elements among the large number of niche parameters influencing stem cell fate. Here we describe a biology-driven computational approach for defining stem cell kinetics in culture in order to gain critical insight regarding culture conditions and intercellular signaling networks and to distill the biological complexity to its essential elements. Through a combination of experimental and *in silico* methods, we examine the balance between self-renewal and differentiation that drives early and late hematopoietic progenitors. We investigate the importance of dynamic feedback associated with cell-secreted biomolecules on lineage specification events in early progenitor populations and demonstrate that changing this feedback alters cell fate. Our analysis suggests that accurately modeling the development of mature phenotypes requires direct contributions from early progenitor populations via differentiation along with the traditional intermediary oligopotent progenitors. While consistent with *in vivo* results, this suggests a revision of our understanding of *in vitro* HSC lineage development and in our approach for engineering optimal culture conditions for HSC biomanufacturing in targeted clinical applications. Finally, we demonstrate the application of the computational model as a predictive tool to identify culture parameters particularly sensitive for regulating HSC proliferation and myeloid lineage specification events.

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Role of Adipose-Derived Stem Cells on Vasculogenesis in 3D Co-Culture

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Adipose-derived stem cells (ADSCs), which can differentiate into adipocytes, chondrocytes, and osteocytes, have been shown to influence wound healing, however much is unclear about the role these cells play in neovascularization. Previous work has shown that ADSCs may be stimulated to express CD31, an endothelial cell surface marker, using EGM2 media. Clinically, techniques such as fat grafting have been used to help promote wound healing. However, understanding the influence of ADSCs on neovascularization may provide novel avenues for stem cell therapy in treating ischemic conditions, such as the chronic non-healing wounds seen in diabetic patients. Utilizing three-dimensional *in vitro* models may provide insight into the *in vivo* cell-cell interactions between ADSCs and vascular endothelial and support cells. We sought to understand the relationships between human derived ADSCs and human endothelial and pericyte cells through co-culture in a cell-adhesive, proteolytically degradable poly(ethylene) glycol-based hydrogel. ADSCs cultured in hydrogels alone were stimulated using EGM2-media and showed increased proliferation and cell spreading, compared to ADSCs cultured in hydrogels and stimulated with ADSC growth media. Co-culture of ADSCs and human endothelial cells in EGM2 media showed the formation of tubule-like networks after 7 days. Additionally, co-culture of ADSCs and human vascular pericyte cells in EGM2 media showed the formation of tubule networks after 7 days. Our findings suggest that ADSCs contribute to neovascularization and may be stimulated to adopt endothelial cell type functions in the appropriate environment.

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A Novel *in Vitro* Model of Human Urothelium with Barrier Function Differentiated from Urine-Derived Stem Cells

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Purpose: An *in vitro* model of human urothelium can provide a multipurpose tool for assessing the effect of drugs on urothelial permeability barrier function and for studying intrinsic alterations in the urothelium of the patients with dysfunctional bladder diseases, cancer, and infection. The objective of this study is to fabricate *in vitro* biological model of human urothelium with permeability barrier properties using urine-derived stem cells (USCs), which can be used to study barrier physiology, disease pathogeneses, and bladder drug efficacy.

Methods: Human USCs were harvested from 6 healthy individuals and then induced to differentiate into urothelial cells on a permeable membrane using five different culture conditions for up to 2 weeks. Non-differentiated USCs were used as a control.

Results: The differentiated USCs expressed urothelial-specific transcripts and proteins (Uroplakin-III, -Ia) as well as general epithelial cell markers (Ck7, Ck13, Ck20 and AE1/AE3). Urothelially-differentiated USCs expressed genes and protein markers (ZO-1 and E-cadherin) of the tight junction. *In vitro* assays using a fluorescent tracer on differentiated cells cultured on inserts demonstrated a 99% decrease in leakage over 3 hours, indicating that the induced USCs could perform barrier functions similar to those of normal urothelial cells. In addition, TEM confirmed the ultrastructural phenotypes of

urothelium differentiated from USCs, including tight junction formation between neighboring cells.

Conclusions: The present study demonstrated that human urothelium differentiated from USCs can efficiently generate urothelium with intact permeability barrier function, which can be used *in vitro* to study urothelial abnormalities present in various urinary disorders.

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Spheroid Culture Combined with 5-Azacytidine Treatment Alters the Developmental Potency of Adipose-Derived Mesenchymal Stem Cells

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Adipose-derived stem cells (ADSCs) are multipotent mesenchymal stem cells located within the microvasculature of adipose tissue. Although ADSCs can differentiate into several cell lineages, they cannot match the differentiation potential of pluripotent stem cells such as ES and iPS cells. The goal of this project is to develop culture conditions that enable ADSCs to more readily differentiate into the myogenic lineage for use in tissue engineering and regenerative medicine applications aimed at restoring damaged and/or diseased skeletal muscle tissue. Previous research in our lab suggests that culturing murine ADSCs as three-dimensional spheroids can induce the expression of genes associated with pluripotency. We hypothesized that the combination of culturing ADSCs as three-dimensional spheroids and treatment with the DNA demethylating agent 5-azacytidine would 1) upregulate the expression of genes associated with enhanced developmental potency and 2) improve the efficiency of myogenic differentiation by ADSCs. Through the analysis of ADSC gene expression via real-time PCR, we found that culturing ADSCs as spheroids in combination with treatment with 5-azacytidine impacts the expression of genes associated with developmental potency and myogenic differentiation. This suggests that these conditions can be used to prime ADSCs for myogenic differentiation. Future work will include further optimization of culture conditions to maximize the myogenic potential of ADSCs. This would allow ADSCs to serve as a plentiful and easily-obtained source of myogenic cells for skeletal muscle tissue engineering and regenerative medicine applications.

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Comparison of Donor-Matched Amniotic Fluid and Placental Mesenchymal Stromal Cells

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Mesenchymal stromal cells (MSCs) are an ongoing focus of cell therapy treatments due to their ability to modulate inflammatory responses, promote angiogenesis, and protect neural tissue. MSCs can be derived from a number of tissue sources including fetal cells from both the placenta and amniotic fluid. There is much debate about the best source of cells to be utilized for stem cell therapy; however, the significant variability among cells from different donors causes difficulty in accurately characterizing the similarities and differences among MSCs derived from various tissues. In this study, MSCs were derived from chorionic villus placenta stromal tissue (PMSCs) and amniotic fluid (AF-MSCs) collected from the same donor, and assessed for growth kinetics, ability to differentiate, secretome, and their functional capacity to promote endothelial progenitor cell tube formation when indirectly co-cultured on matrigel. Our data suggests that PMSCs have greater secretion of immunomodulatory cytokines and angiogenic growth factors than AF-MSCs, but grow less rapidly and have less secretion of neurotrophic proteins. Both cell lines appear to support lasting tube formation at 72 hours, but placental cells show a trend of increasing early tube formation at 4 hours as well. Despite these differences, overall the two cell sources are similar, and therapeutic potential will likely be dependent on the unique clinical need.

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Non-Invasive Cell Tracking with Brighter and Red-Transferred Luciferase for Potential Application in Stem Cell Therapy

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Propose: It is critical to monitor the fate of the grafted cells after implantation and to examine the progress of the implanted cells over time for improving the efficiency of cell therapy. The goal of this study was to determine the safety and efficacy of a novel cell labeling technology on *in vitro* biological function of human placental stem cells (hPSCs) labeled with a red fluorescent protein variant mKATE and a new codon-optimized luciferase from Renilla Reniformis (mKATE-renLUC) using a lentivirus vector. Our methodology is intended to establishing this duel labeling technology for *in vivo* bioluminescence imaging to track the implanted stem cells in the treatment of erectile dysfunction.

Methods: To optimize the transfection protocol, different cell concentrations of hPSCs, types of vectors (mKATE-renLUC, GFP-FLUC), and titers of lentivirus were tested.

Results: The cell viability and cell proliferation of mKATE-renLUC labeled hPSCs were similar to those of non-labelled cells *in vitro*. The cell survival and migration of renLUC-labeled hPSCs were efficiently monitored using IVIS imaging system after these cells were implanted into corporal tissue using an erectile dysfunction athymic rat model. The mKATE-labeled cells were brighter than the GFP-FLUC, which was confirmed within the host penis tissue five days after injection using a fluorescence microscope.

Conclusions: This study demonstrated that the duel labeling technology using mKATE-renLUC provides a safe and effective cell tracking approach with brighter fluorophores and codon-optimized luciferase for stem cell therapy in the treatment of erectile dysfunction allowing us to monitor injected cell noninvasively *in vivo*.

Tissue Chips & Tissue Organoid Models

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Characteristics of Co-cultured Hepatocyte Spheroids in a Microwell Chip Culture

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Hepatocyte spheroid (three-dimensional cell aggregate) culture is a promising technique for tissue engineering and drug screening studies. In this study, we constructed a co-cultured spheroid, which combined hepatocytes with other cells, and evaluated the effects of co-culture on the expression of hepatocyte functions. As a culture platform for generating homogeneous spheroids, we fabricated a culture chip which contained the microwells (500 μm in diameter) on a culture substratum, and its surface was modified with poly-ethylene glycol to create the non-adhesive area. In this study, three kinds of cells, primary rat hepatocytes (Hep), human endothelial cells (HUVEC), and human mesenchymal stem cells (MSC) were used for the spheroid culture, and the spheroid properties were compared in the Hep-spheroid, Hep/HUVEC-spheroid, and Hep/MSC-spheroid conditions. The spheroid formation was promoted with the presence of HUVECs or MSCs. Although the HUVECs scattered within the spheroids, the MSCs coagulated in the center of spheroid. Furthermore, the abilities of protein synthesis and drag metabolism in the co-cultures (Hep/HUVEC or Hep/MSC-spheroids) were higher than those of mono-culture (Hep-spheroid), and especially the functions of Hep/MSC-spheroids were highest under all conditions. These results indicate that the co-cultured spheroids are a promising

technique for the maintenance of hepatocyte functions, and the combination of cell species is important factor for modulation of cell functions.

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Sequential Formation of Vascularized Optogenetic Muscle Tissue in a Micro Device

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Capillary networks surrounding muscle tissue play an important role to not only supply oxygen and nutrients but also to regulate the myogenesis and repair of surrounding skeletal muscle tissues. In spite of this crucial role, strategies of engineering vascularized muscle tissue *in vitro* have thus far been limited. Therefore, engineering vascularized muscle *in vitro* is a high priority for developing transplantable muscle tissues and understanding the pathogenesis of muscle related disease such as muscular dystrophy and *sarcopenia.*

In this study, we model the early stages of 3D vascularized muscle fiber formation *in vitro* using a sequential molding technique to investigate interactions between angiogenesis of endothelial cells (EC) and myogenesis of skeletal muscle cells. Channelrhodopsin-2 induced C2C12 skeletal muscle fiber and 3D vascular structures (ϕ500 μm) were formed at 500 μm intervals in collagen gel. After several days of culture, endothelial cells exhibited angiogenesis, sprouting into the gel and forming robust capillary networks. Additionally, muscle fiber bundle augmented EC sprouting, regulated by the secretion of angipoietin-1 from muscle cells. Myogenesis was also upregulated by the vascular networks, improving muscle contraction via angiopoetin-1/neuregulin-1 signaling. Moreover, continuous training of muscle tissue by optical stimulation significantly induced angiogenesis. We believe this *in vitro* model could be used to help understand the formation of vascularized muscle tissue and testing the interaction between muscle contraction and angiogenesis for tissue engineering and regenerative medicine.

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Organoid Culture of Mesenchymal Stem Cells for Organ Function Regeneration

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Cell therapy using mesenchymal stem cells (MSCs) have been increasing in several degenerative diseases. Even though ongoing research is attempting to the application of MSCs to the clinical trials, further understanding on their efficacy and optimal condition for therapeutic effect are required. Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological functions of tissues. We developed three-dimensional spheroid cell culture system on the hemispherical concave microwell chips for tissue regeneration, for cells in 3D may better mimic the conditions and properties of our body than 2D culture. First, we reported that tonsil-derived mesenchymal stem cells (TMSC) are successfully differentiated into parathyroid hormone (PTH)-releasing cells, demonstrating the therapeutic role in hypoparathyroidism. Scaffold-free TMSC spheroids were engineered in concave microwell plates made of polydimethylsiloxane in control culture medium for the first 7 days and differentiation medium for next 7 days. Second, we present the therapeutic effect of placenta-derived mesenchymal stem cells (PD-MSCs) on a rat model with ovariectomy and compare their effects on the ovary dysfunction according to conventional monolayer 2D culture system and 3D spheroid culture system, restoring ovary function through increased estrogen production as well as gene expressions for folliculogenesis by spheroid forms of PD-MSCs. These findings suggest new insights into further understanding of stem cell-based therapeutic mechanisms for organ function regeneration and should provide new avenues to develop more efficient therapies using biomaterials and new 3D organoid culture system.

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Development of Curved and Branched Blood Vessels Based Microphysiological System to Model Atherosclerosis

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The pathology of many diseases, such as inflammation, atherosclerosis, and autoimmune disorders, is intricately linked with the interaction between immune cells and blood vessels. Tissue constructs engineered from human cells have become important *in vitro* models for disease modeling and drug screening. In this work, we aim to develop an *in vitro* tissue model to study the dynamic relationship between immune cells and blood vessels. We fabricated tissue-engineered blood vessels (TEBVs) from human cells, and engineered a dual-function bioreactor to support long-term culture and facilitate direct real-time imaging of the TEBVs. This perfusion and imaging system was combined with two-photon microscopy to monitor the bioreactions in TEBVs, such as leukocyte adhesion and trans-endothelial migration within the lumen of the TEBVs. The addition of inflammatory cytokines activated the TEBV endothelium, which led to the enhanced adhesion and transmigration of monocytes. We also fabricated branched TEBVs and curved TEBVs with 3D bio-printing, and corresponding bioreactors to study flow induced endothelial activation, and the initiation of atherosclerosis in TEBV. These TEBVs offer a more biomimetic model than conventional systems to study immune cell-blood vessel interactions, atherosclerosis, and potentially cancer metastasis.

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Hydrodynamically Controlled Fabrication of Three-dimensional Microvasculature for Engineering Thick Vascularized Cardiac Tissues

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A major challenge that hinders the practical application of engineered tissues is the inability to form thick, viable tissues. Current methods for manufacturing vascularized cardiac tissues fail to produce large tissue constructs with thickness great than 2 mm.[1,2] Here, we report the fabrication of free-standing, human microvessels with organized heterotypic cell layers using microfluidic method employing hydrodynamic focusing. These microvessels incorporate human umbilical vein endothelial cells (HUVECs) on the luminal surface and human aortic smooth muscle cells (HASMCs) in the wall, respectively, emulating the natural architecture and functions of small diameter blood vessels. After integration in a three-dimensional collagen matrix, the microvessel perfusion is examined by using fluorescent microbeads and the permeability coefficient of microvessel is quantified by using FTIC-dextran. The angiogenic sprouting process is monitored at different time points using confocal laser scanning microscopy. Moreover, the response of the endothelial cells in the microvessels to the mechanical stimuli induced by flow rate change is investigated. The fabrication of human microvessels using hydrodynamic focusing allows for the assembly of engineered tissue constructs in a facile and modular manner. We plan to assemble a thick vascularized cardiac tissue (thickness ≥1 cm) by integrating cardiomyocytes and human microvessels within an extracellular matrix, and investigate the effect of perfused microvasculature on the growth of cardiomyocytes.

Cardiovascular

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Three Dimensional Fabrication of Vasculature with Tropoelastin

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The development of synthetic vasculature is one of the most fundamental yet unmet challenges in clinical regenerative medicine. Current vascular replacement therapy solutions have poor long term patency because they are engineered from materials which cannot adequately emulate the complex functional interplay between mechanical strength, elastic compliance, and biological signalling that is inherent to natural vascular tissue. Elastin is a protein native to vascular extracellular matrix and imparts the appropriate mechanical elasticity and cell signalling properties. Elastin itself is predominantly made from tropoelastin, which has been demonstrated to be an attractive material for vascular engineering due to its ability to reduce thrombogenicity, promote endothelialisation, and to remain conveniently solid during fabrication yet reverting to its natural elastic state when hydrated. Using recombinant human tropoelastin, we have generated a range of freestanding vascular architectures including straight grafts, branching grafts, aortas, and simple vascular beds, achieving fine control over physical design and architecture. Due to the unmet need, our primary focus is to create small vessels of typically 4 mm internal diameter. Our *in vitro* studies validated tropoelastin grafts and show that they support vascular endothelial cell proliferation. Concurrently, we also demonstrated the robustness of the fabrication process by combining tropoelastin with polycaprolactone, enhancing elastic stiffness and burst pressure to emulate the corresponding biological equivalent, and improving suture retention for surgical handling. Data will be presented that validate these foundational results *in vivo* in an ovine carotid artery interposition model to demonstrate the superiority of tropoelastin as a material for vascular engineering.

Biofabrication and Bioreactors

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Application Case Study All-in-one Bio 3d Printer, Invivo From Rokit Inc

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For about recent a year, approximately 70 customers purchased our all-in-one 3D printers for mostly their research purposes. For the last a year, we can feel sudden increasing movement of tissue engineering and biofabrication research using bio 3D printers and we are introducing its applications case studies through the consulting with our customers.

Cell bio-printings with bio-inks is a one big category. Vascularized kidney, cardiac myocyte regeneration, Neuron regeneration for dementia patients, liver function regeneration, Oncology using target therapy, and pancreatic cell regeneration are proposed for tissue engineering researches using our product of 3D bio printers. All the cases include live cell as a printing bio-materials and consist with scaffold design.

Second application category is human skin equivalent (HSE). Skin is relatively easier than other organs for fabrication sue to is structure. Dermis and epi-dermis are not containing large vascular, so many researchers are working on this topic, HSE. One third of our customers were also purchased our 3D bio printers for human skin equivalent. The final applications for HSE are cosmetics test for substitution of animal tests and artificial skin for wound healing for burnt patients.

Third application cases are bone and teeth, hard tissue application. Not just simple or uniform shape of bone-replace scaffold, non-uniform, which is gradually increasing pore size toward outside. This structure may prevent clogging or fouling on the surface and cannot fill the designed structures. In fact, more than half of the studies related to bio-implant is recently focused on scaffolds' internal structure design.

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Optimization of Hydrogel-based Bioink Printability using Rheological Parameters: A Systematic Approach

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The dearth of suitable bioinks is frequently cited as a major limiting factor for the advancement of bioprinting technology. It is well known that the viscosity of a bioink has a direct impact on its printability. Highly viscous materials maintain their structure, but also have decreased cell viability due to high extrusion forces. Complex viscosity is comprised of two discrete components, storage (G') and loss (G'') modulus and their individual contributions to printability are unknown. For this study, gelatin and alginate mixed at various concentrations to obtain hydrogels with a wide range of storage and loss moduli. These hydrogels were then evaluated for the quantitatively defined values of extrudability, structural integrity, and extrusion uniformity. While neither loss nor storage moduli alone were excellent predictors of printability, a lower loss tangent (G''/G') correlated with increased structural integrity while a higher loss tangent correlated with increased extrusion uniformity. Hydrogels with a loss tangent in the range of 0.3 to 0.65 exhibited an excellent compromise between structural integrity and extrusion uniformity. For extrudability, increasing either the loss or storage modulus increased the pressure required to extrude the bioink. A mathematical model relating the G' and G'' to the required extrusion pressure was derived based on this data. Using this approach, a variety of different bioink formulations can be quickly and accurately evaluated for printability. Immediate future work will incorporate cell viability studies to further define printability and will examine additional hydrogels to determine the generalizability this evaluation methodology for the printability of bioinks.

Biomaterial Scaffolds

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Dual Ionic Cross-linked Interpenetrating Network Of Alginate-cellulose Beads With Enhanced Mechanical Properties For Biocompatible Encapsulation

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Spherical hydrogels ('micro beads') made from alginate has been explored and used for biomedical application, delivery of biomolecule and cell transplantation, although restricted mechanical strength and short-term structural cohesion. In this study, controlling mechanical properties and maintaining the long-term structural integrity of spherical hydrogels could be achieved by creating interpenetrating networks (IPN) of alginate and aqueous-soluble cellulose with divalent and trivalent ions. By using various the aqueous-soluble cellulose, with varying concentration of cellulose or types of ions, the moduli of the resulting hydrogels could be controlled in a wide range with a simple yet highly efficient method of engineering alginate beads. We found that processing a dual sequential ionic crosslinking scheme to create IPN of alginate and cellulose with divalent and trivalent ions has synergistic effect to increase moduli and structural stability of hydrogels. Also the aqueous-soluble cellulose is found to undergo crosslinking reaction with trivalent ions more favorably than divalent ions. Especially, the aqueous-soluble anionic cellulose, containing either carboxylate or sulfonate, is easier to control mechanical properties than other types of celluloses. As a result, the IPN alginate-cellulose beads demonstrate enhanced resistance to harsh chemical environment as compared to alginate beads and

suitability for biomedical applications by encapsulating microbial species and therapeutic agents for controlled release.

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Polyaspartamide-based Crosslinker For Controlling The Mechanical Properties Of Hydrogels

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Hydrogels are widely used as cell-culture platforms for various biotechnology applications. To tailor to specific needs, various modification strategies are employed to tune their properties. Herein, a multifunctional polymeric crosslinker based on polyaspartamide is developed, which allows for the adjustment of the type and number of reactive functional groups to fit different reaction schemes and control the mechanical properties of the hydrogels. The amine-based nucleophiles containing desired functional groups are reacted with polysuccinimide to synthesize polyaspartamide crosslinkers. The crosslinking density and the concurrent change in mechanical and properties of the resulting hydrogels are controlled in a wide range only with the degree of substitution. Furthermore, the polyaspartamide crosslinker linked with cell-recognition molecules via the same conjugation mechanism (i.e. nucleophilic substitution) render the hydrogels cell responsive without having to additional processing steps. This polyaspartamide-based crosslinker is expected to provide an efficient and versatile route to engineer hydrogels with controllable properties for biomedical applications.

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3D Culture NSCs in Conductive Scaffold

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Neural stem cells (NSCs), as a self-renewing and multipotent cell population, have been widely studied for never regeneration. Engineering scaffold is one of the important factors to regulate NSCs proliferation and differentiation towards the formation of the desired cells and tissues. Because neural cells are electro-active ones, the conductive scaffold is required to provide three-dimensional cell growth microenvironments and appropriate synergistic cell guidance cues. In this study, two types of conductive scaffolds were obtained for three-dimensional culture of NSCs *in vitro*. A PEDOT-HA/Cs/Gel scaffold was developed by introducing PEDOT-HA nanoparticles into the chitosan/gelatin (Cs/Gel) matrix. HA, as a bridge, not only was used as a dopant, but also combined PEDOT into the Cs/Gel *via* chemical crosslinking. In addition, a poly (3,4-ethylenedioxythiophene)/chitosan/gelatin (PEDOT/Cs/Gel) scaffold was prepared *via in situ* interfacial polymerization, with a nanostructured layer of PEDOT assembling on the channel surface of porous Cs/Gel scaffold. The results demonstrated that these electrically conductive, three-dimensional, porous and biodegradable scaffolds could not only promote NSCs adhesion and proliferation but also enhance NSCs differentiation into neurons and astrocytes with higher protein and gene expression. They will be the promising conductive substrate for NSCs research and neural tissue engineering.

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Comparison of Cell proliferation to 3D Printed Gelatin Scaffolds by different Cross-linking Methods

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Three-dimensional (3D) printing is the powerful techniques in tissue engineering. Gelatin is highly biocompatible and useful 3D printing material. However, gelatin scaffold requires a cross-linking treatment to maintain its structure. N-Hydroxysuccinimide (NHS) is a cross-linking agent that cross-links gelatin, however it has cytotoxicity. To improve its cytotoxicity problem, physical cross-linking method have been studied. We fabricated physically crosslinked 3D gelatin scaffolds by dried heat treatment (DHT). We have confirmed the surface morphology and chemical properties of 3D gelatin scaffolds by using SEM and FT-IR. Swelling degree and mechanical property of each scaffold was identified by water absorption and universal testing machine. The proliferation of human dermal fibroblasts (HDFs) in 3D gelatin scaffolds resulted in DHT scaffold 48% higher than chemical crosslinked 3D scaffold after 7 days of culture.

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The Promotion of Cell Proliferation Induced By Three-dimensional Scaffolds with Optimized Porosity

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Three-dimensional (3D) scaffolds in tissue engineering play an important role in regenerating defective tissue. In order to achieve these goal, the 3D scaffold should be highly porous and with an appropriate pore size, pore interconnectivity, and exhibit a high surface area-to-volume ratio. In particular, the pore size of the 3D scaffold is one of the important factors to determining the regeneration of the tissue as well as affecting the cellular behaviors. 3D printing technology is an appropriate method to fabricate pore size controlled 3D scaffold. In this study, we fabricated pore size controlled 3D gelatin scaffolds by using 3D printing with low temperature processing. The pore size of the 3D scaffold was designed to be 600 ~ 1,200 μm. The desired designed pore sizes of the 3D scaffolds were confirmed by the scanning electron microscopy (SEM) images. The SEM image shows that the pore sizes of the 3D printed scaffolds were reduced by approximately 20% compared to the design. To evaluate the cell proliferation, human dermal fibroblast (HDF) was cultured on the 3D scaffolds with various pore sizes. After 7 days of culture, the HDF on the 3D scaffold with pore sizes of more than 665 μm (G8) showed the higher proliferation rate than that of the 485 μm (G6). These results suggest that the pore size of the 3D scaffold is an important parameter to be considered in fabricating the 3D scaffold.

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Three-dimensional PEG Hydrogel to Probe Cytokine Role in VIC Myofibroblast Activation and Wound Healing Response

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Fibrotic aortic valvular stenosis (FAVS) is a disease marked by stiffening and reduced function of valve leaflets. Valvular interstitial cells (VICs) present within the leaflets transition between quiescent fibroblast and activated myofibroblast phenotypes to regulate homeostasis and repair local injury. The wound healing response initiates with recruitment of fibroblasts, pro-inflammatory cytokines, and growth factors to the injury. This is followed by increased extracellular matrix (ECM) deposition and contractility. FAVS arises when this process becomes dysregulated. To better understand factors that contribute to FAVS, a biocompatible 3D poly(ethylene glycol) (PEG)-based hydrogel platform was employed to investigate wound healing progression and contractility of encapsulated VICs. Hydrogels were prepared using a photoinitiated reaction between norbornene-functionalized 8arm 40kDa PEG macromolecules and thiol-containing enzyme-degradable crosslinkers at a modulus of 1kPa to permit cell spreading and interactions with the matrix via a cysteine-containing CRGDS adhesive peptide. Exogenously delivered cytokines, TGF-b and FGF-2, were introduced over a 10-day

culture period and the subsequent cell response assessed via contraction, gene expression (αSMA, CTGF, COL 1a, COL 3a, FN1), and immunostaining (αSMA, EdU proliferation). Treatment with FGF-2 resulted in increased proliferation while reducing the myofibroblast phenotype, as seen by decreased expression of both CTGF and αSMA. The addition of TGF-b increased the proportion of cells exhibiting the myofibroblast phenotype, increased ECM remodeling, and led to macroscopic contraction of the hydrogel (~20% reduction in surface area compared to control). Subsequently, this 3D culture system provides a unique *in vitro* platform to probe cell-matrix interactions and contractility with respect to FAVS.

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A Supercritical Foaming Method to Create Biodegradable Scaffolds Incorporating Autologous Growth Factors for Bone Regeneration

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Preparation rich in growth factors (PRGF) is an autologous cocktail of proteins obtained from platelet-rich plasma useful in tissue engineering due to the contained growth factors (GF) and cytokines that participate in several healing stages[1]. PRGF has been included in biodegradable scaffolds using a different methods such as electrospinning or foaming, and able to control cell recruitment, attachment and proliferation. In this work, highly porous poly(ε-caprolactone) (PCL) scaffolds containing PRGF were designed using supercritical CO_2 green technology and an architecture similar to bone matrix was tuned. Solvent-free scaffolds with open and interconnected porous structure were produced avoiding harsh conditions that could degrade or remove GFs contained in the PRGF. SEM micrographs revealed the presence of large pores in the structure of the scaffolds and fluorescent staining confirmed the homogeneous distribution of GFs. Sustained GFs release over 15 days had a positive effect on cell attachment and proliferation of mesenchymal stem cells in the scaffolds containing PRGF. Live-Dead staining also revealed the cytocompatibility of PCL-PRGF grafts. Results encourage the exploitation of these scaffolds for the induction of bone tissue formation with an expected higher efficacy in terms of GF-delivery, cost and success rate than current GF-containing devices clinically used.

Acknowledgments: MINECO (SAF2014-52632-R; BES-2012-051889).

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Biofabrication of Scaffolds for Human Induced Pluripotent Stem Cells

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Collagen scaffolds have excellent biocompatibility to most human cells, including human induced pluripotent stem cells (iPSCs). Nevertheless, weak mechanical strength and degradation nature of collagen hinder the use of collagen scaffolds for iPSC differentiation that lasts several weeks. poly (ethylene glycol) diacrylate (PEGDA) is a FDA approved biomaterial. The cytocompatibility of PEGDA treatment to iPSCs remains largely unknown. In this study, we investigated whether a physically stable and iPSC compatible collagen scaffold can be fabricated through PEGDA penetration treatment. We discovered that not only the content but also the molecular weight of PEGDA affects the stability and cytocompatibility of the treated scaffolds. Scanning electron microscopy analysis revealed the PEGDA treatment affects the diameters of collagen fibrils but not the pore size of the scaffolds. The determination of the shear storage modulus of the scaffolds suggested that high molecular weight PEGDA treatment results in more elastic and mechanically stable scaffolds and prevents scaffolds from shrinking in extended iPSC cultures. The Young's modulus of the PEGDA treated scaffolds varies from 110 to 5,500 Pa, suggesting the stiffness of the scaffolds can be finely tuned by PEGDA treatment. Furthermore, we discovered that the stiffness of 2 kDa PEGDA treated collagen scaffolds is about 2.79 kPa, which is close to the stiffness of human pancreatic tissues ranging from 1.15 to 2.09 kPa. This study implies that these collagen-based scaffolds can possess better mechanical cues for biofabricating pancreatic tissues from iPSCs. It will offer a new technology for generating various iPSC-derived tissues and organs.

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Engineered Phage Matrix Stiffness for Osteogenic Differentiation

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Although biochemical cues in extracellular matrices play a critical role in regulating cellular growth and fate, their physical cues as stiffness in guiding stem cells has not been well studied so far. Here, we have demonstrated engineered phage mediated matrix controlling stiffness for various applications and verified that osteogenic differentiation could be controlled by the constructed phage matrix. The stiffness of matrix was controlled by cross-linking the engineered phage displaying RGD and HPQ with different concentrations and compositions of streptavidin and polymer mixture. Our phage matrices, which can be easily functionalized by chemical/genetic engineering, may be used as a convenient tissue matrices platforms for controlling stem cell expansion and differentiation.

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Bioactivation of Ti6Al4V Through Developing Micro and Nano Surface Roughness

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Titanium and its alloys are widely used for biomedical, aerospace and defence application because of its novel properties like low density with high strength, excellent corrosion resistance, and biocompatibility. As Titanium based metallic implants have been widely accepted but implant-bone interfacial bonding is still a challenge due to poor tissue integration with implant. In past decades, researchers have explored various techniques to minimize this failure using different surface modifications using physical, chemical methods and coating with bioactive material to improve interfacial bonding. As surface roughness or surface physics and surface chemistry play a critical role for cell proliferation cell attachment and tissue adhesion. In this study, micro roughness has been generated by creating different shapes of micro pillars/grooves on Ti6Al4V scaffolds to enhance cell adhesion. Further the scaffolds were thermally treated to generate nano roughness on Ti6Al4V surface. Surface morphology of developed architecture on Ti6Al4V sample and cell attachment were evaluated by scanning electron microscopy (SEM). Human mesenchymal stem cell (hMSC) line has been utilized for cytocompatibility as well as cell proliferation property of prepared Ti6Al4V scaffold. MTT and live/dead assay were performed to

evaluate scaffold cytocompatibility. *In vivo* study was carried out in rat model to examine soft tissue adhesion. Preliminary results using SEM showed significant cell attachment and proliferation while MTT and live/dead assay indicated non-toxic nature of the matrix. It was also observed significant difference for thermally treated and untreated Ti6Al4V scaffold in *in vitro* and *in vivo* studies.

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Natural Rubber Latex as Stem Cells Scaffold for Muscle Regeneration and Cell Therapy

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Natural rubber latex (NRL) is a product extracted from rubber trees, with the most common source of NRL is *Hevea brasiliensis.* NRL is a mixing of colloidal rubber particles stabilized by a thin layer of phospholipids and proteins [1]. NRL has several features that make it an excellent scaffold and biomaterial to promote the growth and repair of tissues, skin, and bones, due to its' characteristics, such as biocompatibility, angiogenic potential, flexibility, mechanical stability, surface porosity, permeability and low cost [2]. The present study aimed to assess the potential NRL use as stem cell scaffold, for future application in muscle regeneration and cell therapy, evaluating cell adhesion, proliferation and migration, after the primary culture of rat stem cells in the biomamembranes NRL surface. By means confocal microscopy, scanning electron microscopy, immunocytochemistry and immunophenotyping techniques, we evaluated aspects of cellular interaction with NRL biomembrane surface, such as cell viability, proliferation, migration and adhesion. Our results demonstrate high cell viability, excellent cell adhesion, high cell proliferation and migration rate, besides this it was shown that the NRL biomembrane made a good provisional extracelular matrix demonstrating the great potential NRL as stem cell scaffold.

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Fabrication and Characterization of Sericin Gel and Sponge for its Biomedical Applications

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Silk is naturally occurring biomaterial composed of two biopolymers: fibroin and sericin protein. Sericin is hydrophilic protein that has strong polar side groups and it has been considered as a good material for biomedical applications due to its excellent biocompatibility, biological activities and resistance to oxidation, ultraviolet light and bacteria. However, sericin gel and sponge have poor mechanical properties restricting its applications to biomedical fields. In this study, sericin gels and sponges were fabricated with various sericin concentrations and ethanol contents to improve mechanical properties. Their gelation behavior, rheological properties and structural characteristics were examined. As the sericin concentration increased, the rate of gelation of sericin accelerated and gel strength increased significantly. This occurred up to an ethanol content of 15%. Above that, the rate of gelation and the gel strength decreased. There was no change in the gel-sol transition temperature of sericin with the sericin concentration, although more ethanol addition resulted in a decrease in the starting temperature of thermal gel disruption. The crystallinity index of the frozen and thawed sericin sponge did not change with the sericin concentration, whereas it increased with increasing ethanol content up to 15% and decreased above 15%. As the sericin concentration was increased, the mechanical properties of sericin sponge were remarkably improved. Studies *in vitro* indicated biocompatibility of the sericin sponge.

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Pamam (generation 4) Incorporated Three Dimensional Macroporous Scaffold as an Improved Substitute for Bone Tissue Engineering

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Introduction: The present study, explored the applicability of generation 4 PAMAM in three-dimensional gelatin-CMC-nHAp based macroporous scaffold (P-MGC) for bone tissue engineering application. We hypothesize that by incorporating amine group terminated PAMAM in the macroporous scaffold, will allow greater interaction between cells and scaffold, hence increases the overall biocompatibility of the scaffold.

Methods: The P-MGC scaffold was prepared by high-speed stirring and freeze-dried to generate macroporosity. FTIR, SEM, and UTM were used to characterize the physico-chemical properties, while hMSC differentiated osteoblasts microtissue and HUVEC was used to assess their viability, proliferation, and RT-PCR based gene expression profile. Angiogenic activity was performed using CAM assay.

Results and Discussion: The P-MGC scaffold showed augmentation in mechanical strength and pore size distribution in comparison to non-PAMAM based scaffold. An enhanced expression of osteogenic and endothelial genes was also observed in the P-MGC scaffold. Live-dead staining and alamar blue assay showed an increased adhesion and proliferation rate of osteoblasts microtissue and endothelial cells. CAM assay showed high angiogenic activity on P-MGC scaffold.

Conclusion: Our study demonstrated P-MGC scaffold allow enhanced cellular adhesion, proliferation and osteoconductive and angiogenic activity. This suggests that blending of PAMAM (G4) into matrix provides additional support to bone regeneration and angiogenesis.

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Aptes Functionalized Filter Paper as a Bioactive Scaffold for Bone Tissue Engineering Applications

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Bioactive, functional scaffolds are required to improve the regenerative potential of stem cells for tissue reconstruction and functional recovery of damaged tissues. Here, we report a filter paper-based bioactive scaffold platform for bone tissue engineering applications. The paper-based scaffolds were surface-functionalized with (3-Aminopropyl)triethoxysilane followed by its coating with collagen. The prepared paper scaffolds were compatible with general cell culture and manipulation techniques. They supported efficient cell attachment, proliferation of the human mesenchymal stem cells. The paper based scaffold also supported efficient differentiation of the mesenchymal stem cells into bone cells. Our study suggests that paper possesses great potential as a bioactive, functional, and cost-effective scaffold platform for stem cell-mediated bone tissue engineering.

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3D Printing Hydrogel into Media to Increase Nutrient Availability for Tissue Engineering Constructs

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3D printing cell-laden materials for tissue engineering models has developed quickly over the last 10 years. However, printing organ-level constructs remains a challenge for many reasons, including reduced cell viability for long print sessions. We created a platform to print a crosslinkable alginate hydrogel directly into media to enhance nutrient transport and viability of cells during long 3D print sessions. The objective of this experiment was to optimize the crosslinking process of sodium-alginate and calcium-chloride when extruded through a 3D BioPlotter (EnvisionTEC, Germany). In addition, we assessed feasibility of encapsulating cells in the sodium-alginate solution so that they are incorporated in the crosslinked gel and simultaneously surrounded by media during printing. An experimental protocol was developed to test various concentrations of sodium-alginate and calcium-chloride media. Separate tests were run to investigate the diffusion of dye out of the hydrogels into the surrounding media to determine the diffusion coefficient, indicative of nutrient exchange potential. Extruding sodium-alginate into calcium-chloride produced thin, precise strands. To prevent coagulation of gel around the print nozzle, the surface was coated with a thin metallic sheet, allowing stable multi-layer, insoluble constructs to be printed into media. The diffusion rate of dye was calculated, and supports the theory that nutrient exchange will occur. Future objectives include incorporating live cells into sodium-alginate mixtures, and assessing cell viability in extruded hydrogels over long print durations.

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An Impedance Based Biosensing Platform for Epithelial Cell Scaffold Characterization

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A label free and non-destructive method of assessing the interaction of cell tissue and scaffolds aids in the ability to discern the effective quality and magnitude of any scaffold modifications. Impedance cell spectroscopy is a biosensing method that employs a functional approach to assessing the cell monolayer. The electrical impedance barrier function of a cell monolayer represents the level of restriction to diffusion of charged species between all adjacent cells across an entire contiguous cellular monolayer. The impedance signals from many individual paracellular pathways contribute to the bulk measurement of the whole monolayer barrier function. However, the scaffold substrate must be entirely porous in order to be used with electrochemical cell impedance spectroscopy (ECIS) and cells must be closely situated to the electrodes. For purposes of evaluating cell-scaffold constructs for tissue engineering, non-invasive evaluation of cell properties while seeded on scaffolds is critical. A Transwell-type assay makes a measurement across a semi-permeable membrane, using electrodes placed on opposing sides of the membrane immersed in fluid. It was found that by suspending a nanofiber scaffold across a Transwell aperture, it is possible to integrate a fully functional nanofiber tissue scaffold with the ECIS Transwell apparatus. Salivary epithelial cells were grown on the nanofiber scaffolds and tight junction formation was evaluated using ECIS measurements in parallel with immunostaining and confocal imaging. The trans-epithelial resistance increased coordinate with cell coverage, culminating with a cell monolayer, at which point the tight junction proteins assemble and strengthen, reaching the peak signal.

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Peptide Polymer Scaffolds for Liver Tissue Engineering

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In 2-D culture, hepatocytes are flattened, with a corresponding loss of liver-specific mRNA. In contrast, hepatocytes cultured in 3-D display round, aggregated morphologies and a gene expression profile more similar to *in vivo*[1].

Conjugate scaffolds are of interest to tissue engineers because they are reproducible, require no precious resources (e.g. donated cadaveric material) and allow researchers to customise scaffolds[2]. Tissue engineering exploits polymer chemistry and molecular biology to create scaffolds which promote interaction between scaffold and hepatocyte via the asialoglycoprotein receptor (ASGPR)[3], a cell surface receptor found primarily in the liver[4].

We manufactured a novel peptide-polymer conjugate scaffold exploiting the ASGPR of functional hepatocytes; creating a 3-D environment which promotes cellular adherence. Scaffolds were assessed for biocompatibility by culturing hepatocytes on them and analysing their adherence, survival and enzyme/albumin production.

Directly incorporating peptide sequences which promote the adherence of hepatocytes into scaffolds provides a method of creating customised microenvironments for liver bioengineering and the elucidation of cell scaffold interactions. Peptide:polymer scaffolds represent a viable method of providing a bioactive environment for hepatocytes.

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Assesing Intestinal Organoid Drug Response in a well-defined Microenvironment

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Intestinal organoids have gained relevance as a tool in preclinical drug discovery, disease modeling and basic research. Forskolin-induced swelling of organoids derived from Cystic Fibrosis patients is used in drug-screening. Similarly, intestinal organoids have been used to investigate intestinal wound repair upon prostaglandin E2 (PGE2) stimulation. Matrigel hydrogels in combination with Wnt-3A, R-spondin, and Noggin, is the predominant intestinal medium used in drug discovery. However, Matrigel is ill-defined, varies from lot to lot, and contains numerous bioactive soluble proteins that could affect drug response. Exactly how these factors could impact drug discovery is unknown. Here, we show that a fully-defined synthetic basement membrane can be used to investigate intestinal organoid drug response to similar extent to the ill-defined Matrigel hydrogels. Human-derived intestinal organoids were cultured in the synthetic matrix and Matrigel and their response upon PGE2 and Forskolin was compared. The synthetic matrix consists of a multi-arm polyethylene glycol macromer reacted with free thiols groups of a collagen-derived peptide (which has a high affinity for integrins), and two small peptides sequences that had affinity for collagen IV, lamin, and fibronectin. After six days, the organoids were treated with PGE2 and forskolin in serum free organoid medium. Images were taken

before and after stimulation to measure the organoid diameters processed with FIJI. Intestinal stem cell embedded in the synthetic matrix form organoid similar to Matrigel, after six days. Upon forskolin or PGE2 stimulation, organoids increased in size on average 1.2 to 1.6 times in the synthetic matrix or Matrigel.

Cancer

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Nanoconstruct induced Apoptosis Through Downregulation Of Nucleolin-rtk Pathway In Cancer

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Cell surface nucleolin (NCL) has been shown as a novel cancer target molecule due to its abundance in various cancers including gastric, breast, lung and prostate. In this regard, anticancer drugs targeting NCL have been developed for anti-NCL therapy. Among of them, AS1411, DNA aptamer, is a representative anti-NCL agent that induces apoptosis through destabilization *bcl2* mRNA. Although promising, as1411 has been faced with some limitations such as short circulatory half-life, unexpected immune response, and insufficient anticancer outcome. These drawbacks have been alleviated by modification of aptamer, conjugation with nanoparticle and combinatorial treatment with other anticancer reagents. Here, we suggested the combinatorial treatment of cancer cells using nucleolin targeting aptamer (AS1411) and c-Met aptamer (c-Met apt) simultaneously. To improve delivery efficacy of two aptamers into gastric cancer (MKN-45), we introduced a star-shaped gold nanoparticle (AuNS) as a carrier. AS1411 and c-Met Apt were immobilized onto the surface of AuNS through gold-thiol chemistry. The bi-functional AuNS, c-Met-AS1411 AuNS, can alternately bind to c-Met and nucleolin on the cellular surface, results in induction of anti-cancer pathway via dual pathway of c-Met apt and AS1411. This nanocomplex showed increase of therapeutic efficacy compared with free apts and single apt-functionalized AuNS. Interestingly, mixture of c-Met-AuNS and AS1411-AuNS could not suppress the cell viability as c-Met-AS1411-AuNS decreased at the same apt total concentration. The result indicated that bi-functional AuNS effectively induces apoptosis pathway through interaction of c-Met- and nucleolin-correlated cell death mechanism.

Cardiovascular

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AAV-mediated Therapeutic Strategy for Ischemic Heart Disease

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Recent work in cellular reprogramming of fibroblasts into cardiomyocytes has demonstrated therapeutic potential for cardiovascular disease, in which viruses were utilized as biomaterials for successful transgene delivery. Retrovirus- or lentivirus-mediated transgene delivery into the cardiovascular system was majorly used and is not yet feasible for application to humans. Here, we propose to utilize AAV-mediated gene delivery as a cardiovascular regeneration strategy to generate new cardiomyocytes and limit collagen deposition in the injured heart. This was achieved by inducing synergism of Gata4, Mef2c, and Tbx5 (GMT)-mediated heart reprogramming and thymosin β4 (Tβ4)-mediated heart regeneration in a myocardial ischemia model. AAV-GMT promoted a gradual increase in expression of cardiac-specific genes, including Actc1, Gja1, Myh6, Ryr2, and cTnT, with a gradual decrease in expression of a fibrosis-specific gene, procollagen type I. Vessel growth was enhanced in fibrotic areas in the AAV-Tβ4 co-treatment group compared to the AAV-GMT or AAV-Tβ4 only group; enhanced cell survival was also accompanied by AAV-Tβ4 co-injection. Histological assessment of heart also showed decreased myocardial fibrosis and increased wall thickness in the AAV-Tβ4 co-injection group, with consequent improved cardiac function, as shown by echocardiography. Taken together, the combined AAV-mediated GMT and Tβ4 therapy enhanced the recovery of heart structure and function via enhanced heart regeneration and blood vessel growth in an acute myocardial infarction model.

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Tissue-Engineered Blood Vessels with User-Defined Geometries

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A great need exists for blood vessels for use as vascular grafts in the treatment of cardiovascular disease. Autologous vessels are of limited availability/quality and their harvest is invasive. Synthetic vascular grafts are prone to thrombosis and infection. A tissue-engineered blood vessel, able to grow, remodel and repair *in vivo*, offers an attractive solution to vascular grafting.

Natural vasculature is composed of complex geometries (tapers and bifurcations) and it has been suggested in a number of studies that the geometry of the vascular constructs created during surgery has a strong influence on their performance. The ability to generate tissue-engineered blood vessels with varied geometries would therefore be of great potential benefit.

Here we have developed a method for generating tissue-engineered blood vessels of various geometries. A novel photocurable polymer, poly(glycerol sebacate) methacrylate (PGS-M) was developed for use as a scaffold material to support the growth of the tissue-engineered blood vessels. PGS-M is highly elastic, degrades rapidly and displays excellent cytocompatibility. Using a combination of 3D printing and emulsion templating, porous tubular PGS-M scaffolds of various geometries, including tapers, bends and bifurcations, were produced. These scaffolds were seeded with vascular smooth muscle cells and cultured in a bespoke, pulsatile flow, bioreactor which provided mechanical stimulation to the developing constructs. The vessels generated displayed cellular organisation similar to natural vasculature with an extracellular matrix of collagen and elastin. This method may be adapted to produce blood vessels with user-defined geometries for tailored clinical applications.

Dental & Craniofacial

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3D-Printed External Cages Preserve Shape of Human Tissue Engineered Ear Cartilage *In Vivo*

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A persistent challenge engineering ear shaped cartilage is contraction and deformation during implantation due to weak initial mechanical properties. Previously, we observed that constructs contracted up to 60% *in vivo*. We hypothesize that providing mechanical support by encasing the construct in a ''cage'' during implantation will protect the construct from external forces of surrounding tissue, preserving shape definition without hindering tissue development. Human AuCs were isolated from otoplasty specimens and encapsulated in high density collagen, as either 8mm discs or an ear. External cages were 3D-printed from polylactic acid (PLA) to fit constructs, containing large pores to allow nutrient exchange with the construct. Discs were inserted into cages and implanted subcutaneously in nude mice for 1 or 3 months, and ear constructs implanted in nude rats for 3 months. After 3 months, PLA cages remained stable and the encased discs maintained 95% of the initial diameter. Histology of disc and ear constructs displayed collagen bundling and proteoglycan deposition after 3 months, with disc

constructs also containing elastin fibers. After 3 months, disc constructs showed 47% the compressive stiffness and 59% the proteoglycan content of native auricular cartilage. The cages ability to protect the developing construct indicates that that internal cell traction accounts for only 5% of construct contraction, and that most deformation results from external forces. Collectively, these data indicate the potential for encasing auricular chondrocyte constructs within 3D-printed external cages to preserve shape and structure while still allowing for generation of auricular cartilage tissue.

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Mechanical Evaluation of the SLM Fabricated, Stiffness-matched, Mandibular Bone Fixation Plates

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The standard of the care for the treatment of mandibular segmental defects is the use of Ti-6Al-4V bone fixation plates and screws to immobilize the grafted bone and the host mandible. While Ti-6Al-4V bone fixation plates provide strong immobilization during the healing period, they may disturb the stress distribution in the repaired mandible. The highly stiff Ti-6Al-4V fixation carries a great portion of the load which was previously borne by the mandible, and stress shielding may occur on the surrounding cortical bone. Based on the bone remodeling theory, stress shielding causes bone resorption in the affected region and may eventually lead to the failure of the surgical reconstruction. To address this issue, we have developed a new generation of the patient-specific, porous NiTi bone fixation plates which benefit from stiffness-matching of the adjacent bone. Using the CT scan data of the patient's defective mandible, the geometry for the required bone fixation plates is designed and the stiffness of the surrounding regions is measured. By introducing specific level and type of porosity to the bone fixation plate, its stiffness can be tuned. Finite Element simulations have verified the reduced level of stress shielding on the reconstructed mandible, in case of using the proposed bone fixation plates. Selective Laser Sintering has been used for fabrication of the porous NiTi bone fixation plates with six different levels of stiffness. Finite element simulations and mechanical tests have been done to verify the performance of the fabricated parts resulting from our design and fabrication method.

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Clinical study of Dentin Demineralized Matrix (DDM) combined with BMP2 for socket preservation after extraction

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Objectives: This study compared socket preserving effects between autogenous tooth derived dentin demineralized matrix (DDM) combined with recombinant human bone morphogenetic protein-2 (rhBMP-2), DDM only, and xenogenic bone (BioOss® collagen). We hypothesized that DDM+rhBMP-2 would have better bone forming ability and socket preserving effects than those of others.

Methods: Total 21 participants aged over 19 with one or more of hopeless teeth were included. The participants were excluded if they were pregnant, generalized compromised, immune deficient, or heavy smokers. All participants got informed consent, and were randomly selected into three groups. The 8 participants got socket preservation using xenogenic bone, 6 participants got DDM only, and the other 7 participants got DDM+rhBMP-2 graft. Three dimensional (3D) volumetric, radiographic, and histomorphometric changes between initial and 4 months later were analyzed.

Results: The 3D volumetric analysis showed that DDM+rhBMP-2 group had similar horizontal bone resorption compared to DDM only or xenogenic bone group. Likewise, cone beam CT results revealed that DDM+rhBMP-2 group had similar amount of new bone formation, horizontal and vertical bone loss compared to those of other groups. Histomorphometric results displayed new bone formation was found on DDM+rhBMP-2 group comparable to other groups.

Conclusion: Autogenous tooth derived DDM+rhBMP-2 showed new bone formation and socket preservation comparable to those of xenogenic bone or DDM only group.

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Periodontal Ligament Integration with Cementogenesis Using Modified Fibrin Matrices

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Objectives: Cementum formation on the exposed tooth-root surface is a critical process in periodontal regeneration. Although various therapeutic approaches have been developed, regeneration of integrated and functional periodontal complexes is still wanting. Here, we found that the OCCM30 cementoblasts cultured on fibrin matrix express substantial levels of matrix proteinases, leading to the degradation of fibrin and the apoptosis of OCCM30 cells, which was reversed upon treatment with a proteinase inhibitor, ε-aminocaproic acid (ACA).

Methodology and Results: Based on these findings, ACA-releasing chitosan particles (ACP) were fabricated and ACP-incorporated fibrin (fibrin-ACP) promoted the differentiation of cementoblasts *in-vitro*, as confirmed by bio-mineralization and expressions of molecules associated with mineralization. In a periodontal defect model of beagle dogs, fibrin-ACP resulted in substantial cementum formation on the exposed root dentin *in-vivo*, compared to fibrin-only and enamel matrix derivative (EMD) which is used clinically for periodontal regeneration. Remarkably, the fibrin-ACP developed structural integrations of the cementum-periodontal ligament-bone complex by the Sharpey's fiber insertion. In addition, fibrin-ACP promoted alveolar bone regeneration through increased bone volume of tooth roof-of-furcation defects and root coverage.

Significance: Therefore, fibrin-ACP can promote cementogenesis and osteogenesis by controlling biodegradability of fibrin, implicating the feasibility of its therapeutic use to improve periodontal regeneration.

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Attaching a Hydroxyapatite-binding Domain to Anchor BMP2-derived Peptides onto Bone Graft Materials

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Objective: The osteoinductive factor, BMP2, has been passively adsorbed to commercial bone graft materials to improve osseointegration, however BMP2 disseminates quickly from the graft, resulting in inflammation. Previously we identified a molecular domain of 7 glutamates (E7) which tightly binds to hydroxyapatite, a common graft material. We hypothesize a bioactive peptide derived from BMP2 with an attached E7 domain (E7-BMP2pep) can anchor to hydroxyapatite, increasing bone regeneration without evoking a deleterious immune response.

Methods: Osteoblastic cells were treated with BMP2pep, with or without E7, to measure osteogenic cell signaling. Peptide-coated hydroxyapatite particles were implanted in rat cranial defects and evaluated by H&E and immunohistochemistry to analyze bone formation, presence of T cells (CD3), and vascularization (CD34).

Results: *In vitro* assays showed that the E7 domain greatly improves peptide anchoring to hydroxyapatite without diminishing BMP2pep's capacity to stimulate BMP2-dependent signaling (pSMAD activation) and osteoblastic differentiation (upregulation of alkaline phosphatase, ALP). In fact, cells treated with E7-BMP2pep had equivalent levels of induced ALP expression compared with full-length rBMP2. *In vivo* studies of implanted E7-BMP2pep-coated hydroxyapatite revealed that the peptide strongly stimulated bone formation, and did not hinder vascularization. Furthermore, there was minimal T cell infiltration associated with the E7-BMP2pep group compared with rBMP2-coated samples.

Significance: E7-BMP2pep offers a valid alternative to full-length rBMP2 because it can prolong osteoblastic signaling at the graft site by strongly adhering to hydroxyapatite, resulting in equivalent bone formation compared to rBMP2, without the inflammatory response commonly seen with rBMP2 treatment.

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Polyglutamate Modified VEGF Mimetic Peptides Elicit a Proangiogenic Response

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Introduction: Engineering bone graft materials with gradient release of angiogenic factors such as VEGF is a major research focus, given the need for neovascularization for efficient bone healing. To address this issue, we synthesized the VEGF mimetic peptide, QK, with three different polyglutamate domains (diglutamate, tetraglutamate, and heptaglutamate) that selectively release from Ca^{2+} found within bone grafts based upon the number of glutamate residues. A mixture of these peptides (''Polyglu-QK'') was used to develop a proangiogenic gradient on graft materials.

Methods: Solution fluorescence assays were conducted with FITC-labeled components within Polyglu-QK to quantify release from bone grafts. Polyglu-QK was evaluated for proangiogenic bioactivity through endothelial tube formation assays, scratch test, and activation of signaling proteins.

Results: Individual peptides within the Polyglu-QK mixture are released in accordance with polyglutamate domain length, creating a gradient. Additionally, we confirmed that the polyglutamate domain does not interfere with the bioactivity of QK, as measured by endothelial cell migration, tubule formation, and activation of signaling molecules (pAkt and p-eNOS). Finally, bone graft materials coated with Polyglu-QK elicited a greater angiogenic response in endothelial cells compared with grafts passively coated with rVEGF or QK.

Conclusions: Polyglu-QK offers a new approach for gradient delivery of proangiogenic factors from bone grafting material, thus facilitating bone tissue neovascularization.

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Imaging

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Cell-Based Probes for Real-Time Evaluation of Chondrogenesis

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Chondrogenic differentiation constitutes a multistep program modulated by spatially- and temporally-determined combinations of morphogens. Current assessments of engineered tissues rely on destructive methodologies applied at the end of the fabrication period, making impossible to predict failures early in the process. Therefore, developing a dynamic, multi-modal, non-destructive/non-invasive technology toolset to monitor cell differentiation in real-time is important. An imaging approach is applied to chondrogenic structures serving as platform for testing the biological effect of specific designed interventions. It uses cell-based probes to directly interrogate differentiation events during *in vitro* chondrogenesis. Native promoters of well-established cell-differentiation biomarkers are used to create independent reporters incorporating a traceable signal (Luciferase), to monitor in real-time the progression throughout the differentiation program of Mesenchymal Stem Cells chondrogenesis. This technology will permit a longitudinal reconstruction of the differentiation program and an analytical assessment of phenotypic changes of engineered cartilage. They offer significant advantages to researchers to improve the fabrication algorithms of engineered cartilage, such as: 1) avoid relying on end-point/destructive assessments to determine success/failures; 2) to make fine spatio-temporal correlations between specific interventions and resulting phenotypes; 3) to be able to make adaptations and tune ups ''on the go''; and 4) the possibility of defining release criteria for implantation based on established features and events monitored in real-time. These advantages positively impact both the design strategies and the testing approaches of engineered cartilage, speeding up the translation of implantable constructs to treat cartilage defects.

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Ultrafast Pump Probe Imaging of Melanin For Non invasive diagnosis of Melanoma

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96 Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:''Table Normal''; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:''''; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin:0in; mso-para-margin-bottom:.0001pt; mso-pagination:widow-orphan; font-size:12.0pt; font-family:Calibri; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin;} We integrated the state-of-the-art ultrafast pump-probe microscopy to distinguish between hyperpigmented and malignant melanoma tumor. Multiphoton microscopes are operating based on two-photon induced fluorescence and second harmonic generation. Such methods are only useful for imaging exogenous fluorophores (dyes) and genetically expressed markers, but have more limited utility with endogenous contrast. In ultrafast pump-probe microscopy, two femtosecond laser pulses with MHz repetition interact with the sample. A variety of different linear and nonlinear optical interactions of the two laser pulses with the tissue provide contrast for imaging. This includes refractive-index change (self-phase or cross-phase modulation), two-photon or single-photon absorption, or pump-probe features such as excited-state absorption, ground-state depletion or stimulated emission. Time-resolved pump-probe images and transient absorption spectrum of melanin in different forms of synthetic nanoparticles, in tissue biopsy specimens and dissected tumors, are compared. Our results suggest that the excited state dynamics of melanin in malignant melanoma are significantly different with that in the hyperpigmented tissue. The nature of underlying photophysical processes, such as excited state absorption, ground state bleaching are described. These processes provide chemical information and the molecular level and are compared for melanin pigments in hyperpigmented tissue with malignant melanoma tissue. Therefore, the technique and the biophysical model provides a reliable quantitative optical contrast for non-invasive diagnosis of melanoma.

Matrix & Extracellular Matrix Biology in Regenerative Medicine

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Superior Elastogenicity And Pro-elastogenic And Anti-proteolytic Effects Of Phenotype-selected, Adult Stem Cell-derived Smooth Muscle Cells In A 3-d Collagenous Milieu

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Abdominal aortic aneurysms (AAAs) are rupture-prone dilations of the abdominal aorta that develop from irreversible proteolytic breakdown of the elastic matrix. Towards use in a matrix regenerative cell therapy for AAAs, we showed in 2D co-cultures that bone marrow mesenchymal stem cell-derived smooth muscle cells (BM-SMCs) provide paracrine pro-elastogenic/anti-proteolytic stimuli to AAA SMCs, and identified a specific phenotype (cBM-SMCs) derived on a fibronectin substrate in presence of TGF-β and PDGF that is more elastogenic than even healthy aortic SMCs and show more impressive paracrine effects versus other generated phenotypes. We presently investigate the performance of the cBM-SMCs in a 3D collagenous milieu evocative of the de-elasticized AAA tissue. Rat aortic SMCs, cBM-SMCs and rat AAA SMCs were cultured standalone or together with AAA SMCs within compacted collagen gels for 21 days. Elastic matrix amounts (Fastin assay), fiber formation (TEM, IF), and lysyl oxidase mediated (Western blot, Amplex Red assay) formation of desmosine crosslinks (ELISA) within the elastic matrix, besides contractility (stereomicroscopy) and elastic moduli (Nano-indentation test) of the constructs were significantly higher and metalloprotease protein synthesis and activity (western blot, gel zymography) were significantly lower in standalone cBM-SMC cultures and in cBM-SMC/AAA SMC co-cultures versus controls. Our study strongly supports the utility of cBM-SMCs for cell therapy aimed at restoring elastic matrix homeostasis in the AAA wall towards achieving growth arrest or regression.

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Extracellular Matrix Hydrogels for Mediating Inflammation and EMT

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Fibrosis is the principal factor leading to progression and end-stage of many diseases, oftentimes leading to organ failure. Despite accounting for greater than 45% of deaths in the western world, there are currently no available agents for targeting fibrosis. A common feature of fibrosis across tissues is inflammation and epithelial injury manifesting as pathologic epithelial-to-mesenchymal transition (EMT). Recent data suggests cryptic fragments of extracellular matrix (ECM) can moderate the activation state of macrophages and alter EMT gene expression and mitigate downstream fibrosis. The aim of the present study was to determine the effect of an ECM derived hydrogel (ECMH) upon of innate immune cell activation and the progression EMT. Murine bone marrow derived macrophages were cultured with ECM hydrogel (with and without polarizing cytokines) and phenotype was assessed by flow cytometry. Epithelial cells were cultured on ECMH substrates and EMT was induced by addition of transforming growth factor beta (TGFβ). The effect of ECM on EMT was assessed by matrix metalloproteinase (MMP) activity, gene expression, and immunolabeling. Macrophages treated with ECMH had a diminished pro-inflammatory response without changing expression of anti-inflammatory markers. Epithelial cells grown on ECMH had reduced activity of MMPs alongside altered EMT gene expression. Despite challenge with TGFβ, the cells retained expression of epithelial proteins when grown on ECM hydrogels. Results suggest that ECMH represents a potential therapeutic for pathologic fibrosis as it contains components that can suppress both inflammation and EMT.

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Biomimetic Matrices for Enhanced Stem Cell-Mediated Osteogenesis

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New promising solutions for bone tissue reconstruction have been developed in the last years due to the dramatically increase of the number of bone-related medical conditions that require clinical interventions. The biological process of bone regeneration is very complex and is influenced by multiple factors that regulate osteogenesis, angiogenesis and tissue remodeling. New technologies that accelerate bone healing response without compromising the quality of new bone tissue would be useful tools for clinical applications. Bone non-collagenous proteins, such as osteocalcin (OC) and osteopontin (OPN) have been implicated in a variety of roles, such as cell attachment to extracellular matrix, osseointegration at the biomaterial/tissue interface and bone toughness. Here, we investigate the effect of biomimetic OC/OPN-enhanced collagen matrices on human mesenchymal stem/stromal cells (MSC) proliferation, osteogenic differentiation, angiogenic properties and *in vivo* bone regeneration. Our OC/OPN-enhanced collagen matrices demonstrate accelerated, increased and sustained bone formation response, producing mineralized tissue similar to bone. Additional *in vivo* studies in a critical sized-defect rabbit long-bone model reveals bone formation with active tissue remodeling. Our work shows, for the first time, that selected non-collagenous proteins can be used in concert with type I collagen matrix, to create a novel biomimetic matrix for enhanced cellular activity and bone formation. These findings open avenues for the use of bone non-collagenous proteins as an alternative to other biomolecules, promoting a paradigm shift in the design of regenerative scaffolds.

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Bio-molecular Characterization of Common Organs being used as Tissue Scaffolds

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Introduction and Objectives: Extracellular Matrix (ECM) rich, biologically derived scaffolds are quickly proving more efficacious than their synthetic substitutes in the clinic. Despite the ECM lending known phenotypic heterogeneity when tissue sourcing is varied, bioanalytical methods aimed at teasing apart this compositional diversity are lacking. To fill this gap in knowledge, we aim to provide a method to quantify and characterize ECM proteins, Glycosaminoglycans, and cross-links in organs in order to enhance our understanding of tissue engineered bioscaffolds.

Methods: Tissues representing a unique and diverse array of ECM abundance, composition, and biomechanical properties where chosen for characterization by a novel collection of mass spectrometry techniques, including (1) quantitative proteomics utilizing a stable isotope labeled polypeptide library for absolute quantitation of ECM proteins, (2) determination of glycan composition, and (3) quantitation and characterization of *in vivo* derived cross-links.

Results and Discussion: To extend the general utility of these methods, we determined the unique bio-molecular composition of the ECM within five tissues by (1) quantifying 216 ECM-targeted and cellular proteins, (2) characterizing the glycan composition, and (3) quantifying cross-linking across tissues with varied biomechanical stiffness.

Conclusion: The approaches developed here allows for unprecedented bio-molecular characterization of the ECM and can serve as a catalyst to drive tissue engineering efforts towards the clinic. In addition, these analytical methods can be used to monitor product heterogeneity and establish relationships between clinical outcomes and molecular composition of a scaffold.

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Systematic Review and Meta-Analysis: Efficacy of Volumetric Muscle Loss Treatments in Animal Models

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Background: Volumetric muscle loss (VML) injuries present a complex clinical problem resulting in chronic loss of muscle tissue and strength. Our objective was to perform a systematic review and meta-analysis of the research examining the effectiveness of various approaches for treating VML injury.

Methods: Our primary question was to investigate if ''*In humans and/or animals with VML injury, is treatment better than no treatment at recovering function*''. In total, we identified 2,230 relevant publications and 2,196 were excluded based on the review of title/abstract or full text for not meeting the inclusion criteria.

Results: The overall effect size (ES) was calculated to be 0.78 (95% CI = 0.55-1.00; $p < 0.00000001$), indicating that the treatments employed in the 34 studies, on average, resulted in a significant improvement in function. This overall ES equates to $\sim$15% smaller strength deficit for treated VML compared to untreated (i.e., $-37 \pm 1\%$ vs. $-44 \pm 1\%$). Between-study ES variance was low (Q-$df = 2.8$; $p = 0.34$; $I^2 = 7.5\%$) and none of the moderator variables analyzed (e.g., subject sex, age, and species, thickness and location of VML injury, treatment type, and study design) explained a significant portion of the ES variance among studies.

Conclusion: Overall, our findings indicate that various biomaterial, cellular, and rehabilitative treatments applied at the time of injury in animal models improve muscle function over leaving the VML injury untreated.

Musculoskeletal

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Ovine Satellite Cell Inhomogeneity in the Fabrication of Tissue-Engineered Skeletal Muscle Units

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Volumetric muscle loss (VML) is defined as a loss of skeletal muscle that overwhelms the body's innate regenerative capacity and results in sustained impairment of function. Our work to date has involved the use of satellite cell populations derived from ovine hindlimb muscle to fabricate tissue-engineered skeletal muscle units (SMUs) to treat hindlimb VML. However, many studies have shown that satellite cell populations derived from different muscle sources are heterogeneous and exhibit differences in regenerative capacity, satellite cell density, and gene expression. Thus, when designing a treatment for craniofacial VML, the optimal starting satellite cell population may not be hindlimb muscle. We hypothesized that satellite cell heterogeneity would be reflected in the properties of the resultant SMUs. Thus, we assessed the development, structure, and function of SMUs derived from four muscle sources, including two hindlimb muscles (i.e. soleus and semimembranosus) and two craniofacial muscles (i.e. zygomaticus major and masseter). Immunostaining for MyoD coupled with a BrdU assay was used to evaluate myogenic proliferation of cells 4 days after seeding. Differentiation of myoblasts was measured with immunostaining monolayers for myogenin on day 6. Following 3D construct formation, tetanic force production was measured to evaluate functionality. While the masseter group had significantly fewer myogenin-positive cells/mm^2 than the other muscle groups on day 6, there were no significant differences in force production between groups. Overall, we have demonstrated that it is important to consider the satellite cell source when fabricating tissue-engineered skeletal muscle constructs for varied clinical applications.

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Characterization of Biochemically Induced Rabbit OA Model

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Biochemical induction of OA using collagenase II has several advantageous points in a sense that it does not involve surgery to induce model and the extent of induced cartilage degeneration is almost uniform. However, concerns still existed for biochemical OA model, because they induce abrupt destruction of cartilage tissues biochemically in a short period of time and this might accompany systemic acute inflammatory response, which is rather a trait of RA (rheumatoid arthritis) than being a trait of OA disease. Therefore, instability-induced OA model is widely used than biochemically induced model. In this study, symptoms exhibited by biochemically induced rabbit OA model was investigated to verify if they truly represent OA disease state. Our results showed that cartilages were rapidly degenerated during short period of time, and they did not accompanied with RA-like process based on the fact that the cartilage tissues on non-induced side remained intact. Also there were no significant change in CBC (complete blood count) profile showing a characteristics of OA disease. These study shows that biochemically induced of cartilage degeneration truly represent reliable and uniform OA state.

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N-acetylcysteine Reduces Fibrosis and Improve Both Muscle Regeneration and Function in Compression Induced Injury

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Skeletal muscle regeneration from severe injuries is dependent on the ability of satellite cells to survive, proliferate, and differentiate within the resultant damaged and toxic microenvironment of the injured tissue. Inability of the tissue to regenerate efficiently may result in fibrotic lesions, scar formation and subsequently decreased functional recovery. The full impact of the injured microenvironment on the balance between healthy muscle regeneration and pathologic fibrosis is not completely known. For example, the inflammatory response and wound repair pathways may be differentially regulated at the site of injury, which will affect extracellular matrix protein synthesis, neovascularization, and antioxidant activity. Historically, antioxidants have been used as a treatment for severe pathologic fibrotic diseases such as idiopathic pulmonary fibrosis because when added to standard theraphy they preserve lung capacity. Here, we show that treatment with the antioxidant N-acetylcysteine (NAC), decreased fibrosis and enhanced muscle regeneration and functional recovery in a model of compression injury of skeletal muscle in rats. This data suggests that NAC treatment post skeletal muscle injuries may be a viable option for the prevention of long-term fibrosis, scar formation and muscle dysfunction.

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Extracellular Matrix Formation and Meniscal Phenotype in Co-cultures Of Meniscal Cells and Peripheral Blood-derived Mesenchymal Stem Cells

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To establish 2D and 3D co-culture systems of MFC and peripheral blood MSC and test the effects of cell ratios on matrix formation and meniscal phenotype. MSC and MFC were co-cultured in various

ratios. Conventional 2D monolayers and 3D micromass pellet co-cultures were prepared. Cell morphology was analyzed by confocal microscopy. The phenotype of meniscal cells was analyzed based on matrix production were assessed based on deposition of glycosaminoglycans (GAG). In 2D monolayer cultures, adding MSC to MFC cultures maintained the MFC phenotype, based on expression of type I and III collagens, and it increased their matrix formation, based on GAG deposition. Mixing the cells in equal parts (50:50) led to the highest expression of type I and III collagen; levels of sulphated GAG did not vary significantly with co-culture ratio. In 3D micromass pellet cultures, adding MSC to MFC monocultures reduced pellet size and GAG production: matrix formation positively correlated with the proportion of MFC in the co-culture. Adding MSC cells to MFC also changed the pattern of collagen expression. Co-cultures expressed lower levels of type II and III collagen than did MFC monocultures, though all co-cultures expressed these collagen types at much higher levels than type I. Only MSC monocultures expressed type X collagen. In 2D co-cultures, mixing the cells in a 50:50 ratio optimizes the meniscal phenotype and leads to good matrix formation. In 3D co-cultures, matrix production positively correlates with the proportion of MFC.

Neuronal Tissue

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Novel Nerve Conduits with Wall-Encapsulated Cells Improve Peripheral Nerve Regeneration

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Engineered nerve conduits have the potential to aid peripheral nerve regeneration across functionally debilitating segmental defects. Although incorporation of cells secreting neurotrophic factors within these designs should confer additional beneficial effects, cell delivery lacks control, generally limited to injection into the conduit lumen or surface adsorption after fabrication. We report here a single-step method to encapsulate cells into the walls of a nanofiber conduit during fabrication through the use of a composite methacrylated gelatin/polycaprolactone scaffold, allowing for strict control of cell number and spatial distribution away from the lumen. The resulting structure is flexible, and mechanical testing verifies excellent suture retention strength for implantation. Immunofluorescence staining *in vitro* shows that the encapsulated cells respond to the nanofiber topography with concentric distribution of cells visualized within the conduit walls. Enhancement of neurite extension, up to twice the length of controls, was seen in chick embryonic dorsal root ganglia cultured on these constructs. *In vivo* implantation of this novel conduit into a 1 cm sciatic nerve gap in rats confirms this effect displaying complete bridging of the gap at 6 weeks, with strong S100 Schwann cell staining throughout the regenerated nerve and into the distal nerve stump, in contrast to the highly limited S100 staining in the distal portion observed in the acellular control. Given these results, this new conduit fabrication strategy presents a promising new avenue in the nerve conduit arsenal to study and maximize the potential of cell application in peripheral nerve repair.

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Production Assistance for Cellular Therapies

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Development Of The Tonsil-derived Mesenchymal Stem Cell-therapeutics For Hypoparathyroidism Treatment Using Fibrin Sealant As Injectable Scaffold

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The incidence of hypoparathyroidism has been increasing along with the increasing thyroid surgery. The conventional treatment was oral calcium administration with vitamin D, however, this treatment could not mimic the physiologic effect of the native parathyroid hormone(PTH). We previously reported the possibility of cell therapeutics which was differentiated from the tonsil-derived mesenchymal stem cell (TMSC). For stable hormone release, the ideal scaffold material which facilitates cellular engraftment is mandatory. The fibrin sealant has got FDA approval and widely used. The purpose of this study was to development the cell therapeutics and to evaluate its' feasiblility. As *in vitro* study, cell viablility test was performed. As *in vivo* study, 50 Spraque-Dawley rats was divided into four groups (2 experimental group and sham group and fibrin-sealant only injected group). After parathyroidectomy, 10^6 Cells were administrated with fibrin sealant at dorsum by subdermal injection in experimental group. Intact PTH, calcium, and phophrous was measured by venous sampling. The TMSC and fibrin sealant has feature as gel after injection *in vitro*. The cell survived more than 4 weeks *in vitro*. Serum PTH level decreased to non-detectable level as postoperative 3 days and began to restore at 21 postoperative day. Immunofluorescence microscopy revealed that PTH and CHGA positive cells were remained in Cell therapeutic injected area. TMSC-derived hypoparathyroidism cell therapeutics demonstrated its validity in PTH level restoration.

Regenerative Medicine Transplantation

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Tooth Pulp Stem Cells: Treatment of NASH or Cirrhosis in Swine

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Liver cirrhosis or Nash was by transplantation of hepatocyte-like-cells derived from human exfoliated deciduous tooth (SHED) stem cells. SHED was separated by magnetic sorting with CD117 antibody. For hepatic differentiation, CD117 + SHED were grown in DMEM supplemented with insulin-transferrin-selenium-x (ITS-x), embryo-tropic-factors(ETF) and hepatocyte-growth-factor (HGF) for 5days: IMDM supplemented with ITS-x, ETF, HGF, dexamethasone and oncostatin for another 11 days. Nude rats or Swine were employed for this study. Carbon tetrachloride (CCl_4) or specific meal was administrated for certain period. Hepatocyte-like-cells (2×10^6 cells/animal) were transplanted into the spleen. The vehicle was injected to the positive control group. Non-cirrhosis-models were used as negative control group. Animals were sacrificed 4 weeks after the transplantation. Immunocytochemistry observation of the hepatically differentiated cells strongly demonstrated positive staining for albumin, IGF-1, α-feto-protein, HNF4α and CPS-1. The histopathological analysis, HE and Masson's trichrome staining, indicated a significant decrease of fibrous tissue in the transplantation group with comparing to the positive control group. Healthy liver tissues were recovered by the transplantation. Moreover, serological test results revealed signif icance differences between the groups. Serum ALT levels of the test group dramatically decreased to one third compared to the positive control group. Activities of albumin, bilirubin, BUN, HA levels were also recovered. The xenotransplantation of hepatocyte-like cells from human tooth transplanted into the liver with severe failure demonstrated their capacity to preform positively because of drastic decreasing fibrous tissues. Together, hepatocyte-like cells derived from SHED are a potential source for treating chronic liver injuries such as cirrhosis.

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ecm Hydrogel Injection For The Treatment Of Stroke

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Stroke is the leading cause of adult disability and a significant effort is underway to develop therapies to repair the damaged tissue. Biomaterials composed of mammalian ECM promote constructive tissue remodeling with minimal scar formation in peripheral tissue and organs. The biodegradation and functional effect of injecting a large volume of a 8 mg/mL ECM hydrogel into the brain are unknown. We aimed to determine if biodegradation occurs and if ECM remodeling will affect the behavioral deficits of animals with stroke damage. Two weeks post-stroke, Magnetic Resonance Imaging-defined lesion volume equivalents of ECM was injected into the lesion cavity of stroke rats. A battery of behavioral tests were performed at pre-treatment, 1, 4, and 12 weeks post-treatment for control, untreated and ECM-treated groups. Retention, gelation, and biodegradation of the ECM, as well as host cell invasion and phenotype were analyzed at 12 weeks post-injection using immunohistochemistry. Behavioral tests indicated a functional impairment that was not affected by the injection of a large volume of ECM into the cavity. ECM showed a robust gelation and retention in the lesion cavity with a 30% decrease in volume over 12 weeks. A significant host cell invasion into the ECM hydrogel was seen within the hydrogel. ECM hydrogel therefore can be readily injected and retained within the lesion cavity, while promoting an acute endogenous repair response, without deleterious effects.

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Osseointegration with PEKK 3D Technology TETRAfuse in an Ovine bony defect model

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Introduction: High performance thermoplastics becoming key biomaterials for load bearing implants in spine and reconstructive surgery. A comparative evaluation of osseointegration with smooth PEEK, titanium-coated rough PEEK and PEKK 3D Technology (TETRAfuse) was performed in an ovine bony defect model. The hypothesis was that surface topography influences bone apposition and implant interlock.

Methods: Total of 36 cylinder shaped implants were randomly placed into distal femora of six sheep bilaterally for 8 and 16 weeks. Micro CT, SEM, histology and biomechanics were performed. Periprosthetic Bone Area (% PBA) and Appositional Bone Index (ABI) were quantified by accredited pathologist. Peak force (N) was obtained from push-out test. Statistical analysis was performed using Tukey, One-way ANOVA.

Results: Microscopy demonstrated new viable bone surrounding all implants. Periprosthetic bone area increased by 8 weeks and stabilized thereafter. Bone apposition significantly increased to 3D PEKK and ti-coated PEEK but decreased to smooth PEEK implants by 8 weeks. Excellent osseointegration was achieved with 3D PEKK and ti-coated PEEK implants. Smooth PEEK showed ''spot welding'' osseointegration with limited mechanical interlock due to interposing fibrosis. 3D surface allowed for progressive bone ingrowth. Average push-out peak force (N) significantly increased in 3D printed and ti-coated groups by 8 weeks (Tukey, One-way ANOVA), when smooth PEEK showed no overtime increase.

Conclusions: Both, TETRAfuse Technology and rough titanium coating provides biomechanical advantage of early and persistent implant stabilization in the cancellous bone. The histological and biomechanical results demonstrated that material properties and surface topography determine push-out strength and bone growth potential.

Regenerative Pharmacology

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Injectable Hydrogel For osteogenic differentiation Human Periodontal Ligament Stem Cells

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We prepared a covalently bone morphogenetic protein-2 (BMP2)-immobilized hydrogel that is suitable for osteogenic differentiation of human periodontal ligament stem cells (hPLSCs). BMP2 covalently immobilized on an inectable hydrogel (MC-BMP2) was prepared quantitatively by a simple biorthogonal reaction between alkyne groups on BMP2-OpgY and azide groups on MC-N_3. *In vivo* osteogenic differentiation of hPLSCs in the MC-BMP2 formulation was confirmed by histological staining and gene expression analyses. Histological staining of hPLSC-loaded MC-BMP2 implants showed evidence of mineralized calcium deposits, whereas hPLSC-loaded MC-Cl or BMP2-OpgY mixed with MC-Cl, implants showed no mineral deposits. Additionally, MC-BMP2 induced higher levels of osteogenic gene expression in hPLSCs than in other groups. In conclusion, the injectable in situ-forming MC-BMP2 hydrogel investigated here may be used for noninvasive administration of therapies for debilitating orthopedic conditions.

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A 3D Printed Bioreactor with Electrospun Scaffold as a Millifluidic Renal Tubule Model

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Chronic kidney disease is a major global health problem effecting millions of people, kidney tissue engineering provides an opportunity to better understand this disease and has the capacity to provide a cure. Two-dimensional cell culture and decellularised tissue have been the main focus of this research thus far with promising results [1,2]; although, these methods are not without their shortcomings. We have designed a dual flow bioreactor to be used with electrospun scaffold, delivering an even shear stress across the surface. Experiments show that as a result of stokes flow, 2 different media types can be used without mixing allowing for the co-culture of cell. Adjustment on the head of the head height of the media reservoir allows for different flow rates to be used in each chamber, allowing for different shear stresses to be delivered to either side. The bioreactor maintained a co-culture of endothelial and epithelial cells, and demonstrated the influence of fluid induced shear stress. This bioreactor is an excellent tool as a model of kidney tubules, but has applications in any area where a dual environment with a controlled shear stress is needed.

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Investigations into Potential Mechanisms of Action of A Cell-based Treatment for Severe Alcoholic Hepatitis

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Only liver transplant extends long-term survival for severe alcoholic hepatitis (sAH), characterized by decompensated hepatic function from chronic steatosis, inflammation, oxidative stress, and cholestasis. The ELAD System is an investigational (phase 3) cell-based liver treatment using VTL C3A cells, with ancillary delivery device components, to treat sAH. Numerous studies involving proteomics, metabolomics, gene expression, and *in vitro* signaling were completed, elucidating several hypothesized mechanisms of action in which C3A cell-secreted factors could provide benefit to this target population. The C3A cell secretome contains numerous proteins

associated with immune modulation, regeneration, oxidative stress, angiogenesis, transport function, hemostasis, and homeostasis. C3A cells increase expression of anti-inflammatory IL1-receptor antagonist (IL-1Ra) in response to pro-inflammatory cytokines, and IL-1Ra is significantly higher in ELAD-treated vs. control subject plasma. Amphiregulin activates the EGF receptor, soluble Fas blocks Fas-mediated apoptosis, and glutathione helps reduce reactive oxygen species (ROS), all to promote survival in hepatocytes. VEGF and other unidentified factors protect endothelial cells by blocking LPS-induced apoptosis and H_2O_2-or ethanol-induced ROS. Our research shows that C3A cells produce energy-rich molecules (e.g. creatine phosphate), consume fatty acids through beta-oxidation, and detoxify through a dynamic cytochrome P450 enzyme system. Subjects on the ELAD System have reduced levels of bilirubin, decreased levels of primary bile acids and increased levels of secondary conjugated bile acids. Further, C3A cells secrete transport proteins such as albumin, lipoproteins, and transferrin. Cell-based treatments offer advantages over monotherapies due to their ability to target multiple cell types through multiple mechanisms and dynamically respond to their environment.

Respiratory, Urologic and Gastrointestinal

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Personalized Models of Distal Airway Epithelial-Stromal Unit in COPD

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Background and Objectives: Distal airways (diameter <2 mm) represent the primary site of pathologic changes in chronic obstructive pulmonary disease (COPD), the 3rd leading cause of death in the U.S. Although clinical and pathological characteristics of COPD are well known, no effective disease-modifying therapies for targeting distal airway remodeling are currently available, largely due to heterogeneity of pathologic phenotypes and absence of reproducible models of human distal airways. The objective of this study is to establish patient-derived ''personalized'' organotypic models of human distal airways, by co-culturing epithelial basal stem cells (BSC), autologous fibroblasts, endothelial cells isolated from the same distal lung region of subjects with and without COPD, into functional, organ-level tissue.

Methods and Results: A protocol was established to isolate-propagate epithelial basal stem cells, fibroblasts and endothelial cells from the distal airways of normal and COPD lung donors. Heterogeneous cellular and molecular phenotypes in the human distal airways were characterized using immunofluorescence and single cell RNA sequencing. Patient-specific distal units were reconstructed by co-culturing BSC and autologous stromal cells using an air-liquid interface-based airway wall model and a bronchosphere-based 3D distal airway organoid assay.

Conclusion: Isolated epithelial and stromal cells from distal airways of subjects with and without COPD can be assembled into functional, organ-level tissue which mimics the architecture of human distal airways and, in patients with COPD, reproduces several distal airway remodeling phenotypes. Patient-specific models of distal airway epithelial-stromal cross-talk established in this study can be used to identify candidate pathways that mediate disease-relevant airway remodeling.

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In vitro Generation of Mouse Colon Crypts

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The lumen of the colon is made up of densely packed invaginated structures called crypts with a distinct basal-luminal cell organization. Appropriate maintenance and function of the colonic epithelium plays a role in a host of medical issues including cancer, drug absorption and bacterial infections. Unfortunately, primary colon stem cells do not grow under standard cell culture conditions, making research into these issues difficult. Recently, 3D organoid culture of colon stem cells has been shown to produce structures with a crypt-like organization, but organoids offer limited luminal access for *in vitro* study. Our lab has developed a novel platform for the culture of mouse colon crypts in a way that mimics normal physiology. Molded hydrogels provide the matrix cues and mechanical properties necessary to form an epithelial layer in the distinct crypt pattern seen in the mouse colon. Optimized chemical gradients across the basal-luminal axis maintain stem cells at the base of the crypts and a layer of continually repopulated differentiated cells at the lumen. This system offers the unique opportunity to observe the differentiation process of colon stem cells as well as study the effects of various perturbations to normal physiologic conditions.

Skin & Wound Healing

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Effective Cutaneous Gene Delivery through Dissolvable Microneedle Mediated Intradermal Delivery of Adeno-Associated Viral Vectors

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Cutaneous gene delivery has emerged for treatment and prevention of a broad range of diseases. Virus-mediated gene transfer has proven to be a more efficient and long-lasting alternative to non-viral gene expression. Specifically, adeno-associated virus (AAV) has gained much recent attention due to its low immunogenicity and long-term expression. However, the full potential of AAV-based cutaneous gene delivery has not been achieved due to (a) limitations in clinical deployment strategies beyond parenteral injections and (b) a knowledge gap in the ability of different AAV serotypes to transduce skin cells. Therefore, it is necessary to identify effective AAV serotypes for cutaneous gene delivery and to provide more effective means for enabling reproducible and efficient delivery of these vectors to patients. To this end, we first investigated the transduction efficiencies of six commonly-used AAV serotypes in *in vitro* cultured murine skin cells and identified that AAV serotypes AAV6 and AAV2 result in the highest transduction efficiencies in keratinocytes and fibroblasts, respectively. Next, we evaluated the use of dissolvable microneedle arrays (MNAs) for effective intradermal delivery of those AAV vectors to the skin. MNAs that incorporate the AAVs were created from a biodissolvable material combination Carboxymethylcellulose/Trehalose. Results demonstrate that MNA-embedded AAVs retained their infectivity. MNAs also effectively delivered 85% of their content to living human skin within 20 minutes. Importantly, MNA-delivered AAV effectively transduced murine skin cells *in vivo*, driving robust fluorescent protein expression at localized application sites. The findings presented here could significantly increase our ability to conduct reliable cutaneous gene therapy.

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Manufacturing Tissue Based On Mechanical Properties Its Native State

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Translation of regenerative medicine research from the bench to the patient is slow-moving as the result of factors such as federal approval, cost, and most frequently reproducibility. In traditional manufacturing environments, parts are comparatively measured based on initial material properties and quality of the final product, therefore improving reproducibility, which leads to lower costs and approval of products. This research aims to use a novel device to expose native biologic tissue to uniform pressure in an orbicular manner to determine mechanical properties. The orbicular pressure device (OPD) provides sufficient grip of tissue up to 9 psi, where attachment sights are not the point of failure in the tissue. Therefore, yielding true responses of the material and not capabilities of the device. During initial testing the main response variable was surface area and measured a 40% increase during seven days while exposed to pressure in the OPD. However, the OPD has the ability to provide a complete mechanical mapping of skin, the tissue of this research and other tissue types in the future. Future experiments aim to measure stress, strain, and modulus of elasticity, thickness, surface roughness, tensile strength, and yield strength. These results will provide a more complete picture of skin to aid in regenerative medicine research as it transitions to a manufacturing like process and therefore improving reproducibility.

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Tissue Engineered Polyurethane/Alginate as a Novel Wound Dressing

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Tissue Engineered Polyurethane/Alginate as a Novel Wound Dressing Sohini Sen [1], Bishnu P Sinha [2], Tapan K Mandal [2], Piyali Basak [1] [1]School of Bioscience & Engineering, Jadavpur University, 188 Raja S.C. Mullick Road, Kolkata 700032 [2] Department of Pharmacology and Toxicology, West Bengal University of Animal and Fishery Science, 37, Khudiram Sarani, Kolkata 700037

Introduction: Burn wound is sensitive issue worldwide. Polyurethane has been used as wound healing agent, due to biocompatibility, swell ability. Composite scaffolds were fabricated using polyurethane, alginate, recombinant epidermal growth factor (rEGF). Cell seeded scaffolds were evaluated for *in vivo* wound healing.

Materials and Methods: Isophoron diisocyanate based polyurethane was blended with alginate, rEGF; lyophilized to get porous scaffold followed by FTIR, SEM characterization. HUVEC, NIH 3T3 cells were co-cultured onto scaffold. Cell proliferation was estimated upto 72 h by MTT assay. Cell seeded scaffolds were applied on burn wound of Wistar rats. Gross wound healing including histopathological findings were recorded 3 to 15 days post wounding with control.

Results: FTIR, SEM results confirmed its synthesis, porous structure. In MTT assay, 96% cells were viable with exact morphology on scaffold. Cell seeded scaffold showed highly satisfactory wound healing response when applied *in vivo* within 14-15 days.

Discussion: Since cells seeded matrix showed abrupt healing capacity compared to bare polyurethane/alginate, it may be concluded rEGF incorporated, cell seeded scaffolds can serve as highly promising wound healing agent.

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Soft Tissue

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Bioreactor for Joint Segmentation Model to Investigate Toxicants/Teratogens

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In the US, alone or a combination of genetic defects and environmental insults cause about 3% of children born each year to have a major structural birth defect by their sixth birthday. The most common birth defects are skeletal abnormalities. Investigating such abnormalities is typically carried out in an animal model. However, this suffers from poor translation of the findings to humans. An *in vitro* model of human tissue can be hugely beneficial. The goal of this project is to develop three-dimensional organotypic culture models to investigate limb development and its susceptibility to toxicants/teratogens. Towards this, we are developing a biomechanical stimulus-induced joint segmentation model in a bioreactor system. The bioreactor system consists of a tissue holder containing microscale anchors for forming a tissue under investigation in straight channels. The three phases of limb development: cartilage development, hypertrophy and joint formation, is modeled in the reactor system. The first two phases are achieved by exogenous biochemical factors, whereas the joint formation/segmentation is achieved by a combination of chemical factors and mechanical stimulus. The mechanical stimulus is introduced by an actuator the subjects the tissue to undergo flexion. In this presentation, we demonstrate the efficacy of the mechanical stimulus to effect joint segmentation.

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Mechanical Behavior and Failure of Polydisperse Semi-Flexible Polymer Networks

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The classical theories for elasticity of polymer networks are built upon the assumption of network monodispersity; That is, the network is comprised from sub-chains of equal length. However, the crosslinking in polymers and biopolymers is often a random process and thus, the resultant networks are likely to be polydisperse. The effect of structural polydispersity on mechanical behavior of polymer networks is not well understood. The main goal of this research is to show how network polydispersity controls the mechanical behavior and ultimate properties of crosslinked filaments subjected to finite deformation. We propose a continuum micromechanical model based on the Langevin statistics of individual chains. The mechanical strength of network is controlled by finite-extensibility of filaments. Shorter filaments break at relatively smaller stretches. The progressive failure of the filaments continues and eventually determines the ultimate strength of the network. The predicted stress-stretch behaviors are in reasonable agreement with experimental data.

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Mechanical Properties of Human Liver

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Hepatocytes are known to respond to the mechanical stimuli of their surrounding microenvironment[1]. Liver tissue engineering aims to create a niche environment for the survival and function of hepatocytes, however little is known regarding the mechanical properties of the human liver[2].

A human liver was obtained approximately 12 hours post-mortem, kindly donated by the deceased and their relatives. Tensile and compression testing was conducted on the tissue along the X, Y and Z axis.

Results demonstrate that the Z-axis Young's modulus is 2.5 times greater than both X and Y for all strain bands represented, indicating a spatial influence upon the mechanical characteristics of the human liver, and thus upon the hepatocytes within. This data is of value to the field of liver tissue engineering when considering the factors to be addressed when designing niche microenvironments for the optimal survival and function of hepatocytes.

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1. Grant, R., Hay, D. & Callanan, A. Tissue Eng. Part A 23, 650-662 (2017).

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Stem Cells

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Regulation Of Cell Behavior By Bioactive Materials for Tissue Engineering

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It is known that the chemical composition and nano-structure are two critical factors of biomaterials which could affect cell behavior and tissue regeneration. We have designed and fabricated calcium phosphate and silicate based bioactive ceramics and composites with different chemical composition and micro structures, and demonstrated that the chemical signal released from the silicate based biomaterials and microstructural environment of calcium phosphate bioceramics stimulated stem cell behavior such as proliferation and differentiation indicating that ''tissue inducing biomaterials'' with certain chemical composition and surface structure may be designed for tissue engineering applications.

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Peripheral Nerve Regeneration Using Schwann Cells Differentiated From Human Tonsil-derived Mscs in a Charcot-marie-tooth Disease Mouse Model

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The hypothesis of this study was that transplantation of Schwann cells (SCs) into atrophic muscles in lower limbs would enable neuromuscular regeneration in a mouse model of hereditary peripheral neuropathy. We investigated the potential of Schwann-like cells differentiated from human tonsil-derived MSCs (T-MSCs) for neuromuscular regeneration in *trembler-J* (Tr-J) mice, a model of Charcot-Marie-Tooth disease type 1A (CMT1A), which involves hereditary motor and sensory peripheral neuropathies. T-MSCs differentiated toward Schwann-like cell with increased expression of Schwann cells (SCs)-related markers *in vitro*. *In situ* transplantation of T-MSC-SCs into the caudal thigh muscle in Tr-J mice enhanced motor function proved by a rotarod test and measurement of sciatic function index. Morphology of the sciatic nerve and skeletal muscle recovered without the formation of teratomas. The sciatic nerves in the T-MSC-SCs-transplanted mice revealed regenerative morphology in myelin sheaths and axons. Transplantation of T-MSC-SCs could enable neuromuscular regeneration in patients with CMT1A. Further studies such as the assessment of neurotrophic factors and regeneration potential of neuromuscular junctions might provide more insight into the outcomes of transplantation of T-MSC-SCs for patients with Charcot-Marie-Tooth disease.

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A New Molecular Target to evaluate and modify Mesenchymal Stem cell-Chondrogenesis Towards Articular Cartilage

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Mesenchymal Stem Cells (MSCs) derived cartilage significantly differs from articular cartilage (AC). We aim to use a novel AC molecular fingerprint as a platform to evaluate and modify molecular events during MSC chondrogenesis. We hypothesize that mechanistic processes during chondrogenesis can be modulated by transcriptional regulatory elements systematically selected from a robust molecular analysis of native AC. We performed whole-genome expression analysis on de-identified AC from young cadaveric knees, or adult MSC cultures. AC is dissected from de-identified specimens (n = 5) procured from consented guardians of donors. MSC cultures from de-identified donors (n = 5) were established and differentiated as previously described[1]. Total RNA was prepared using standard procedures and whole-genome expression analysis was performed using an Illumina platform. MultiExperiment-Viewer was used for data analysis. The transcriptomal profile of AC was globally distinct from MSC-derived cartilage with over 500 genes highly expressed in AC and not expressed during MSC chondrogenesis. Differences were minor during initial stages (first 7 days) of chondrogenesis. This suggests that osteochondral fate of MSC-derived cartilage may be re-routed during earlier stages. We identified key transcription factors that could be molecular inductors of AC. A specific molecular phenotype of high-quality AC was identified as a gold standard for tissue-engineered AC. This data will be useful for the design of successful clinical application using MSCs and the generation of a robust read-out profile of AC novel markers.

Reference: 1. Correa, D. *et al. Osteoarthr. Cartil.* **23,** 443-453 (2014).

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Engineered Phage Matrices Facilitate Angiogenic Differentiation Of Adipose Derived Stem Cells

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Although stem cell niche plays a vital role in stem cell differentiation towards different lineages, an artificial stem cell niche achieved so far is not successful to fulfill the complex microenvironment of the stem cell. Here, we demonstrated engineered hybrid phage matrices that possess cell adhesive and angiogenic peptides with a suitable scaffold by formulating polyacrylamide hydrogel incorporating phage in different stiffness to guide adipose derived stem cells (ASC) and could achieve higher stiffness favoring osteogenesis and lower stiffness favoring adipogenesis. In this study, we present a specific phage based angiogenic matrices by modulating physical and biochemical cues in differentiation of ASC, providing convenient artificial stem cell niche.

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A Phenotype-Specific Gene Circuit to Regulate Chondrocyte Maturation

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Mesenchymal stem cells have great potential to be used for cartilage repair; however, cartilaginous tissues formed by MSCs typically have inferior mechanical properties compared to native cartilage, as these cells tend to mature to hypertrophy in which they stop producing and start degrading structural matrix macromolecules, like aggrecan and type II collagen. Preventing the maturation of MSC-derived chondrocyte using the growth factors is often compromised by their short half-life and conflicting effects on heterogeneous cell populations. To allow individual cells to resist maturation as needed, we have developed a gene circuit containing a tunable promoter (CXpcis) that is designed to activate exclusively in pre-hypertrophic chondrocytes and inhibit the activity of Runx2, one of the key drivers of hypertrophy. The Col10a1 basal promoter was placed upstream of Runx2 short-hairpin RNA. The kinetics of Runx2-shRNA expression was regulated with the inclusion of different copies of cis-enhancer upstream of the basal promoter. Human and mouse chondroprogenitors that stably express CXpcis-shRunx2 circuits or doxycycline-inducible shRunx2 were assessed for early and late chondrogenesis markers in monolayer and pellet cultures. The synthetic Col10a1 promoter can specifically target chondrogenic cells with tunable kinetics by varying the copies of cis-enhancer. Using the inducible shRunx2 system, we verified that the timing of Runx2 silencing significantly affects chondrogenesis, matrix accumulation, and hypertrophy, highlighting the necessity of phenotype-specific RNAi expression in therapeutic applications. CXpcis-shRunx2 circuits allowed cells to grow into larger chondrogenic aggregates, accumulate a higher amount of proteoglycan-rich matrix, and decrease Runx2 expression over five-week culture period in pellet culture.

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Dynamic Migration Of Dental Pulp Stem Cell - derived Chondrogenic Cells In Collagen Hydrogels

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The biological approach targets therapy that preserves the intervertebral disc (IVD) function by regenerating the degenerated and damaged nucleus pulposus (NP). Biomaterial hydrogels that mimic the NP extracellular matrix (ECM) can serve as stem cell carriers for cell transplantation and a matrix for replacement of the degenerative NP. Stem cells derived from dental pulp have received growing attention because they have a similar differentiation capability as other mesenchymal stem cells (MSCs) and they are relatively easy to obtain. Type II collagen, as a major component of NP, has been fabricated as a hydrogel and studied for the growth of NP cells and MSCs. The migration of transplanted cells within the collagen hydrogels or NP is critical because migration allows the cells to replace those lost in degenerated NP. In this study, we differentiated dental pulp stem cells (DPSCs) into chondrogenic cells. The cells were grown in type I and type II collagen hydrogels, and the cell motility in these materials were determined by time-lapse imaging. We found DPSC-derived chondrogenic cells migrated dynamically in these hydrogels. Quantification of cell migration revealed the the higher motility in type I collagen hydrogel than type II collagen hydrogel. Our study explored the differential cell motility of DPSC-derived chondrogenic cells in these collagen hydrogels.

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In Vitro Osteogenesis of Periosteal Tissue through Endochondral Ossification

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Introduction: The cambium is a highly cellular layer of the periosteum containing progenitor cells which are involved in the process of fracture repair. Previous work has shown that *in vitro* endochondral ossification can be induced from these cells, but is limited to young animals using tissue adjacent to the growth plate. In this study, we have explored the potential of adult-derived rib periosteum, not near a corresponding growth plate, as a more clinically relevant tissue/cell source for bone tissue engineering.

Materials and Methods: Adult rabbit rib periosteum was harvested (ACC 563) and 4mm×4mm explants were cultured in maintenance, chondrogenic, or osteogenic media for 4 weeks. Periosteal cells were isolated by egression or Ficoll separation, expanded, and then cultured in osteogenic or chondrogenic media. All cultures were analyzed by histology.

Results: Untreated explants showed minimal presence of alkaline phosphatase (ALP), whereas chondrogenically treated explants showed the presence of proteoglycans and minimal ALP. Osteogenically treated explants showed more extensive ALP and proteoglycans with mineral deposits in the cambium. Isolated cells (osteogenic or chondrogenic induced) showed no evidence of ALP or mineralization, irrespective of isolation method.

Discussion and Conclusion: *In vitro* endochondral ossification of rib periosteal explants can be promoted indicating that this site may be a viable source for bone tissue engineering. The inability of isolated cells to directly become osteogenic indicates that additional endochondral ossification stimulation is necessary. Further work with isolated cells will include a chondrogenic pre-culture/template prior to osteogenic induction.

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SaCas9 Mediated Genome Editing in Mammalian Cells

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Introduction: CRISPR/CAS9 system has shown a promising potential therapeutic application as a genome engineering tool. In this study, we investigate CRISPR/CAS9 system to correct mutations in HBB (hemoglobin beta subunit) gene causing Sickle cell anemia (SCA) and IL2RG (interleukin 2 receptor subunit gamma) causing Severe Combined Immune Deficiency (SCID-X1).

Materials and Methods: A lentiviral vector (pSin-EF2-IL2RG-HBB-GFP) for GFP reporter cell line was made including *IL2RG* and *HBB*. The reading frame of GFP could be restored only if Cas9 targets and cuts these genes leading to deletions or insertions. Adeno-associated viral serotype 6 (AAV6) vectors expressing SaCaS9, template and locus specific guide sequences were designed. We also assessed the ability of inserting a larger fragment by adding extra GFP to the template. We evaluated these systems in HEK293T cells, GFP reporter cells and human Hematopoietic Stem cells (hHSCs).

Results: SaCaS9, guide and the template delivered in AAV serotype 6 could transduce HEK293T and hHSCs with 40% efficiency with 10% HR for SCA. However, HR rate decreased to 0.3% when foreign GFP cDNA sequence was inserted. Similarly inserting an IL2RG cDNA into the locus without removing the previous gene for SCID-X1, showed low HR efficiency (0.3%) despite an Indel rate of 30%, suggesting that inserting a larger fragment decreases the efficiency of HR dramatically.

Conclusion: AAV6 can efficiently deliver SaCas9, guide RNA and template into hHSCs with high rate of HR as long as the template does not differ much in terms of size or sequence with target sequence in the genome.

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Improving the Osteogenic Potential of Human Adipose Tissue-derived Mesenchymal Stem Cells using a Cytoskeletal Modifying Drug for Bone Tissue Repair

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Strategies for tissue regeneration utilize adult mesenchymal stem/stromal cells (MSCs) that can be obtained from bone marrow- and lipo-aspirates. Human adipose tissue-derived MSCs (hAMSCs) can be harvested in large quantities required for cell-based tissue-engineering approaches, but are generally considered to be less osteogenic than bone marrow MSCs. Herein, we tested a new molecular strategy to improve the osteogenic differentiation ability of hAMSCs using Cytochalasin D (CytoD), a secondary metabolite derived from molds. We show that CytoD, which reorganizes actin cytoskeleton, is a potent osteogenic stimulant as reflected by significant increases in alkaline phosphatase activity, extracellular matrix mineralization and osteoblast-related gene expression (e.g., RUNX2, ALPL, SPARC and TGFB3). RNA sequencing (RNA-seq) analyses of MSCs revealed that acute CytoD treatment (24 hr) stimulates a broad program of osteogenic biomarkers and epigenetic regulators. CytoD decreases mRNA and protein levels of the Polycomb chromatin regulator Enhancer of Zeste Homolog 2 (EZH2), which controls heterochromatin formation by mediating trimethylation of histone 3 lysine 27 (H3K27me3). These findings conclude that CytoD is a potent osteogenic stimulant that can be used to precondition hAMSCs to attain an osteogenic phenotype for skeletal tissue engineering applications.

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Regulation of Cell Reprogramming in 3D Microenvironment Interactions

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After the introduction of induced pluripotent stem cells (iPSCs), various methods to reprogram the somatic cells into pluripotent state have been investigated. Along with transducing agent and supplemented chemical substances to improve reprogramming, cell culture substrates were highly explored. Recent study have been shown that chemically defined PEG hydrogel enhances reprogramming by promoting mesenchymal-to-epithelial transition (MET) as well as epigenetic remodeling. However, cell/microenvironment interaction on cell reprogramming were not clearly defined. In this study, we look at various 3D microenvironments to find possible candidate to improve reprogramming cells significantly, and investigate its interaction that are involved with. Selected microenvironments were used to encapsulate the reprogramed cells by photopolymerization system, and analyzed its pluripotency, MET, and epigenetic state. Throughout many hydrogel systems, hyaluronic acid hydrogel microenvironment highly improves reprogramming efficiency by regulating pluripotency, MET, and epigenetic plasticity. These results show that this facile system may produce more purified reprogrammed cells using 3D microenvironment hydrogel.

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Cyclic Forces Induce Rapid Differentiation of human embryonic stem cells

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Mechanical forces play critical roles in human embryonic stem cell (hESC) fate decisions during development. Investigation of the intrinsic mechanosensitivity of hESCs is limited by the difficulty in applying spatiotemporally controlled forces to cells with subcellular resolution. Here, we used an ultrasound technique, acoustic tweezing cytometry (ATC), to exert cyclic forces to hESCs by acoustic actuation of integrin-bound microbubbles. We found that ATC-mediated cyclic forces applied to a subpopulation of hESCs induced global responses in hESC colonies, including increased cellular contractile forces, enhanced calcium activities, downregulation of the pluripotency transcription factors Oct 4 and Nanog, and cytoplasmic YAP localization, leading to initiation of rapid differentiation and characteristic epithelial-mesenchymal transition (EMT) events that depend on focal adhesion kinase activation, cytoskeleton tension, and myosin II activities. These results reveal robust mechanoresponses of hESCs to integrin-targeted cyclic forces and efficient orchestration of integrin and E-cadherin in EMT and differentiation.

Tissue Chips & Tissue Organoid Models

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Advanced Self-organized Hepatic Microtissue: Liver Organoid Development That Can Regulate Hepatic Function According To Hepatic Progenitor Maturity And hASC Co-culture

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Liver tissue engineering has an attention in regenerative medicine for the treatment of liver failure and *in vitro* model for liver toxicity test. Classically, adherent cells have been expanded as monolayer cultures; recently, numerous studies have discussed the advantage of 3D cell aggregates, demonstrating that 3D aggregate or spheroid cultures can improve the differentiation. Most cells are present three-dimensionally while in contact with the neighboring cells surrounded by extracellular matrices in a tissue. Mesenchymal cells are directly involved in the production of ECMs in the natural tissue. In our study, induced hepatocytes were co-aggregated with hASCs utilizing a biofunctional matrix for self-organizing tissue. Hepatic phenotypes of induced hepatic progenitor in immature stage were reduced in 3D culture compared to in 2D culture. When being co-cultured with hASCs both in 3D and 2D cultures, the hepatic phenotypes decreased compared to hepatocyte alone. Interestingly, these features showed an opposite tendency to enhance hepatic function in response to increased maturation of the induced hepatic progenitor cells. Furthermore, we have found that conditioned media from hASC in 3D and 2D cultures can also reduce or increase hepatic function in ambivalently depending on the maturity of miHeps. This result provides clues that liver function of 3D co-cultured miHeps can be regulated through secreted factors from hASC. Overall, our study provides insights into the development of a more biologically advanced liver organoid that can more accurately mimic the physiological characteristics of real human liver tissue through regulation of maturity in hepatocyte source and co-culture of hASC.

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Generation and Optimization of Renal Organoids from Whole Kidney Cells for Drug Screening

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Introduction: Kidney is the essential organ in the process of drug metabolism. Renal organoids made from embryonic stem cells and iPS cells have been reported before. We hypothesize such renal organoids have approximate characteristics with normal kidney so as to have the potential applications in drug screening.

Methods: Human renal cells were isolated from unused donor kidneys and characterized. Whole cells from kidneys were expanded to form organoids by seeding in Hanging Drop plates. Morphology of organoids were observed by microscopy and H.E staining. Proliferation curve was generated by ATP measurement. Live/Dead staining was applied for viability assay. Functional tests were carried out using ELISA kits for erythropoietin produced in hypoxia condition. Also, the γ-Glutamyltransferase activity in normal renal organoids and drug-treated organoids were compared.

Results: Cells obtained from whole kidney could form solid organoids. Proliferation curve and Live/dead staining demonstrated organoids grew well *in vitro*. In function assay, we found the renal

organoids assembled from passage 1st kidney cells secreted EPO protein in hypoxia condition. Additionally, aspirin and penicillin G were used as nephrotoxic drugs to test kidney function measured by GGT level which presented amino acid transformation.

Discussion and Conclusions: Human whole kidney cells demonstrate the ability to form functional organoids which may ultimately be developed into an efficient research and clinical application for drug screening.

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Biomimetic Cardiac Hypertrophy Model by Engineering Continuous Beating 3D Cardiac Microtissues

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Cardiac hypertrophy is accompanied with a number of heart diseases, including ischemic heart disease, hypertension, valve disease, and heart failure, which are associated with high morbidity and mortality. Cardiomyocytes in cardiac hypertrophy respond to hemodynamic stress, decrease cardiac beating and increase in their size, resulted in cardiac remodeling. However, underlying molecular and cellular mechanisms are not well known yet. Recent studies reported that injured cardiac fibroblast-derived mediators may act in autocrine and paracrine communications between fibroblasts and cardiomyocytes to induce cardiac remodeling. Here, we investigated a potential paracrine exosome crosstalk between cardiac fibroblasts and cardiomyocytes. We engineered functional cardiac spheroids of human iPS cell-derived cardiomyocytes with synchronous beating in the heart by co-culturing human endothelial cells and human fibroblasts at the approximate ratios of *in vivo*. Then, we confirmed that these engineered continuous beating 3D cardiac microtissues could be utilized as 3D myocardial hypertrophy model by spheroid size, gene expression level, immunofluorescence staining and the beating rates. These findings demonstrate that injured cardiac fibroblast secrete exosomes as paracrine signaling mediators and affect cardiomyocytes, resulted in cardiomyocyte hypertrophy. We suggest that this biomimetic three-dimensional cardia hypertrophy system is a promising alternative to an animal experimental model for studying cardiac disease, as well as a drug screening system for finding therapeutic targets.

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Development of Human Pancreatic Islet Organoids from iPSCs

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Previously, we reported the generation of islet-like cell clusters from mouse embryonic stem cells. Building upon these successes, we herein report the successful biofabrication of human pancreatic islet organoids from iPSCs. While islet transplantation is promising for diabetes treatment, it has not been available to a majority of patients due to the scarcity of donors. The generation of islet organoids entailing mature beta cells has yet been demonstrated. In this work, we demonstrated the feasibility of developing human islet organoids from iPSCs. We showed that a 3D microenvironment is critical to the generation of pancreatic endoderm and endocrine. The organoids formed consisted of pancreatic alfa, beta, delta, and pancreatic polypeptide (PP) cells. A high level co-expression of PDX1, NKX6.1, and NGN3 in these organoids suggests the characteristics of pancreatic beta cells. More importantly, most insulin-secreting cells in the organoids did not express glucagon, somatostatin, or PP. The expression of mature beta cell marker genes such as Pdx1, Ngn3, Insulin, MafA, and Glut2 was detected in the orgnoids. Insulin-secretory granules, an indication of deta cell maturity, were detected in these cells. Glucose challenging experiments suggested that the organoids are sensitive to glucose levels due to their elevated maturity. In conclusion, we showed the generation of islet organoids from iPSCs. The further optimization of the process will enable the biofabrication of islets. The fabricated islets can be used for diabetes treatment, drug screening/toxicity validation, and pathological study.

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In Vitro Characterization of Murine Colonic Epithelial Stem Cells under Physiological Conditions

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As links between the gut microbiome and human health continue to emerge, the establishment of a relevant *in vitro* model of the gut is becoming increasingly urgent. To develop a simple *in vitro* model for gut research, primary murine colonic epithelial cells were cultured under conditions physiologically comparable to the *in vivo* environment. These conditions included low oxygen tension and low glucose concentration. Cells formed monolayers composed of all cell types observed *in vivo* including stem cells, proliferative cells, goblet cells, and enterocytes. After a 24 hour exposure to low oxygen and glucose, a significant drop in proliferative activity was observed. Cell survival in these conditions suggests that this model can be used for the study of interactions between the gut epithelial cells and anaerobic bacteria. The successful *in-vitro* co-culture and analysis of primary colonic cells with bacteria will represent a significant contribution to gut microbiome research

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Exploring Normal and Malignant Hematopoietic Stem/Progenitos Cell-Niche Interactions Using a Novel Bone Marrow-on-a-Chip Platform

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Under normal conditions, hematopoietic stem/progenitor cells (HSPC) reside within specific bone marrow (BM) niches. These are comprised of an array of different cell types located strategically to provide myriad chemical signals and physical interactions that maintain HSPC. In human myeloid malignancies, the BM niche is remodeled by malignant cells, which displace resident HSPC, and create self-reinforcing malignant niches that drive disease progression, chemo-resistance, and relapse. We engineered a microfluidic platform, inside of which 4 individual hyaluronic acid-based niche constructs were patterned *in situ*. The corresponding niches contained BM-derived mesenchymal cells (Stro-1+; MSC), arterial endothelium (CD146+NG2+; AEC), and sinusoidal endothelium (CD146+NG2-; SEC). A fraction of the Stro-1+cells were induced to undergo osteogenic differentiation, to generate osteoblasts (OB) for the fourth niche. U937 (lymphoma), MOLM13 (leukemia), and normal $CD34^+$ cells were fluorescently tagged and independently perfused into the system. At day five post infusion, immunocytochemistry demonstrated the continued expression of appropriate phenotypic markers by each respective niche. U937 cells exhibited a marked predilection for AEC over the other niches, and normal $CD34^+$ cells engrafted preferentially within the SEC and OB niches. Our studies establish the feasibility of recreating and testing various BM microenvironments-on-a-chip, derived from the same donor, and demonstrate that this novel system can be used to study preferential interactions of normal and malignant HSPC with distinct niches.

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A Complex Model for Studying Human Vascular Disease in a High-Throughput Microfluidic Platform

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Introduction: Current drug discovery pipelines rely heavily on simple *in vitro* cell cultures that fail to recapitulate the complexity of human tissue. This project aims to develop a complex model of human vascular biology that incorporates multiple cell types, extracellular matrix (ECM), and fluid shear stress in a novel, high-throughput microphysiological system (MPS). Although applicable across multiple diseases, here we focus on modeling perivascular fibrosis, a significant healthcare burden for which no FDA-approved drugs currently exist.

Materials and Methods: Human ECs were co-cultured in Draper's bilayer MPS with multiple support cell types (smooth muscle cells or pericytes). The co-cultures were maintained up to 7 days and assayed for viability, proliferation, permeability/barrier function, and immunostaining for tissue specific and functional markers. Fluid shear stress (0.025 - 10 dyn/cm^2) was applied using a syringe pump. As an initial model for disease, pericytes were activated using TGFβ1.

Results: The configurability of the MPS allowed for permutation of multiple EC and support cell types co-cultured across a range of ECM compositions and shear stress profiles to differentially control EC phenotypes as monolayers or microvascular networks. Proof of concept studies have also demonstrated activation of pericytes on various ECM substrates for future incorporation into the MPS model.

Discussion and Conclusions: The vascular MPS provides a versatile platform for integrating multiple cell types with key biophysical cues. This complex system will provide a more physiologically relevant *in vitro* model for human vascular diseases.

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An *in vitro* Continuous Primary Intestinal Epithelial Monolayer for Evaluating Xenobiotic Transport and Metabolism

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Following oral xenobiotic administration, one of the main sites of absorption occurs in the small intestine, where the compound is subject to numerous transporters and the effects of metabolism. For example, cytochrome P450 3A4 (CYP3A4) is the dominant drug metabolizing enzyme in the human small intestine and P-glycoprotein (P-gp) is expressed at high levels in the epithelial cells of the intestine. One approach to the evaluation of intestinal metabolism of xenobiotics is the use of tumor cell lines, such as Caco-2, MDCK, and LLC-PK1, although these cells lack or have low expression of CYP3A4. To address such limitations, a cellular monolayer derived from human primary small intestinal stem cells was cultured on a biomimetic scaffold. The measured trans-epithelial electrical resistance (>100 Ω cm^2), lucifer yellow permeability (<2×10^{-7} cm s^{-1}), and positive tight junction staining (E-cadherin) indicated a monolayer possessing appropriate tight junctions. The expected gene expression for transporters and metabolic enzymes was revealed by RNA seq. Targeted quantitative proteomic analysis via LC-MS/MS provided measurable levels of CYP3A4 and P-gp. Rhodamine 123 was used as a substrate to evaluate P-gp transport indicating highly active efflux function ($P_{app,\ efflux}/P_{app,\ influx} = 22$). This tissue model is promising as a means to evaluate xenobiotic absorption and metabolism, which would greatly facilitate screening of compounds for pharmacologic efficacy or toxicity.

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Generating Functionally Mature iPSC-Hepatocytes Through Innovative High-Throughput Bioengineered Culture Platforms

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Mature hepatocytes produced from induced pluripotent stem cells (iPSCs) could afford renewable and precise predictive models for personalized drug screening. Unfortunately, current iPSCs-derived hepatocytes largely lack functional metabolic and protein activity. By combining innovative engineering platforms with biological throughout discovery strategies, we have designed a streamlined cell differentiation and processing protocol to generate highly functioning iPSC-derived hepatoblasts and hepatocytes. This process utilizes unique stage-specific defined biochemical environments within a custom high-throughput culture platform that incorporates precision pump mechanisms to imitate physiologically-relevant biophysical and biomechanical hepatic parameters, including stage-specific precision flow patterns and nutrient replenishment schemes. We have reproducibly generated iPSC-Hepatocytes from 3 distinct iPSC lines that surpass most industry and academic levels of established functional adult hepatocytes metrics, including protein levels of a1αT, albumin, cyp3a4, cyp1a2, and cyp2b6, NAT2, and UGT1a1 that are within at least 50% of primary hepatocyte gold standards, while displaying reduced levels of immature AFP and cyp3a7 levels. Expression levels of most of 30+ mature genes and markers are also at similar levels to primary hepatocytes. We are currently applying these cells toward specific liver disease tissue-on-a chip organoid models, including NASH and inflammatory-based diseases, as well as investigating the robustness of this innovative maturation protocol towards larger arrays of IPSC lines. By utilizing advanced bioengineering hardware to generate biological discovery we have generated highly functional iPSC-Hepatocytes to advance personalized hepatic regenerative medicine.

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Bioproduction of a Universal, Chemically-Defined, and Xeno-Free Cell Culture Media

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Introduction: Animal-derived serum supplemented into cell culture media, while effective in supporting cell proliferation and phenotype maintenance in primary cell culture, lacks batch consistency and cannot be fully chemically defined. These deficiencies result in variable performance among serum lots. Substituting bioregulatory serum factors with human sourced factors or small molecule bioregulatory factors would allow for the development of standard media formulations that could simplify clinical manufacturing process development and accelerate the translation of regenerative medicine therapies to clinical deployment.

Materials and Methods: Media formulations were evaluated on human primary cells, derived from each of the three embryonic germ layers, using standard cell culture techniques. The performance of prototype media formulations in terms of promoting cell proliferation was determined using the IncuCyte S3 Live Cell Imager, while immunofluoresence was performed to confirm maintenance of cell phenotype.

Results and Discussion: The first-generation media formulations proved to be particularly supportive for human primary cells derived from mesoderm and ectoderm, in some cases outperforming the cell suppliers recommended media that contained undefined supplements. These media contained either the undefined but well characterized supplement, human platelet lysate (HPL) or our bovine pituitary extract replacement cocktail. The media not only increased proliferation rates of mesoderm and ectoderm-derived cells, but also prevented changes in immunophenotype. A second-generation media containing defined substitutes for the most prominent bioregulatory constituents of HPL has been formulated and is currently undergoing testing. Finally, several

synthetic molecules have been identified that provide support for human primary cell proliferation and phenotype maintenance.

Conclusions: These data are being used to inform the development of a prototype standardized medium for regenerative medicine clinical manufacturing. The future vision for the media is to have a standardized and well defined base medium for cells derived from each of the three embryonic germ layers. This will provide a standardized cell platform for the clinical manufacturing of regenerative medicine therapies.

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Development of nanoparticles from a biofabricated fructose polymer

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Introduction: A few polysaccharides have been reported for nanoparticle development in regenerative medicine [1]. Levan is a biocompatible polyfructan, but its potential has not been fully investigated. Here, we developed levan-based nanoparticles (LNPs) as a novel nanobiomaterial.

Methods: Levan was produced by *Bacillus subtilis* var. *Natto* cultivation for 23 h using a 5L-bioreactor (pH 7, 37 °C, 950 rpm) and characterized by thin-layer chromatography, FTIR spectroscopy and ^{1}H NMR/^{13}C NMR. LNPs were produced by nanoprecipitation [2] and characterized by particle size, zeta potential and transmission electron microscopy.

Results: The biofabricated levan was formed only by fructose. The FTIR spectrum showed characteristic bands such as that corresponding to the glycosidic bond. The ^{13}C NMR spectrum exhibited six strong resonance signals, with the signal δ106.71 (C-2) relative to the quaternary anomeric carbon. LNPs sizes varied with the increase in levan concentration: 226 nm, 250 nm and 324 nm for 0.5, 5.0 and 10.0%, respectively. The polydispersity of LNPs was found to be narrow at low polymer concentrations and it widened with increasing levan concentration. The zeta potential varied from −7.9 mV to −12.9 mV. LNPs were spherical with negligible aggregation at low polymer concentrations.

Discussion and Conclusions: Levan produced by *Bacillus subtilis* var. *Natto* was able to form nanoparticles intended for drug delivery and tissue engineering with adequate size, charge and morphology.

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Exploiting Macrophages For Vessel Development

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Under certain conditions, macrophages can dramatically enhance and support angiogenesis. Generally, there are two main classifications of macrophage phenotypes: M1, thought to be pro-inflammatory and M2, thought to be pro-tissue healing. We are developing scaffold materials that offer the opportunity to control macrophage phenotype locally, thus providing a pathway to exploit the beneficial actions of macrophages. Our scaffold is a 3D poly(ethylene glycol)(PEG)- based hydrogel that has been modified to support cell-adhesion (via incorporation of the cell adhesive peptide RGDS) and to be proteolytically degradable (via incorporation of GGGPQGIWGQGK, a matrix metalloprotease-2 and -9 sensitive peptide). This bioactive scaffold supports the spontaneous formation of blood vessel networks when endothelial cells (ECs) and support cells are encapsulated into the 3D matrix. To investigate the influence of macrophage phenotypes on vessel formation, macrophages were stimulated towards a M0, M1 or M2 phenotype and then encapsulated with ECs into the 3D matrix. We found that M0 + ECs enhanced vessel volume by nearly two-fold when compared to ECs alone. M2 + ECs enhanced vessel volume by nearly three-fold while M1 + ECs reduced vessel volume to one-tenth of the ECs alone. IL-4 and IL-13, known stimulators of the M2 phenotype, can be covalently immobilized into the scaffold materials for local stimulation of the M2 phenotype. Thus, the bioactive scaffold can be used for local control of macrophage phenotype in order to enhance vessel formation.

Undergraduate Posters:

Undergraduate Poster Session

Monday, December 4, 2017 7:00 am - 8:00 am

Undergraduate Poster: U1

Microfabrication of Posts to be used with Bioengineered Muscle Tissues for High Throughput Screening of Inflammatory Modulators

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Current platforms make use of bioengineered muscle tissues (BMTs) that contract around two flexible posts. However, they rely on a single testing unit per well and the contraction of the muscle cells around the posts can take up to a week or longer depending on the cell type. The present project focused on creating multiple smaller posts within the same well to increase contraction speed. Overall, the goal was to provide faster hydrogel contraction and multiple readouts from a single field of view using a standard microscope. We developed two post designs: 5 or 16 posts within single well in a 24-well plate. Molds are micro-milled from aluminum and polydimethylsiloxane (PDMS) used to cast the molds. Prior to seeding, PDMS posts were plasma treated and coated with bovine serum albumin. Molds were seeded with different hydrogel gels containing different types of muscle cells. We also tested the effects of platelet-like particles (PLPs) and dermal fibroblasts on the speed contraction. All posts were seeded at concentration of 20,000 cells/μL. Smaller posts required less time to contract. Imaging results found that both fibrin and collagen served as effective hydrogels, with comparable tissue formation times. PLP addition was also shown to improve hydrogel contraction when combined with fibrin hydrogels. By expediting preparation of the posts and tissue formation, the new methodology cuts down BMT formation 2-3 days. The smaller design also allows us to test more conditions under a single field in a microscope.

Acknowledgments: We thank the Comparative Medicine Institute's Summer Interdisciplinary Summer Initiative for their support throughout the project. Also, we thank the CMI and NC TRACS (5SOKR141616) for their funding. I would also like to thank the Park Scholarships for their guidance throughout my undergraduate career.

Undergraduate Poster: U2

Laser triggered thermoplastic shape memory polymeric particles encapsulating gold nanoparticles for biomedical applications

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Shape memory polymers (SMPs) are materials that can be modulated in shape, then returned to their original shape when externally

stimulated, in an entropy driven process called the shape memory effect (SME).[1] This feature can be utilized in polymer micro and nanoparticles for biomedical applications, as the optimal shape for a particle *in vivo* often changes with respect to time. For example, ellipsoidal particles resist nonspecific uptake.[4] Modulation of shape therefore allows for modulation of therapeutic properties.[2] This study sought to optimize SMP particles for rapid shape switching at biocompatible temperatures. The particles were synthesized via bulk emulsion from poly(D,L-lactic acid) (PDLLA), encapsulating gold nanoparticles. They were then cast into a PVA film, and stretched in an automated device at a low temperature of 65C, and a high temperature of 90C.[3] Shape reversion was tested via bulk heating, then by laser irradiation, mediated by inductive heating of the encapsulated gold particles. The shape memory particles were also tested *in vitro*. They were added to macrophages, which were then exposed to short-term bulk heat at 42C, after which particle uptake was assessed. The SME is observed in 65C stretch particles for both bulk heat and laser irradiation, while the 90C stretch particles maintained their shape. Confocal microscopy of macrophages exposed to the particles showed an increase in uptake after bulk heat for particles stretched at low temperatures, over a range of doses. SMP based particles represent an innovative therapeutic platform, with promising applications in drug delivery and beyond.

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- Correspondence may be sent to: green@jhu.edu.

Undergraduate Poster: U3

3D Printing Elastin-Like Protein (ELP) with Oxygen-Sensing Boron Nanoparticles

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Homogenous oxygen delivery when designing 3D printed bioscaffolds has remained a critical unsolved challenge impeding cellular viability and effective implantation[1]. With oxygen diffusion limited to 200 μm *in vivo*, oxygen perfusion to cells imbedded within the scaffold must be monitored to prevent hypoxia and promote cell growth[2,3]. In order to monitor oxygenation over time in 3D bioprinted scaffolds, we designed a strategy to incorporate oxygen-sensing boron nanoparticles (BNPs) into a printable bioink. A solution of elastin-like protein (ELP), a synthetic protein that mimics the properties of the protein elastin, was mixed with Xantham Gum (XG) at 8% (w/v), to produce a sheer-thinning, printable hydrogel. The BNPs are dual emissive; they emit fluorescence and phosphorescence when excited by ultraviolet light. The fluorescence to phosphorescence ratio (F/P) quantitatively indicates oxygen levels, which can be represented as a colorimetric map. The BNPs were mixed with the ELP-XG hydrogel and 3D printed as a ''cross-hatch'' structured scaffold, which was immediately crosslinked using tetrakis(hydroxymethyl) phosphonium chloride (THPC). Ratiometric images of the bioscaffold with embedded BNPs revealed different oxygenation levels throughout the scaffold. The oxygen-sensing capacity in the 3D-printed construct was verified by flowing either oxygen or nitrogen over the construct, which caused increases or decreases in the F/P ratio, respectively. We successfully incorporated oxygen-sensing nanoparticles into a 3D-printable ELP-XG bioink and validated the scaffold's ability to indicate fluctuations in oxygen levels. Future work will validate this strategy for monitoring oxygen levels in 3D-printed constructs containing live cells.

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Undergraduate Poster: U4

Fe^{3+} and Photocrosslinkable Tissue Adhesive Intended for Surgical Wound Healing

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There is a growing need for sealants in the facilitation of surgical operations in situations where traditional suturing may prove unsafe, impractical or ineffective. Fibrin, cyanoacrylates, and aldehyde-based tissue adhesives offer a greater flexibility in usage, but there are inherent biocompatibility and toxicity risks involved that restrict the use of these products in clinical applications. Hydrogels have been proposed as viable alternatives, but it has been a great challenge to develop a hydrogel which forms rapidly and has strong adhesion on wet surfaces. In this study, we engineered a double-crosslinked tissue adhesive (DCTA) comprised of a dopamine-conjugated gelatin methacryloyl (GelMA) biopolymer. Fe^{3+} ions are used for rapid crosslinking followed by a long-term acting photocrosslinking mechanism composed of the photoinitiator Eosin Y, co-initiator triethanolamine (TEA) and the co-monomer poly(N-vinylcaprolactam) (VC). GelMA was formed in a direct reaction of methacrylic anhydride with gelatin. Dopamine was grafted onto GelMA using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and *N*-Hydroxysuccinimide (NHS) coupling chemistry, the catechol groups of which can be oxidized to form quinones that are capable of providing strong adhesion to nucleophile groups on tissue surfaces. This novel DCTA exhibited an adhesive strength >100 kPa higher than fibrin-based glues when performing lap-shear tests on wet porcine skin[1]. The DCTA also displayed an elastic modulus of >200 kPa that makes it an ideal candidate for gluing flexible soft tissue. This newly developed adhesive hydrogel is highly promising for internal wound closure, hemostasis, and preventing infections post surgery.

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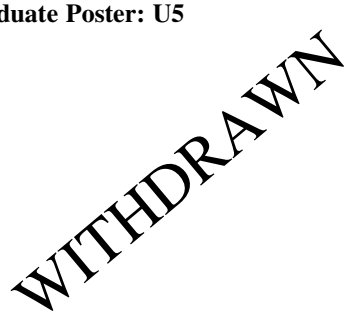
Disclosures: The author has nothing to disclose.

Undergraduate Poster: U5



Undergraduate Poster: U6

Heuristic Computational Modeling of Nutrient Role in Fusion of Cellular Spheroids Useful for Scaffold-Free Biofabrication

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Cellular spheroids are useful as tissue models and as building blocks for biofabrication. These cellular aggregates are in general non-perfused, and thus susceptible of nutrient deprivation, with little-explored effects on their suitability for further 3D assembling. This is particularly relevant for complex tumor models, developed for drug discovery and personalized treatments. We modeled the impact of glucose availability on spheroids fusion rate and on larger-scale ensuing structure, with emphasis on incorporation of tumor spheroids among the normal cell spheroids in the constructs. We used a tumor spheroid model with a bi-dimensional diffusion field for glucose, implemented in the multiscale modeling platform CompuCell3D. We also performed fusion experiments with actual tumor cell spheroids, at normal and low nutrient concentrations. Our fusion model normal and/or tumor spheroids uncovered an unexpectedly small dependence on the amount of glucose over an order of magnitude span in the early phase of the process, until either proliferation (in high glucose), or cell death (in low glucose), modified the size of the cellular construct. These observations were concordant with the tumor spheroids experiments. Moreover, simulation of constructs built from one tumor spheroid bordered by eight other normal cell spheroids revealed a dynamic structure-destabilizing effect of the tumoral core, as dependent on the glucose levels. This modeling approach provides heuristic insights into the behavior of cell spheroids, such as the role of nutrients in tissue engineering constructs, and for better understanding of their biological properties, for basic science, biofabrication and pre-clinical applications.

Undergraduate Poster: U7

Enhancing Tissue Adhesives with Small Molecule Additives

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Every year, over 50 million people undergo surgeries that require invasive stitches or sutures to achieve tissue healing. Consequently, unsightly scars and complications associated with stitches and sutures remain a problem. As such, biodegradable tissue adhesives, such as biopolymer aldehydes and fibrin gels have been employed to close surgical wounds. Unfortunately, the currently available adhesives have several issues such as cytotoxicity and lack of mechanical durability. To optimize this system, we developed a novel adhesive with a base of hyaluronic acid (HA) functionalized with small molecules. HA is a biocompatible polysaccharide that plays a large role in connective tissue *in vivo,* and is widely used as a biodegradable scaffold for tissue engineering. We synthesized small organic molecules (10) with varying hydrogen bonding functional groups that will covalently modify thiolated HA via a thiol-alkyne click reaction. In this study, a variety of alkyne containing imines, amines and amides were prepared and characterized by NMR and mass spectrometry. The small molecules were modularly added into the HA hydrogel systems and tested for cytotoxicity as well as shear and tensile properties to assess the levels of adhesiveness and rigidity. The integration of catechol functionalized molecules with a bioactive HA backbone resulted in a 4-6 fold increase in adhesive properties at minimal concentration while simultaneously reducing cytotoxicity. Functionalization of HA with catechol groups leads to significant increase in adhesive properties. Ongoing studies are focused on enhancing the mechanical properties by diversifying the functional groups on the small molecules and amplifying the concentration of small molecule additives. Successful development of an improved tissue adhesive would aid wound healing applications and decrease previous complications.

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Undergraduate Poster: U8

Human Macrophage Response to Pathogens Isolated From Diabetic Foot Ulcers

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Diabetic foot ulcers are a major clinical problem exacerbated by prolonged bacterial infection. Macrophages, the primary innate immune cells, are multifunctional cells that regulate diverse processes throughout the multiple phases of wound healing. Previously studies have associated certain wound pathogens with healing outcome in mice (1). To better understand the influence of pathogen species on human macrophage behavior, we cultured primary human monocyte-derived macrophages in media conditioned by pathogens isolated from 8 diabetic foot ulcer patients for 24 hours. Gene expression and protein analysis showed that both pro-inflammatory M1 and wound resolving M2 phenotypes were upregulated to different extents by 6 species of pathogens. Interestingly, macrophages individually cultured with each pathogen induced high levels of expression of CCL22 and MARCO causing macrophages to most closely align with the M2 phenotypes compared to the M1 phenotype. Interestingly, *Corynebacterium amycolatum*, *Corynebacterium striatum*, and *Pseudomonas aeruginosa* conditioned media promoted increased secretion of both the wound resolving cytokine PDGF-BB and pro-inflammatory cytokines vascular endothelial growth factor (VEGF), interleukin 1β (IL1β), and tumor necrosis factor α (TNFα) suggestive of a fibrotic response in comparison to phenotypic controls unactivated M0 and M1 macrophages. Therefore, macrophages respond uniquely to the secretions of different pathogen species, producing irregular behavior. To correct this irregularity chronic wound treatments should specifically address macrophage deficits based on the dominant pathogens to promote wound healing and skin regeneration.

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Undergraduate Poster: U9

Optogenetics for the Maturation of hiPS-CMs

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The necessity for *in vitro* models of the human heart grows as the burden of cardiovascular disease continues to be the leading cause of

patient mortality.[1] Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are a promising tool due to their potential applications for disease modeling, drug testing, and regenerative medicine. However, hiPS-CMs are less mature than their native counterparts. Electrical stimulation has been used to mature cardiomyocytes over the past decade; however, such stimulation regimes are associated with the presence of an electrical field as well as charge injection, the relative effects of which are not well understood.[2,3] Cardiomyocytes expressing channelrhodopsin-2 (ChR2), a light-responsive ion channel first transduced into mammalian cells in 2005, would allow for optical pacing as a means for stimulation and maturation.[4] ChR2-expressing hiPSCs were created via lentiviral transduction, and differentiated into cardiomyocytes as previously published.[5] Cardiomyocytes were subsequently digested and seeded in collagen-fibrin hydrogel constructs. The tissues were stimulated using a ramped stimulation protocol using a custom Arduino-driven LED system for fourteen days. The maximum capture rate (MCR) was periodically evaluated to set stimulation parameters. The MCR was quantified using custom image processing software and conduction velocity was analyzed by optical mapping of impulse propagation. Sarcomeric alignment was quantified through immunostaining. Here we show that optically stimulated cardiomyocytes exhibit increased ultrastructural organization, enhanced Ca^{2+} handling through increased MCR, and improved conduction velocity. We demonstrate that optical pacing of transgenic hiPS-CMs leads to functional changes linked to cardiac maturation.

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Undergraduate Poster: U10

Design, Development and Verification of a Technique to Manufacture Locally Strain Variant Electrospun Scaffolds for Ligament Tissue Engineering

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Anterior cruciate ligament (ACL) rupture is a burden of sports people globally, with approximately 130,000 reconstructions each year in the US alone.[1] Current treatment involves an autograft from either patella tendon or hamstring tendon, with the concomitant result of donor site morbidity, surgical cost and time as well as increased risk of infection. Scaffold based alternatives such as hyaluronan[2], silk[3], polymer[4] have been studied previously but are yet to replace allografts as the gold standard for ACL reconstruction. The structure and integration of the scaffold is a key consideration, with the architecture of the bony plug distinct from the ligament. We have designed and developed a polymer scaffold to better mimic the local strain variance seen in native ligaments, with an architecture that better represents the ACL and gold standard treatment a bone-patellar tendon-bone autograft. Polymer scaffolds were electrospun onto a specially designed rotating mandrel, programmed to stop at precise intervals increasing deposition in certain areas. Analysis of results from mechanical testing illustrates a significant variation in both scaffold thickness and consequently strain behaviour along the scaffold length in agreement with that seen in native ligaments. Stress-strain behaviour for the scaffold as a whole is shown to be very similar to scaffolds of uniform thickness with potential for closer matching behaviour through manipulation of manufacturing parameters. Thus, the novel technique of strain variant scaffold manufacture for ligament tissue engineering has great potential.

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Undergraduate Poster: U11

Catechol-functionalized Pectin Hydrogel as Mucosa Adhesive and Therapeutic Delivery Agent for the Treatment of Peptic Ulcer

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The treatment of peptic ulcer disease is typically mediated by acid suppressant such as proton-pump inhibitor (PPI) or by mucosal protective agent such as sucralfate. However, acid inhibitor may not only attenuate the anti-infection ability but also lead to malnutrition or vitamin B12 deficiency. Of note is that mucosal protective agent has problems of weak protectability and short duration of action. Prolonged treatment with PPIs may accelerate the development of atrophic gastritis, a risk factor of stomach cancer. To address these problems, multifunctional pectin hydrogel is developed to serve as tissue adhesive, acid-resistant wound barrier, and therapeutic drug controlled delivery vehicle. For future clinical administrations, multifunctional pectin hydrogel can be delivered through the endoscopy route without affecting intragastric pH levels and can adhere to the ulcer site for a long duration. Pectin was modified with catechol functional groups to act as tissue adhesive and in-situ gelation hydrogel for protecting ulcer wound area against the attack of gastric fluid. Gelatin nanoparticles were embedded in pectin hydrogel to function as drug delivery carrier for accelerative ulcer healing. Catechol was successful modified on pectin with desirable conjugation rate. The hydrogel exhibited sol-gel transition behavior and adhesive property to underlying tissue surface after oxidation treatment. Possessing with strong mechanical property and stability in acid solution, the hydrogel was particularly suitable for surgeons to manage peptic ulcer. Catechol-functionalized pectin hydrogel demonstrates that it is a promising mucosal protective agent and has potential of being applied to chronic wounds, gastric and duodenal ulcers and other mucosa related injury or diseases. Ongoing research works are to assess the drug release profile and cytoprotective potential of this hydrogel.

Undergraduate Poster: U12

Co-Transplantation of Neural-Progenitor Cells With Interstitial Cells Of Cajal To Treat Gastroparesis

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Gastroparesis is delayed gastric emptying without known mechanical obstruction. Symptoms and pathologies are heterogeneous among cases; however, most patients diagnosed have a reduction of gastric neurons and interstitial cells of Cajal (ICCs) upon histological investigation. Therefore, this study aims to determine the efficacy of cell therapy, using ICCs and neural-progenitor cells (NPCs), at restoring functionality to the disease model. Rat stomach was harvested following ablation of neurons and ICCs via chemical treatments to make gastroparesis tissue model (GTM). Murine small intestine NPCs and ICCs were isolated and fluorescently tagged with fluorescent protein. The cells were delivered to GTM. Cell delivered GTM were evaluated using qPCR and immunohistochemistry (IHC), and functionality was analyzed using organ bath studies. The ICCs and neural cells were successfully depleted after chemical treatment. The GTM showed a 70% decrease in ANO1 and 83% decrease in beta-III tubulin expression compared to control. NPCs/ICCs injected tissues showed a 225% increase in ANO1 expression and a 384% increase in beta-III tubulin expression compared to control. IHC confirmed the survival and functional differentiation of ICCs and NPCs in the diseased tissue model. The contraction and relaxation activity improved by 84% and 57%, respectively, within seven days, further confirmed that the NPCs and ICCs were able to re-establish connections to the smooth muscle cells in the tissue. These findings suggest that cell therapy using an injection of NPCs and ICCs can be used to treat neurodegenerative disorders of Gut.

Undergraduate Poster: U13

Electrospun Polymer Fibers Infused with Cellulose Nanowhiskers: A Micromechanics Approach

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Calcium sulfate ($CaSO_4$) and calcium phosphate ($CaPO_4$) bone cements have low toughness due to the brittle nature of the material. Our objective is to use a micromechanics approach to develop discontinuous fiber-reinforced Engineered Cementitious Composites (ECCs)[1] with significantly improved mechanical strength and toughness. In this work, we apply the ECC approach for injectable bone cement in order to improve strain to failure and load-bearing capacity without brittle fracture. Atomic Force Microscopy (AFM) showed that addition of cellulose nanowhiskers increased the surface roughness of the fibers, which is expected to improve the fiber-matrix interfacial strength. X-Ray Diffraction (XRD) confirmed the presence of cellulose nanowhiskers within the cement matrix. Nanoindentation generated force-displacement curves, which revealed increased Young's Modulus for fiber-reinforced cements. For the pressed (pore-free) fiber-reinforced cements, Young's Modulus increased by as much as a factor of 2 (22.8 ± 3.2 GPa vs. 11.7 ± 2.2 GPa), and the elastic recovery decreased by more than 10%, suggesting the cement is stiffer, but also tougher than the unreinforced cements. Predictive micromechanical modeling/simulation was used to demonstrate how factors such as cellulose nanowhisker content, fiber aspect ratio, and fiber length, impact the critical volume fraction needed to induce strain hardening and to serve as a baseline for initial cellulose nanowhisker concentrations. This modeling predicts that the addition of cellulose nanowhiskers will increase the fiber Young's Modulus improving the cement matrix. This ECC behavior can produce a composite cement with increased toughness while promoting the formation of natural bone.

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Undergraduate Poster: U14

Bioprinted Blood Brain Barrier Model for Drug Screening

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Neurodegenerative disease accounts for one million new diagnoses[1] in the United States every year, with 50 million pre-existing cases.[2] Treatment is limited due the lack of an *in vitro* model of the blood-brain barrier (BBB) capable of high-throughput drug screening. We propose that our bioprinted model incorporating all six human brain cell types (astrocytes, pericytes, microglia, endothelial cells, oligodendrocytes, and neurons) will ameliorate this. The purpose of this research is to develop a human-based, physiologically relevant, bioprinted microvessel BBB model for drug screening. Viability was determined via Live-Dead analysis. Neuronal maturation markers were localized via immunofluorescent staining. Cells were printed via the integrated tissue and organ printing system (ITOP) in fibrin-hyaluronic acid hydrogel. Sectioning was achieved using a microtome and subsequently stained via H & E analysis. Live-Dead analysis demonstrated the maintenance of viability for up to 21 days as well as cellular maturation and differentiation. The presence of neuronal maturation markers was confirmed. Sectioning of tissue revealed the presence of an open lumen. Live-Dead analyses indicate long-term culture applications. Normal protein expression in the hydrogel was confirmed via immunohistochemistry. Our bioprinted blood vessel model possesses a lumen resembling blood vessels of the blood-brain interface and, with high viability and reproducibility, holds potential as a platform for drug screening.

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Undergraduate Poster: U15

Bioengineering of vascular myocardial tissue; a 3D bioprinting approach

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Cardiovascular tissue engineering holds significant promise to restore the loss of structure and function after heart damage. However, clinical attempts at myocardial tissue grafting have shown limited success primarily due to the insufficient vascularization and poor control over the scaffold structure. Here, we *hypothesize* that recapitulating physiologic vascular architecture and blood flow in the 3D bioprinted constructs would allow for optimal cardiac cell function in 3D tissue. Our *goal* was to determine the effect of vasculature design and flow on hiPSC-CM and EC viability and function in 3D printed myocardium. Our results demonstrated the feasibility of printing viable and functional human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes (CMs) and endothelial cells (ECs) using gelatin methacrylate (gelMA) as bioink. A reverse engineering approach was utilized to create the vascular network; hiPSC-CMs were encapsulated in gelMA and printed into perpendicular mesh network and the remaining (negative) space was utilized as an interconnected 3D vascular network and perfused with ECs. To form endothelium onto the printed channels, we subsequently used a custom-printed bioreactor to perfuse ECs into the microchannels. Optimal flow conditions and design parameters to achieve hiPSC-CM viability,

contractile function, and maturation were identified. Outcomes of this research establish the design principles and lead to the creation of the first 3D bioprinted, patient-specific, vascular myocardium which can be broadly applicable to other tissues and organs.

Undergraduate Poster: U16

Effects of Bioactive Molecules on Skeletal Muscle Development In 3d Bioprinted Muscle Constructs

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Patients with volumetric muscle loss (VML) experience profound structural and functional impairment. The current standard of care, autologous muscle graft, is often limited by unavailability of suitable host tissue and poor grafting efficacy which prevents functional restoration of muscle mass. Bioengineered functional skeletal muscle has potential to fill this clinical void. To engineer skeletal muscle tissue, several biofabrication techniques have been previously applied. However, these techniques have faced inadequate fiber alignment for functionality with clinically-relevant size for treating VML. To overcome this limitation, we fabricated skeletal muscle constructs using 3D bioprinting of human muscle progenitor cells (hMPCs). The constructs showed a high degree of muscle fiber alignment. To achieve functional recovery *in vivo*, further development of the constructs is needed. In this study, we investigate the effects of bioactive molecules on muscle development. The conditioned medium factors from the human neural stem cell (hNSCs) were tested in 2D hMPC culture and 3D bioprinted muscle constructs. Muscle development was evaluated after 5 days of differentiation by immunostaining with myosin heavy chain (MHC). Our results demonstrated that the treatment of Fibroblast Growth Factor-2 (FGF-2) and Hepatocyte Growth Factor (HGF) significantly increased the development of skeletal muscle. In the future, studying the synergistic effects of these factors with other factors may produce an even more pronounced effect on muscle development. This finding may be beneficial in working toward improving development of bioprinted muscle constructs which may contribute to functional restoration in VML injury *in vivo*.

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