Session Number: 1
ASMB Guest Symposium: The Extracellular Matrix in Morphogenesis and Repair
Tuesday, December 3, 2019, 10:00 AM - 11:30 AM

1 - Presentation Of The Integrin Binding Domain Of Fibronectin Impacts Lung Cells Phenotype

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Purpose/Objectives:** The switch between α5β1 and αvβ3 integrins engagement due to unfolding of the integrin binding domain (IBD) of fibronectin (Fn) has been theorized for a long time but was observed in vitro and murine models only recently. Since Fn is largely present in the extra cellular matrix (ECM) of fibroproliferative diseases like idiopathic pulmonary fibrosis or cancer, further understanding the downstream effects of this differential integrin binding could elucidate disease mechanisms. Specifically, integrin α5β1 (and α3β1 in epithelial cells) requires the regular conformation of Fn's IBD, while αvβ3 can also bind the unfolded conformation. Moreover, cells can generate forces sufficient to cause such unfolding. **We hypothesized that the change in Fn conformation guiding integrin enrichment affects lung cells, pushing them towards a secretory and disease relevant phenotype.
***Methodology:** We tested this premise by engineering Fn fragments containing the IBD site that mimicked either the strained or regular conformation. Additionally, these fragments were designed to be covalently bound via thiol chemistry to a variety of substrates in order to preserve their structural integrity. Lung fibroblasts and epithelial cells were plated on soft gel substrates of various stiffness functionalized with either Fn fragment and their changes in phenotype were measured via immunofluorescence and qPCR.*
***Results:** Even on soft substrates (2kPa for healthy lung tissue), cells plated on the unfolded IBD Fn fragment engage αvβ3 and display increased nuclear translocation of transcription factors associated with a secretory phenotype, with MRTF and YAP/TAZ among others. Furthermore, upregulation of mRNA related to fibroproliferative diseases (α-smooth muscle actin, Fn with extra domain A) was observed. Remarkably, shRNA knockdown of α5β1 of cells on full length Fn pushes the cells towards the disease-related phenotype we determined on the unfolded IBD mimicking fragment, confirming that the engagement of α5β1 is necessary to outcompete αvβ3 signaling in healthy conditions.*
***Conclusion/Significance:** Differential integrin engagement due to Fn IBD unfolding appears to affect cell phenotype. Current efforts are focused on cataloging the different proteins associating with α5β1 or αvβ3 during specific engagement via mass spec, and evaluating the efficacy of a novel αvβ3 inhibiting antibody on the progression of murine lung fibrosis. This work contributes to defining the link between changes in the ECM and cell behavior in the context of fibroproliferative diseases.*

2 - Synthetic Platelet Microgels Containing Fibrin B Knob Targeting Motifs Enhance Clotting Responses

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***Purpose/Objectives:** Native platelets perform several roles in wound healing, including binding to fibrin at injury sites and promoting clotting. During clot formation, fibrinogen peptide sequences known as knobs A and B bind to fibrin holes “a” and “b” on neighboring fibrinogen molecules to form an insoluble fibrin network. Platelets interact with fibrinogen through the cell surface receptor GPIIb/IIIa. Upon formation of a platelet-fibrin mesh, activated platelets utilize actin-myosin machinery to contract and pull on the bound fibers in a process known as clot retraction. Clot retraction stabilizes the clot and increases fibrin density, facilitating the role of the clot as a provisional matrix supporting subsequent stages of wound healing, including cellular infiltration, tissue repair, and remodeling. When platelet activity is impaired, as occurs in cases of traumatic injury or certain diseases, uncontrolled bleeding and/or deficient wound repair can result. To that end, our lab has previously described the development of synthetic platelet-like particles capable of promoting clotting and improving wound healing responses through the coupling of highly deformable hydrogel microparticles to various fibrin binding elements, including a fibrin-specific single domain variable fragment (sdFv) antibody and a full length IgG anti-fibrin fragment E antibody. However, the use of antibody and antibody fragments is more expensive than other approaches such as peptides, potentially hindering the translational potential of these particles. Therefore, we investigated if utilizing a fibrin-binding peptide instead of antibodies influences the previously identified platelet-mimetic properties of our synthetic platelet design. Here we analyzed clot contraction and stability, and then characterized the effects of these fibrin-targeting particles (FTPs) on bleeding times *in vivo*.
***Methodology:** Ultralow crosslinked (ULC) poly(*N*-isopropylacrylamide-*co*-acrylic acid) microgels were synthesized via precipitation polymerization and covalently coupled to a peptide (AHRPYAAK) that mimics the sequence of the fibrin B knob to create FTPs. Control non-targeting particles (NTPs) were formed through covalent coupling to a non-binding peptide sequence (GPSPFPAK). FTPs, control NTPs, or unconjugated ULCs were incorporated into fibrin clots, and clot structure under each condition was assessed using confocal microscopy. The ability of each particle type to stem bleeding *in vivo* was assessed using a mouse liver laceration model.
***Results:** When incorporated into fibrin clots, FTPs were found to significantly increase clot density at an optimal concentration of 2 mg/ml. Upon treatment with FTPs, *in vivo* bleeding decreased relative to controls. Furthermore, quantification of blood loss over 10 minutes illustrated that FTP treatments resulted in blood loss similar to that seen in rats treated with clinically-used prothrombin complex concentrate.
***Conclusion/Significance:** These results indicate that FTPs are capable of recapitulating the platelet-mimetic properties of previous designs while utilizing a less costly, more translational design.

3 - The Role Of Extracellular Matrix Developmental Age On Cardiac Fibroblast Remodeling Response

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***Purpose/Objectives:** Cardiac injury leads to fibrosis in adults but not necessarily in young animals. Prenatal and early neonatal cardiomyocytes (CMs) are capable of functional restoration. Our lab has shown this is partially due to age-dependent changes in cardiac extracellular matrix (cECM) composition,

with fetal ECM promoting greater CM expansion versus adult ECM [1]. Further, research has shown developmental age of cardiac fibroblasts (CFs), cells responsible for cECM turnover and structural support, impacts their behavior, with adult CFs upregulating features of pro-fibrotic remodeling in engineered cardiac tissues compared to tissues supplemented with fetal CFs [2]. We speculate age-dependent ECM-CF interactions play a role in stimulating expression of a pro-fibrotic or pro-regenerative phenotype in these cells. If so, incorporating engineered cardiac tissues with cECM that stimulates regenerative behavior in CFs could represent a method to improve functional outcomes. Our hypothesis is that since regenerative remodeling is highest during prenatal development, fetal ECM may provide cues to stimulate fibroblasts to adopt a more pro-regenerative phenotype.
***Methodology:** CFs were isolated from fetal, neonatal, and adult Sprague-Dawley rats. RNA sequencing of adult, fetal, and neonatal fibroblasts was performed with the goal of identifying age-specific gene expression differences in CFs. Decellularized, solubilized cECM solutions from fetal and adult porcine cardiac tissue were prepared using published methods [1]. CFs were seeded on cECM coatings to assess age-dependent impacts of cECM on CF transition to a myofibroblast phenotype. Image analysis was utilized to quantify expression of α-smooth muscle actin and Ki67. Atomic force microscopy (AFM) and scratch assay analysis was utilized to assess cECM effects of CF stiffness and migration, respectively.
***Results:** RNA-seq revealed gene expression differences between fetal and neonatal CFs, in addition to a major expression shift from fetal/neonatal to adult stages. In particular, fetal cECM appeared to decrease neonatal CF progression to a pro-fibrotic phenotype versus adult cECM. Additionally, AFM data suggested that ECM age had variable effects on CF stiffness and morphology. Scratch assay migration studies indicated that adult cECM appeared to amplify neonatal CF migration in culture versus fetal cECM.
***Conclusion/Significance:** Overall, developmental age of cECM appeared to impact CF behavior. Current work is applying these results in fibrin-cECM constructs to determine how age-dependent CF/cECM interactions impact tissue properties. Further, ongoing work seeks to identify matrix metalloproteinase (MMP)-derived cECM fragments with bioactive effects on CFs and CMs, to determine if specific peptides are responsible for the observed effects.
Acknowledgements: This work was supported by grants from the US Department of Defense (#W81XWH-16-1-0304 to LDBIII), NSF (NSF#1603524 to LDBIII) and AHA (Predoctoral Fellowship #18PRE33960362 to LRP). References:1. Williams, C., et al., Acta Biomater, 2014. **10**(1): p. 194-204.
2. Li, Y.A., Huda; Bursac, Nenad, Acta Biomaterialia, 2017. **55**: p. 120-130.

5 - Methods For Isolation Of Matrix Bound Nanovesicles

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***Purpose/Objectives:** Isolation and evaluation of liquid-phase extracellular vesicles (e.g. exosomes) have been of great interest due to the potential use of these nanovesicles as biomarkers and therapeutic agents. Recently, a subset of nanovesicles embedded within the extracellular matrix (ECM) has been identified (Matrix bound nanovesicles -MBV). MBV have distinct surface markers and intravesicular cargo with the potential to recapitulate biological effects of ECM bioscaffolds. These

tightly bound nanovesicles are potent regulators of cell function. Harvesting MBV from the ECM requires the use of enzymes and/or mechanical forces to solubilize the ECM components and dissociate the MBV from matrix structural components, followed by isolation and purification. These methods would ideally minimize disruption of the MBV lipid membrane and associated surface ligands and preserve their intravesicular cargo. The present study assessed various methods for MBV harvesting and evaluated the properties of the isolated vesicles.
***Methodology:** A combination of four ECM solubilization methods and four isolation approaches were used to harvest MBV from urinary bladder matrix bioscaffolds. The solubilization was performed using one of three enzymatic methods: Collagenase (COL), Liberase (LIB), and Proteinase K (PK); or a non-enzymatic method (KCL). MBV within the solubilized ECM, where then isolated using one of four methods: ultracentrifugation (UC), ultrafiltration (UF), density barrier (DB) or size exclusion chromatography (SEC). Subsequent analysis included RNA and protein quantification, MBV imaging, size distribution and particle concentration analysis, detection of surface markers and cargo proteins, and their bioactive potential to promote proliferation of perivascular stem cells (PVSC).
***Results:** Results showed that although each of the evaluated methods facilitated the harvesting of MBV of similar size, the different methods had a notable effect upon yield, purity, protein markers and bioactivity. Regardless of the isolation process, all enzymatic methods produced a higher yield of MBV compared to the non-enzymatic method. COL provided the highest yield followed by LIB and then PK. All isolation methods promoted similar yields, except DB which was consistently lower. The purest samples (high particle to protein ratio) were obtained with DB and SEC, followed by UC, and lastly UF. COL and LIB, but not PK and KCL, were shown to preserve the markers CD81, LOX1. Bioactivity assay results showed the least PVSC proliferation with UC isolated MBV. Moreover, the solubilization methods COL, LIB, and KCL promoted PVSC proliferation, whereas PK derived MBV were cytotoxic. The harvesting methods that provided the highest yield along with low protein and enzymatic contamination were COL and LIB followed by SEC. Despite the low yield, samples isolated using KCL-UC and KCL-SEC had very low protein contamination and were bioactive.
***Conclusion/Significance:** This study highlights the effects of the MBV harvesting methods upon yield, MBV properties and bioactivity.

6 - Keratin'S Modulation Of Protein Aggregation And Autophagy Pathways May Underlie Its Cytoprotective Effects.

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***Purpose/Objectives:** Keratin is a biomaterial with a wide range of potential tissue engineering applications. Previous studies suggest that keratin promotes cell proliferation and may mitigate damage in stressed cells; however, its mechanism of action is unknown. Our lab's work in human embryonic kidney (HEK293) cells supports the notion that keratin is cytoprotective. Additional studies in other labs and our own, suggest a tentative link between keratin's protective effect and cell recycling pathways. Here, we aimed to expand on the link between keratin and autophagy and protein aggregation to better understand the mechanism by which keratin may protect cells from damage.
***Methodology:** Cell viability assays were used to confirm keratin's cytoprotective effects and determine optimal concentrations for keratin dosing. To track protein aggregation and subsequent clearance via

the ubiquitin/proteasome pathway, we transfected HEK293 cells with a plasmid containing the GFP-250 gene that gives rise to a misfolded protein. Upon stress, cells activate the protein aggregation pathway to initiate removal of GFP-250. Aggregation and removal in the presence and absence of soluble keratin was tracked via fluorescent microscopy. Alternatively, to track keratin's effect on autophagy, we transfected HEK293 cells with a dual reporter plasmid containing the gene for an autophagy protein LC3 fused with GFP and mCherry fluorescent tags. Progression through the autophagy pathway can be observed by tracking fluorescent puncta, indicating that the pathway is turned on, and subsequent color changes of the puncta. GFP denatures in the acidic environment of the lysosome during late autophagy. Therefore, during early autophagy, puncta are both green and red and, in late autophagy, puncta display as only red. Confirmation of transfection data was performed by tracking endogenous expression of ubiquitin and through western blotting for LC3.
***Results:** HEK293s treated with crude keratin (a mixture of keratin fractions) enhanced cell viability at an optimal concentration of 0.0015mg/mL. An isolated fraction of gamma keratin displayed optimal activity at 1mg/mL. In the first 30 minutes after stress, crude keratin significantly reduced the number of GFP-250 aggregates, indicating that keratin promotes rapid removal of protein aggregates. Crude and gamma keratin also induced earlier induction of autophagy. Specifically, nutrient starved cells showed an expected, gradual increase in LC3 puncta formation over time, whereas, keratin-treated nutrient starved cells displayed signs of autophagy induction (presence of puncta) an hour prior to those without keratin.
***Conclusion/Significance:** Our results suggest a relationship between keratin and recycling pathways in cells. These pathways are critical to homeostasis and to cell survival in times of stress. Keratin has broad potential as a biomaterial and cell scaffolding material. Keratin's utility may be further enhanced by fine tuning its ability to induce autophagy so that it exerts not only structural/physical properties, but also cytoprotective properties in tissue-engineered constructs.

8 - Restoration Of Ovarian Function By Tissue Engineering

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***Purpose/Objectives:** Preservation of female fertility has been one of the main clinical and social challenges for the last two decades, especially when women are exposed to chemotherapy treatments that may impair their fertility. Alongside the existing therapeutic approaches, there is a need to create a broad alternative, especially for the population of patients for whom existing treatments are not suitable, as young girls.
***Methodology:** Herein, we developed specialized three-dimensional (3D) cultivation systems for the *in vitro* and *in vivo* maturation of primordial ovarian follicles. These systems contain triggers leading to signaling of different natures, all envisioned to facilitate follicle maturation. These smart cultivation systems are based on macroporous alginate scaffolds, whose numerous advantages are well known, amended with various types of triggers. These triggers include peptides for cell adhesion of the ovarian supporting cells, such as the ECM-derived peptides RGD, YIGSR or their combination, and affinity-bound bone morphogenetic protein-4 (BMP-4), a specific growth factor that is dominant in the first stage of follicular growth.

***Results:** ECM-derived-peptide and pristine alginate scaffolds enabled porcine follicular maturation in a native fashion into multi-layered secondary follicles, within 4 weeks of cultivation. On day 21, the YIGSR-modified scaffold was shown to have a significant effect on follicle growth, in comparison to the pristine and RGD- modified scaffolds, and furthermore the effect of the RGD/YIGSR- modified scaffold was superior to that of the RGD scaffold. By day 28 differences were no longer significant, presumably due to ECM secretion as shown by collagen staining. During 8 weeks in culture, GDF-9 and PCNA gene expression were increased in both pristine and RGD/YIGSR groups. Affinity-bound BMP-4 significantly contributed to follicular maturation, as evident by the 5-fold increase in the number of developing follicles and enhanced estradiol secretion in these cultures compared to the non-bounded BMP-4. After 21 days in culture, an increase in GDF-9 and AMH gene expression, was statistically significant when BMP-4 was affinity bound, compared to all other scaffold groups. Ultimately, when evaluating the ability of our system to serve as a transplantable artificial ovary, xeno transplantation of the follicular constructs supplemented with additional angiogenic factors, showed that follicles reached antral size and secreted hormones at levels leading to restoration of ovarian function in ovariectomized severe combined immunodeficiency (SCID) mice.

***Conclusion/Significance:** Altogether, our work establishes the grounds for the recapitulation of the ovarian microenvironment for follicle culture both *in vitro* and *in vivo*, by employing macroporous alginate scaffolds. In the future, this bio-inspired approach can be further tailored, to meet the needs of human follicle maturation.

Session Number: 2
Strategies for Tissue Microvascularization
Tuesday, December 3, 2019, 10:00 AM - 11:30 AM

9 - Induction Of Designed Micro-vascular Network With 3D Bioprinting

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***Purpose/Objectives:** Generation of bio-mimetic vascular network is a critical challenge in tissue engineering. Without proper micro-vessels, adequate delivery of oxygen and nutrients cannot be achieved due to the limit of mass transfer into tissues. Also, highly organized microvascular networks exist in metabolically active tissues which greatly affect the viability and function of parenchymal cells. Therefore, engineering the biomimetic micro-vascular structure is a key element for functional artificial tissue fabrication. A variety of recent approaches have been introduced to produce a designed vascular network, but there is a limit to control the pattern of capillary network having a diameter of several ten micrometers. Here, we developed a micro-vascularization method that can construct the vascular network on the scale of tens to hundreds of micrometers with 3D bioprinting technology.
***Methodology:** The 3D bioprinting system consisted of multiple cartridges was used to accurately pattern the vascular structure and utilize the various materials and cells. Human umbilical vein endothelial cells (HUVEC) and normal human dermal fibroblasts (NHDF) were used to generate the vascular structure. The vascular channel of several hundred micrometer scale was created by directly printing of sacrificial gel containing endothelial cells and molding the fibrin gel over the printed structure as a scaffold. The micro-vessels of several ten micrometers were generated through the directional control of angiogenic sprouting by the chemotaxis effect. To induce the formation of micro-vessels in the desired pattern, the bridge gel was utilized which have higher diffusion rate of angiogenic factors and lower matrix stiffness compared to the fibrin scaffold.
***Results:** Endothelilalized hollow vascular channel and lumenized micro-vessels were formed in the printed structure. The sprouted micro-vessels from vascular channel showed directionality to the fibroblast region secreting the angiogenic factors. Various patterns of capillary structure can be formed by control the bridge gel pattern. Also, the hepatic lobule-like vascular structure which has bio-mimetic pattern and scale can be constructed. When primary mouse hepatocytes were seeded into the printed structure, albumin and urea secretion were significantly increased compared to control group without vascular network. In chick chorioallantoic membrane (CAM) assay, the blood perfusion through the engineered micro-vascular network was observed. The implanted vascular structure served as a pathway for the blood flow while maintaining its pattern of micro-vascular network.
***Conclusion/Significance:** In this study, we developed a technique to induce the formation of micro-vascular network in desired pattern within the tissue constructs. Our approach allowed to fabricate the bio-mimetic pattern of vascular structure. The patterned vascular network enhanced the functionality of hepatocyte and showed the perfusability ex vivo. Our strategy has great potential to be utilized as an effective tool for producing tissue-specific vascular structures in tissue engineering.

10 - Tuning Of Matrix Proteolytic Degradation And Cell Adhesive Peptide Ligand Concentration Synergistically Enhance 3d Vascular Sprouting

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***Purpose/Objectives:** Microenvironmental conditions, such as integrin adhesion ligand concentration, matrix stiffness and degradation rate are known to play important roles in modulating 3D cell response including vascular sprouting required for engineering vascularized tissues. Studies exploring the combined effects of these matrix properties on vascular sprouting have been limited.
***Methodology:** In this work we utilized a statistical design of experiments (DOE) approach with a full factorial design to screen interactions of these properties using highly tunable proteolytically degradable, cell adhesive synthetic poly (ethylene glycol)-based hydrogel scaffolds to probe 3D vascular cell response.
***Results:** Variations in biochemically conjugated monoacrylate and diacrylate monomer concentration led to the formation of scaffolds with a range of elastic moduli (0.8-4kPa), immobilized concentration of the RGD cell adhesive peptide ligand (0.7mM-3.5mM), and proteolytic degradation rate, the latter varying from slow degradation (26hrs for complete gel degradation in 0.1 mg/ml collagenase) and enhanced degradation (4hrs for complete gel degradation in 0.1 mg/ml collagenase). A significant reduction in vascular sprout length and junction number was found to occur in the case of scaffolds exhibiting slow degradation and high modulus (4kPa) regardless of cell adhesion ligand concentration. In the case of the faster degrading scaffolds, vascular sprout length and junction were dependent on immobilized concentrations of RGD across the range of moduli investigated. Faster degrading scaffolds resulted in significant increases in vascular sprouting as compared to those exhibiting slow degradation kinetics, the former dependent on high cell adhesive ligand concentration. To better elucidate the combinations of matrix stiffness, degradation and cell adhesion ligand concentration on 3D vascular sprouting, a regression model was postulated from DOE analysis and coefficients extracted to describe the effect of each factor as well as their interactions. DOE analysis revealed proteolytic degradation rate to be the most prominent factor in enhancing vascular sprout formation, with no significance was noted in terms of cell adhesive ligand concentration with variations in modulus significantly reducing vascular sprouting parameters. Interestingly, the interaction between proteolytic degradation and cell adhesive ligand concentration predicted significant enhancements in vascular sprouting, while the interactions between degradation and modulus or between RGD concentration and modulus had no significant effect.
***Conclusion/Significance:** The use of DOE enables a framework for efficient screening of combinatorial effects of matrix cues on cell behavior while minimizing the number of experiments required for optimizing cell responses required for vascularization of engineered tissues.

11 - Spatial Variations In Degradation, Elastic Modulus, And Cell Adhesion Peptide Ligand Concentration Influence 3d Vascular Sprouting

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***Purpose/Objectives:** Neovascularization is highly dependent on gradients of soluble and immobilized extracellular matrix signals. While several studies have elucidated the effect of growth factor gradients on this process, the role of spatial variations in cell adhesion ligand concentration, matrix stiffness and proteolytic degradation on guided vascular cell response remain unclear.
***Methodology:** Using a previously developed polymerization technique, ascending frontal polymerization (AFP), hydrogel scaffolds with decoupled gradients of immobilized RGD cell adhesion peptide ligand concentration (0-3.5mM), elastic modulus (800-4000 Pa) and proteolytic degradation (total degradation time from 4hrs to 26hrs) were created in the presence of cells. Cell-laden gradient scaffolds were created by using programmable syringe pumps to control the delivery of the of specific precursor solution components during the free-radical polymerization process. Scaffolds with (1) immobilized gradients of RGD were formed by varying the molar ratio of PEG monoacrylate-RGD to non-functional PEG monoacrylate while maintaining the total concentration of monoacrylate monomers in the precursor constant; (2) spatial variations in elastic modulus gradient induced through variations in the MMP-sensitive PEGDA crosslinker concentration, and (3) gradients in proteolytic degradation achieved by adjustments in the molar ratio of two types of PEG diacrylate crosslinkers, one containing a single MMP-sensitive peptide sequence and another containing two repeats of the peptide resulting in crosslinkers of high and low MMP sensitivity, respectively. Vascular spheroids composed of a co-culture of HUVECs and SMCs were added to the feed stream of precursor solution during the scaffold fabrication process and encapsulated throughout the different regions within the scaffolds along the gradient (10-15 spheroids per gel).
***Results:** RGD gradient scaffolds led to significant increases in vascular sprout length and number towards the gradient and in regions where the spheroids were positioned closer to the gradient. In hydrogels embedded with gradients of elastic modulus and uniform concentration of immobilized RGD, spheroids did not exhibit directional sprouting responses towards the gradient, but modulus gradients led to spatial variations in sprouting parameters with significant increases in sprout length in regions of low modulus as compared of high modulus. Scaffolds possessing gradients of proteolytic degradation and uniform RGD concentration and modulus resulted spatial variations in sprout length with longer sprouts in regions exhibiting faster degradation as compared to those prone to slower degradation kinetics.
***Conclusion/Significance:** Our findings demonstrate that this gradient fabrication platform can be used to create scaffolds with multiple types of gradients necessary for modulating guided vascularized tissue formation and holds potential for engineering complex tissue interfaces where spatiotemporal gradients in biochemical composition and mechanical properties play a critical role.

12 - The Role Of Hemodynamics And Viscoelasticity On The Pre-vascularization Of Thick, Bioengineered Tissues

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***Purpose/Objectives:** Deep, chronic wounds are characterized by the inability to efficiently repair cutaneous defects and restore tissue function, causing permanent disfigurement, debilitation, or even death. Nearly 6.5 million Americans are burdened by non-healing wounds, with an annual cost of $25 billion for treatment. The delivery of an autologous flap, with intact and patent vasculature, to the

wound site provides a potential solution for the reconstruction of non-healing wounds. However, these invasive surgeries are limited by donor-site morbidity, prolonged post-operative debilitation, and donor tissue availability. Tissue engineering strategies can be used to address this clinical concern through the biofabrication of micro-vascularized neo-pedicles. We examined the use of a mechanically-robust polymer, gelatin methacryloyl (GelMA), to assess the role of viscoelasticity and hemodynamic flow on *de novo* angiogenesis and long-term cell maintenance within voluminous, bioengineered tissues.
***Methodology:** GelMA was prepared at 99% (GelMA100) and 50% (GelMA50) efficiency and verified by H-NMR[1] analysis. 5% GelMA100 acellular constructs (15x15x9mm^3) containing a straight channel (D=0.75mm) were photo-crosslinked (λ=325nm; t=5min) and perfused to assess the diffusion rate of Trypan Blue in the presence and absence of a pseudo-endothelium (t=3hrs). The material properties for GelMA constructs were determined by the swell ratio and the compressive modulus (n=3). HUVEC/MSC-laden 5% GelMA constructs were cultured in hypoxic-conditioned MSC medium, supplemented with pro-angiogenic cytokines, for 14 days, to assess cell viability and microvessel formation.
***Results:** This work demonstrated that GelMA permits prolonged perfusion through a fabricated lumen and the diffusion of small molecules within the bulk material. However, the presence of a pseudo-endothelium attenuates the rate of diffusion, which may hinder bulk cells from access to adequate levels of nutrients and oxygen for long-term survival. Under the appropriate pro-angiogenic conditions, GelMA50 supports cell viability and augments *de novo* sprout formation in comparison to GelMA100. Although the swell ratio of GelMA50 was significantly greater than GelMA100 as predicted, the compressive moduli did not demonstrate a significant difference between the two polymers. This phenomenon may be due the viscoelasticity of GelMA, rather than its stiffness. Further analyses on the average pore size and shear moduli may be necessary to verify this claim. It should also be noted that a decrease in methacrylation efficiency decreases the mechanical robustness of GelMA. Therefore, the combined use of GelMA and a high-swelling polymer, methacrylated hyaluronan, may be required to achieve long-term structural integrity, while maintaining the appropriate swell ratio and stiffness to facilitate angiogenesis.
***Conclusion/Significance:** GelMA is a mechanically robust and biocompatible polymer that permits *de novo* angiogenesis, prolonged perfusion, and diffusion through thick constructs. However, the degree of GelMA methacrylation must be tuned to elicit the desired angiogenic response within bioengineered tissues.

13 - Neovessel Invasion Through Tissue Interfaces Is Stromal Cell Dependent

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***Purpose/Objectives:** Rapid vascularization of engineered tissues upon implantation is a major barrier to their success. Without a rapidly established blood supply, tissues will necrose and die. Host neovessels must grow through the interface with the newly implanted tissue and create a network that is fully integrated between host and implant. Understanding the mechanisms that guide vessels across the interface between two tissues and inosculate with the host circulation is essential for improving engineered implant viability.
***Methodology:** To study these mechanisms, we developed an experimental tissue interface model. A

collagen gel “core” is embedded in a “field” of fresh collagen, resulting in two distinct regions. Second harmonic generation imaging and scanning electron microscopy (SEM) verified the formation of a thin, high-density layer of collagen at the interface between the core and field. We have previously shown that microvessels (fragments of intact capillaries, arterioles, venules) isolated from adipose tissue will sprout, grow, and connect when embedded in a collagen matrix. To model neovessel growth across a tissue interface, microvessels were incorporated into the core region of the model with or without stromal cells, or subfractions of stromal cells. Crossing events, where microvessels grew across the interface, were quantified.
***Results:** Without additional stimuli, microvessels grow and sprout within the core collagen region, but rarely cross this high-density interface, instead growing along the aligned collagen fibrils. However, when microvessels are mixed with stromal cells in the core (stromal vascular fraction), neovessels crossed the interface and began to populate the field. SEM imaging indicated that these crossing events do not grossly alter or degrade the interface structure. Further investigation revealed that stromal cell-mediated interface crossings are VEGF-A dependent. In the presence of a VEGF-A trap, neovessel crossing events were significantly reduced. However, VEGF-A alone was insufficient to stimulate crossing. Finally, using magnetic-particle depletion, we determined that stromal macrophages are necessary for this process. Microvessels were incorporated in the core with the complete stromal cell fraction or with stromal cells lacking $CD11b/c^{+}$. An increase in crossing events was visible only when $CD11b/c^{+}$ cells were present.
***Conclusion/Significance:** These studies indicate that stromal cells play a critical role in guiding neovessel growth during angiogenesis across tissue interfaces. We propose that stromal cells, particularly tissue-resident macrophages, migrate across an interface, laying down a spatiotemporal gradient of VEGF. This gradient guides neovessels across the tissue interface. Our findings suggest that inclusion of a vascular precursor and stromal macrophages within a fabricated tissue would promote tissue engraftment following implantation and accelerate graft perfusion. The authors gratefully acknowledge NIH R01 (NHLBI HL131856) as our funding source.

14 - 3d Hydrogel System To Promote Distinct Arterial-venous Specification For Tissue-engineered Vasculature

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***Purpose/Objectives:** Engineering a highly perfused, well-formed vasculature is critical to the success of large-scale tissue constructs. Previous work in this field has demonstrated success in establishing basic lumen-like structures lined with endothelial cells but failed to produce results that encompass the complexity of multilayered vasculature and the differences between arterial and venous tissues. A physiologically relevant vascular construct will help elucidate key differences between arterial vs. venous development, provide critical insight into vascular pathologies (e.g. arteriovenous malformation), and mechanisms of angiogenesis. In this work, we determine the biophysical parameters and dynamic culture conditions that drive the vascularization and arterio-venous distinction of endothelial progenitor cells (EPCs) in culture.
***Methodology:** Methacrylated gelatin (GelMA), a highly biocompatible material polymerizable under ultraviolet light is used as the substrate biomaterial. Substrate stiffness is varied in order to mimic physiological stiffness of arteries (40 - 60 kPa) and veins (5-10 kPa). A bioreactor capable of supporting

cell culture, high velocity fluid flow, as well as in situ cell monitoring was designed using a CAD modeling software (SolidWorks) and 3D printed using a stereolithography printer (EnvisionTEC). EPCs are seeded on the surface of GelMA substrates (2D) or encapsulated within (3D) and cultured under static or dynamic conditions, and in presence of vascular endothelial growth factor (VEGF, 100 ng/mL). Cell phenotype and gene expression for arterial-venous distinction is monitored over 14 days for markers specific to arteries (EFNB2, Hey2, Cx40), veins (COUP-TFII, EPHB4), and vascularization (CD31).
***Results:** Mechanical testing revealed stiffness of 7.5wt% GelMA being comparable to veins (6.5±1.4 kPa) and that of 15wt% comparable to arteries (31.2±1.7 kPa). Immunofluorescence staining for Actin composition indicated negligible differences in cell spreading between the two substrates. However, the presence of VEGF led to a 4-fold increase in cell surface area for the softer substrates that remained consistently higher compared to the stiffer substrates. Similarly, elevated expressions of COUP-TFII (venous marker) and Cx40 (arterial marker) were observed in presence of VEGF with no noticeable differences across the soft and stiff groups. Gene expression analysis indicated a downregulation of both arterial (EFNB2, Hey2) and venous (EPHB4, NR2F2) markers by day 14 for both soft and stiff substrates. Under dynamic conditions (shear stress of 0.1 Pa), cells aligned in the direction of flow and exhibited a higher expression of both arterial (Cx40) and venous (COUP-TFII) markers after 7 days compared to static cultures. Interestingly, 3D cultures of EPCs led to a significant increase in CD31 and Cx40 expression compared to 2D cultures.
***Conclusion/Significance:** EPC phenotype was examined in response to variations in substrate stiffness, VEGF, and shear stress as an indicator of arterio-venous specification. Preliminary results indicate a higher impact of shear stress and VEGF, and not substrate stiffness for the arterio-venous markers examined. Current work investigates the combinatorial effects of fibroblast co-culture of EPCs in the presence of VEGF and bioreactor conditions for microvasculature development. Through this work, we aim to establish the influence of biophysical parameters on the mechanisms of arterial and venous formation for a fully mature vasculature.

Session Number: 4
In Vitro and Translational Studies in Neural and Spine Engineering
Tuesday, December 3, 2019, 10:00 AM - 11:30 AM

15 - Tissue Nano-transfection Promotes Localized Delivery Of Therapeutics To The Peripheral And/or Central Nervous System Via Minimally Invasive Methods

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***Purpose/Objectives:** Nerve damage and neuropathies can contribute to loss of sensation, pain, systemic complications, and weakness. Cellular reprogramming, facilitated by localized *in-vivo* gene delivery to nerve tissue, presents an encouraging approach for these conditions. However, delivery of targeted therapies to nerves poses challenges because of the unique anatomical architecture, with axons of motor and sensory neurons extending over 1 meter from their cell body, spanning both the central (CNS) and peripheral nervous system (PNS). Nevertheless, current delivery methods face many practical and translational challenges, including heavy reliance on viral infection, stochasticity, lack of specificity, and cellular damage. Our reprogramming method bypasses the reliance on stems cells (embryonic or induced-pluripotent) and viral vectors, circumventing off target effects such as tumorigenesis and systemic immune concerns. We have developed a novel non-viral tissue nano-transfection (TNT) chip platform that can be used to deliver therapeutic cargo to nerve tissue at both levels (*i.e.,* peripherally and centrally) via the use of solid state nanochannels, coupled with nano-electroporation and nano-electrophoresis.
***Methodology:** Via a combination of cleanroom-based manufacturing techniques, such nanochannels were fabricated. This novel chip platform was then used to controllably deliver a wide variety of cargos to the CNS and PNS of mice, including CRISPR/Cas9 components and plasmid DNA for gene modulation. Nano-electroporation conditions were optimized by delivering labeled plasmids at different voltages and pulse lengths. Delivery efficacy and retrograde transport from PNS to CNS was evaluated via immunofluorescence microscopy and qRT-PCR at different levels, including peripheral nerve bundles, the dorsal root ganglion (DRG), and the spinal cord (SC). Electrophysiology measurements were used to evaluate potential alterations in functionality post-TNT.
***Results:** Tissue sections collected shortly after transfection revealed successful cargo delivery following a short-lived (<100 ms) pulsed electric field across the nanostructured platform. Fluorescence intensity demonstrated up to ~ 50,000 fold changes with respect to controls when varying voltage alone, and differences across groups of up to 20x when varying the duration of nano-electrophoresis. Fluorescence revealed negligible axon degeneration compared to non-treated controls. Immunofluorescence analysis and qRT-PCR confirmed tissue transfection and strong CRISPR/Cas9 and plasmid DNA activity at the peripheral nerve level, with varying degrees of expression/activity at the DRG and SC levels depending on the nano-electroporation conditions. No significant behavioral changes (e.g., paw clenching, gait perturbations) were noted in treated mice.
***Conclusion/Significance:** Our nanostructured platform, with the use of non-viral cargo, showed the ability to efficiently transfect peripheral and central nerve tissue in a targeted and controlled manner.

Ongoing studies are focused on modulating tissue repair via induced tissue plasticity following TNT-based delivery of reprogramming factors.

16 - Enriching For Distinct Subtypes Of Mouse Embryonic Stem Cell Derived V1 Interneurons

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***Purpose/Objectives:** Spinal Cord Injury (SCI) is a debilitating condition that can result in significant loss of motor function and reduction in the quality of life for approximately 250,000 to 500,000 people worldwide every year. Therapeutic options are limited due to the inhibitory scar environment and downstream complexity of neural networks that control motor function. Recent advancements in cell transplantation have spurred interest in deriving various spinal interneurons for transplantation and in vitro modeling of neural circuits. Our laboratory has established methods for producing purified populations of spinal neurons by inducing mouse embryonic stem cells (mESCs) into motoneurons, V3 INs, and V2a INs. Although, there exists a method to produce the inhibitory V1 IN populations, there has been limited work conducted on generating enriched populations of specific subtypes of V1 INs. The goal of this research was to evaluate the induction of mESCs to V1 INs in order to drive specific subtype generation.
***Methodology:** mESCs were induced using a 2-/4+ methodology, wherein the first two days they are cultured in suspension without morphogens as they become embryoid bodies. The following four days EBs are induced with varying concentrations of sonic hedgehog agonist and retinoic acid to specify ventral and caudal (spinal) identity, respectively. Optimal concentration was initially determined by measuring the effect on gene expression of the definitive marker for V1 INs, Engrailed 1 (En1), and a cranial neuronal marker, Otx2. Further evaluation of the induction methods involved evaluation of various subtypic markers of V1 INs (FoxP2, MafA, Sp8, and Pou6F2) over the course of induction.
***Results:** We found low nanomolar concentrations of sonic hedgehog agonist had the strongest impact on En1 gene expression and V1 IN cell type. Additionally, varying the concentration of retinoic acid between (0.01 and 2 μM) had no impact for driving En1 gene expression, though necessary for spinal identity. By performing flow cytometry we found that we consistently produced >70% β-tubulin III+ cells and 45% of those neurons were En1+ with less than a 1% positive for definitive markers for V0 or V2a INs. Immunocytochemistry indicated that we produced significantly more FoxP2+ V1 INs, contrary to what is reflected in the literature using a slightly different protocol. To understand when different subtypes became specified, we evaluated gene expression of the various subtypes of V1 IN over the course of induction. FoxP2 gene expression peaked at day 6, however, we were unable to detect MafA gene expression until day 8. Previous work has suggested that the γ-secretase inhibitor DAPT is capable of driving the formation of MafA+ V1 INs through unexplained mechanisms, and down regulates FoxP2+ V1 IN specification. Interestingly, our data suggests that DAPT does in fact down regulate the gene expression of FoxP2, but DAPT alone was incapable of driving MafA gene expression.
***Conclusion/Significance:** This work demonstrates that production of multiple phenotypes of V1 IN is possible. By producing a methodology for generating enriched populations of FoxP2+ V1 INs, it enables future work into evaluating functional in vitro neural circuits.

305 - T Cells Regulate Il-4 Within Acellular Nerve Allograft Repaired Nerves To Promote Regeneration Of Myelinated Axons

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***Purpose/Objectives:** Clinical repair of peripheral nerve injury often requires the use of bridging materials for a gap, such as acellular nerve allografts (ANAs). ANAs, which are decellularized nerve grafts taken from cadaveric donors, has seen steady increase in its clinical use to treat nerve injuries. During regeneration in this context, the immune system plays a critical role. Previously, our studies showed that T cells are important for robust axon regeneration across ANAs. However, how T cells promoted nerve regeneration across ANAs was not elucidated.
***Methodology:** ANAs were generated using a previously published chemical detergent protocol. These ANAs were used to repair sciatic nerve gaps in Rag1KO (lacking adaptive immunity), IL-4GFP, IL-4KO, and WT (control) mice. As well, in select WT mice with nerve repaired using ANAs, monoclonal CD4 and IL-4 antibodies were used to deplete CD4 T cells and neutralize IL-4, respectively. Leukocyte responses to these conditions were quantified using qRT-PCR, flow cytometry, and immunofluorescence analysis. Nerve regeneration was quantified using histology, immunofluorescence analysis, and functional outcome metrics.
***Results:** In Rag1KO mice, which lack both T and B cells, regeneration of myelinated axons across ANAs and functional recovery were significantly hampered compared to WT. As well, myelin debris were increased within the graft, suggesting defect in myelination. To understand this outcome, leukocyte responses were considered before considerable axon regeneration across ANAs. While both T and B cells were absent, Rag1KO ANAs also contained fewer eosinophils compared to WT ANAs. Macrophage number or M2 polarization was not affected. Furthermore, gene expression analysis revealed that Th2 related cytokines, including IL-4, were reduced in Rag1KO vs WT ANAs. Therefore, the role of endogenous IL-4 was considered. Mice lacking IL-4 (IL-4KO) had reduced myelinated axon regeneration across ANAs, increased myelin debris, and functional recovery compared to WT. Based on the significance of IL-4 during regeneration, its source was identified using IL-4GFP reporter mice. These revealed a correlation between IL-4 expressing cells and T cell accumulation within ANAs. However, eosinophils, rather than T cells, were the primary source of IL-4 within ANAs. But, depletion of CD4 T cells reduced accumulation of IL-4 expressing eosinophils in ANAs. Therefore, these results demonstrated a CD4 T cell regulatory role in IL-4 expression. Depletion of either CD4 T cells or IL-4 led to significantly reduced regeneration of myelinated axons. Finally, the targets of IL-4 were considered. Co-culture of Schwann cells with neurons with exogenous IL-4 increased myelin basic protein expression vs control (no IL-4), which suggests endogenous IL-4 could target neurons and their axons or Schwann cells in vivo to exert its regenerative effects.
***Conclusion/Significance:** T cells promote regeneration in part through recruitment of IL-4 expressing eosinophils. IL-4 promotes robust myelinated axon regeneration across ANAs and functional recovery.

18 - Assessment Of A Peripheral Nerve Extracellular Matrix Derived Hydrogel For Improving Functional Recovery Following Nerve Reconstruction

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***Purpose/Objectives:** In the US, peripheral nerve injury (PNI) occurs in 3% of all trauma cases and affects an estimated 20 million people increasing by 250,000 annually with a cost of $150 billion[1-4]. Without intervention, peripheral nerves show a slow and lacking regenerative response following injury, making surgical intervention an imperative[5]. When interventions are performed, satisfactory motor recovery only occurs half of the time[6]. Furthermore, chronic denervation for prolonged periods results in irreversible loss of function[7,8]. Creating a therapy to both increase the regeneration rate and the extent of function regained following nerve reconstruction is of great clinical interest. Our goal is to translate a novel immunomodulatory ECM-derived hydrogel into clinical settings by first testing it in animal models.
***Methodology:** We developed a novel peripheral nerve-specific extracellular matrix (PNM) hydrogel and have shown that it increases constructive remodeling of injured peripheral nerves. The present study evaluates the PNM hydrogel's efficacy in 3 rat sciatic injury models of increasing severity. Injury types evaluated include nerve crush, transection, and sub-critical (8mm) gap, with experimental groups designed to compare end-to-end repair and autografting, the clinical gold standards, to PNM hydrogel. Measures included kinematic assessment, electrophysiological measurement, and histological analysis up to 12 (crush) and 24 (transection and gap) weeks.
***Results:** Results demonstrated increased rate and magnitude of recovery with application of PNM gel. The crush model achieved positive control values for muscle kinematics at 4 weeks post-injury as opposed to 8 weeks in the non-PNM comparison. Furthermore, the crush model also demonstrated a significantly increased number of axons and compound mean action potential (CMAP) over non-PNM groups, reaching 80% of control values as opposed to 50%. The gap model showed significant increases in kinematic metrics at 8 and 16 weeks over non-PNM conduit repair. Axon counts and CMAP for PNM treatment showed significant increases over conduit without PNM and showed similar values as seen in autograft repair, reaching 70% of control with only 40% for conduit without PNM. The crush (least severe) and gap (most severe) injury models demonstrate both a clear regenerative advantage for PNM application and equivalence to the autograft gold standard. However, results from the transection (moderately severe) model are less clear. Kinematic assessment following transection injury showed recovery up to 10 weeks followed by a plateau until 24 weeks in all groups. CMAP values were increased over negative controls, but did not demonstrate any differences in treatment types. We believe that the large variations observed are due to a synkinetic response in nerve repair from the end-to-end surgical technique used. Studies have shown that the transection injury type is, counter-intuitively, the most difficult injury modality to heal.
***Conclusion/Significance:** This strategy has demonstrated superior recovery compared to transection injuries repaired end-to-end. While we can conclude, overall, that our PNM gel increases nerve regeneration in most injury types, our next step will be to conduct a small gap model to demonstrate the PNM gel's efficacy in this commonly used surgical technique to overcome the difficulties present in a transection-type injury.

Session Number: 5
3D Bioprinting in Engineering Tissues and Organs I
Tuesday, December 3, 2019, 1:00 PM - 2:30 PM

19 - Processing Temperatures For The Development Of Polymeric Formulations For Extrusion-based Three-dimensional Printing With Growth Factors

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***Purpose/Objectives:** Performing three-dimensional printing with synthetic polymers often requires heating of the materials to reduce their viscosity. These processing temperatures may render bioactive molecules included in the printing formulation inactive. In this study, poly(propylene fumarate), a photocrosslinkable synthetic polymer with mechanical properties comparable to those of bone and biocompatible degradation products, is used for extrusion-based three-dimensional printing.
***Methodology:** In order to lower the temperatures required for the printing process, this polymer is supplemented with its monomer, diethyl fumarate, at various weight percentages, reducing the viscosity of the resulting mixture. A photoinitiator is also included to enable ultraviolet photocrosslinking using an accessory head of the commercial three-dimensional printer.
***Results:** Subsequently, the temperatures required for preparing the material formulation, transferring it to an extrusion printing cartridge, and printing with the material to fabricate multi-layer scaffolds are minimized. Formulations with at least 20 wt% diethyl fumarate were processable at temperatures tolerated by growth factors, and formulations with at least 10 wt% diethyl fumarate could be transferred and/or printed at or below physiologic temperature.
***Conclusion/Significance:** Our findings illuminate the choice of a printing formulation that allows incorporation of bioactive molecules such as growth factors. Consequently, we are currently investigating the growth factor bone morphogenetic protein-2 for printing within poly(propylene fumarate)-based scaffolds. Applying release supernatants from these constructs to cellular assays involving W-20-17 murine bone marrow stromal cells will be used to verify maintained growth factor bioactivity under the temperature conditions imposed during material printing and processing.

20 - The Effect Of Peptide-tethering Bioinks On The Differentiation Of Human Dental Pulp Stem Cells In The Bioprinted Dental Construct

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***Purpose/Objectives:** Tooth loss is a significant health issue affecting millions of people in the US and worldwide. Artificial dental implants are the current gold standard tooth replacement; however, they do not exhibit many properties of natural teeth and can be associated with complications leading to implant failure. Thus, a bioengineered tooth bud has been proposed as an alternative tooth replacement option. To facilitate this effort, the fields of Tissue Engineering and Regenerative Medicine have made great progress in 3D bioprinting technologies capable of producing tissue constructs using various cell types, biomaterials, and/or bioactive molecules. In this study, we fabricated a bioengineered dental

construct containing human dental pulp stem cells (hDPSCs) using 3D bioprinting capability. We hypothesized that a bone morphogenetic protein (BMP)-mimetic peptide-tethering bioink could accelerate the differentiation of hDPSCs in the bioprinted dental constructs.
***Methodology:** We used a thiolated BMP-mimetic peptide that was directly conjugated into a gelatin methacrylate (GelMA)-based bioink. We examined the rheological properties, printability, and the structural stability of the bioprinted dental constructs. Moreover, we evaluated the biological properties and hDPSC differentiation in the bioprinted constructs by measuring cell viability and proliferation, calcium content, and immunofluorescence (IF) for dentin sialophosphoprotein (DSPP) and osteocalcin (OC).
***Results:** The peptide conjugation into the GelMA-based bioink formulation was successfully performed. We determined that greater than 50% of the peptides remained in the bioprinted construct after 3 weeks in cell culture conditions. DPSC viability was >90% in the bioprinted constructs after the printing process. Alizarin Red staining showed that the BMP-mimetic peptide-tethering bioink bioprinted dental construct group exhibited the highest calcification as compared to the other groups (growth medium and non-BMP peptide groups). In addition, IF analyses showed robust expression of DSPP and OC in the BMP-mimetic peptide-tethering bioink. These results strongly suggested that BMP-mimetic peptide-tethering bioink could accelerate the differentiation of hDPSCs in the bioprinted dental constructs.
***Conclusion/Significance:** We have developed a novel BMP-mimetic peptide-tethering bioink for bioengineering dental tissue constructs. This bioink formulation provided printability as well as a dental-specific microenvironment that supported hDPSC differentiation. 3D bioprinting strategies combined with the peptide-tethering bioink formulation has great potential to successfully bioengineer dental tissue constructs, including bioengineered tooth buds, for use in future Regenerative Medicine and Dental applications.
Acknowledgments: This study was supported by NIH/NIBIB (1P41EB023833-346 01) and NIH/NIBIB (R01DE026731).

21 - 3d Bioprinting A Contractile Ventricle Using Human Stem Cell-derived Cardiomyocytes

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***Purpose/Objectives:** Myocardial infarction is a leading cause of death due in large part to the limited regenerative capacity of the adult heart. Human embryonic and induced pluripotent stem cell-derived cardiomyocytes have provided a new way to rebuild the myocardium, but these cells are phenotypically immature and difficult to organize into functional muscle. Tissue engineering promises to replace scar with aligned, contractile cardiac tissues, but to date has been limited to simple geometries and fabrication processes that lack scalability towards more complex organ-scale constructs. Here we report the development of a 3D model of the left ventricle using the Freeform Reversible Embedding of Suspended Hydrogels (FRESH) 3D bioprinting technique.
***Methodology:** FRESH is a 3D bioprinting approach specially developed for cells and hydrogels, and works by printing within a temperature sensitive support material that is gently melted away after the printing process. We used FRESH in a dual material strategy that printed collagen type I bioink as a structural material and a high-density cellular bioink composed of human embryonic stem cell-derived cardiomyocytes (hESC-CMs) as the contractile component. The ventricle was designed on the scale of an

embryonic heart as an ellipsoidal shell 8 mm from base to apex and 7 mm at its largest diameter.
***Results:** The ventricle was printed with high fidelity, and after 7 days in culture had visible. synchronized contractions. A dense layer of interconnected and striated hESC-CMs was found throughout the ventricle, confirmed by immunofluorescent of sarcomeric alpha-actinin. The ventricles had a baseline spontaneous beat rate of ~0.5 Hz and using field stimulation could be captured and paced up to 2 Hz. Calcium imaging of the spontaneous contractions revealed propagation of calcium waves with conduction velocities of ~1 cm/s. Finally, we observed wall thickening of ~13% at 1 Hz pacing and a decrease in cross-sectional area of the ventricular chamber, suggesting we were able to achieve similar contractility to native myocardium.
***Conclusion/Significance:** Together, these results demonstrate that collagen and hESC-CMs can be bioprinted together into ventricular constructs that exhibit key functional metrics of that heart, including synchronized contraction, action potential propagation, and wall thickening.

22 - 3d Bioprinted Dermoepidermal Human Skin Equivalent As An Experimental In Vitro Model For The Evaluation Of Antibacterial Topical Treatments

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***Purpose/Objectives:** Human skin equivalents (HSEs) serve as in vitro platforms for permeability, sensitivity and toxicity studies, among many others, of new drugs, cosmetics and topical treatments [1]. Due to the higher accuracy mimicking native environments than conventional 2D cell culture models, HSEs have been successfully implemented as part of preclinical evaluation protocols [1]. This has led to an alternative to help to optimize and reduce the use of animals for experimentation. Here, we aimed at fabricating a dermoepidermal HSE model via 3D bioprinting and at standardizing a protocol for its in vitro infection with Staphylococcus aureus. Moreover, the infected HSE model was used to evaluate an antibacterial topical consisting of a magnetite-polyethylene glycol (PEG)-buforin II (BUF-II) nanobioconjugate dispersed on an oil-based emulsion.
***Methodology:** For fabricating the HSEs, primary human dermal fibroblasts were embedded on an extracellular matrix (ECM)-based hydrogel and subsequently bioprinted via extrusion to form 10mm x 10mm x 0.5mm constructs. Printbead temperature was maintained at 37ºC during the printing process to facilitate crosslinking of the bioinks. After printing and crosslinking, the constructs were submerged on fibroblast growth media and incubated at 37ºC, 5% CO2 and 95% relative humidity for 7 days. Culture media were replaced every 2 days. Next, human epidermal keratinocytes were manually seeded over the bioprinted constructs and incubated in the absence of medium during 60 min to allow adhesion. Resulting tissue constructs were submerged on epidermalization medium and incubated for 6 days before raising them to an air-liquid interface to promote complete maturation. Histological and immunohistological assays were performed to observe the integrity of the dermis and epidermis layers, as well as stratification of the epidermis. To infect the resulting HSEs with S. aureus, tissue constructs were first wounded with a razor blade and then pierced with the needle at the edge of the wound at a low angle and then pushed toward the wound center, where bacteria were finally ejected [2]. Infected HSEs were then incubated at 37ºC for 24 hours to allow bacterial adhesion. This was followed by placing the different formulations of the antibacterial topical on top of each infected wound and subsequently incubating for another 24 hours the treated tissue constructs. Finally, to determine the extent of the antibacterial effect of the topical treatments, colony forming units were quantified via SEM and TEM

imaging.
***Results:** Results showed complete stratification of the epidermal portion of the HSEs and a well defined basal membrane that maintained dermis and epidermis separated. Furthermore, bacterial colonization of the tissues was confirmed with histology of Gram stained portions of the constructs. The developed topical treatment demonstrated considerable antibacterial activity without significantly affecting cell viability on the HSEs.
***Conclusion/Significance:** Current work demonstrates the value of engineered HSEs as platforms for the reliable in vitro testing of novel drugs and topical treatments before performing experiments on animal models.
References:
[1] Y. Yousuf, S. Amini-Nik, M. Jeschke. Skin Tissue Models, 2018. [2] J. Shepherd, I. Douglas, S. Rimmer, L. Swanson, S. MacNeil. Tissue Engineering Part C Methods, 15, 3, 2009.

23 - Development Of An Air-brush Hand-held Biopen For Skin Tissue Biofabrication.

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***Purpose/Objectives:** The manufacture of artificial tissues, and their application in clinical practice, have become one of the most effective solutions to alleviate the negative effects of large burns in humans and for helping to recovery in wound healing. Recent publications in skin tissue engineering centres on the synthesis of three-dimensional (3D) polymer scaffolds which contains functional biomolecules to which cells are introduced, leading to scaffold/skin constructs that can be used for skin regeneration. These approaches had benefited from recent 3D bioprinting manufacturing process. 3D biofabrication technology is increasing in tissue engineering and seems to make possible new approaches because of several reasons. First of all, the capability of generating spatial free designs help to mimic all human structures, second the design of new material favors cell growth, and the creation of structures in layers aid to create a more biological approach. A problem of 3D biofabrication is complexity of the 3D process itself for in-patient skin tissue regeneration. To solve this problem for skin tissue engineering, we have developed an airbrush-based biopen for skin tissue biofabrication.
***Methodology:** We have validated the biopen by spraying multiple layers of biomaterials containing living cells, such as photocrosslinkable Gelatin-methacryloyl (GelMA), in a biologically relevant range of concentrations (5 - 15% w/v) and alginate/calcium chlorite. The air-brush biopen was loaded with a bio-ink (1) comprised of GelMA containing HUVEC cells and a bioink (2) GelMa containing fibroblast to recreate hypodermis and dermis, respectively.The bioink was then sprayed onto the surface and solidified by a UV light. To reduce the potential cell damage induced by prolonged UV exposure, we have used lithium phenyl-2,4,6-trimethyl-benzoylphosphinate (LLAP) which is shown to have minimal cytotoxicity. For the alginate based bioinks, we have sprayed layers of alginate bioink/calcium chloride, alternatively, to promote crosslinking of alginate. Cell survival after different spraying conditions (spray pressure, biofabrication speed, volume of bioink, drops size), UV light exposure (exposure time and intensity) and photoinitiator concentration were evaluated.
***Results:** The Biopen has been validated for skin tissue biofabrication. In particular we have biofabricated dermis and epidermis and we have studied cell viability after bioprinting for the different

printing parameters involved.
***Conclusion/Significance:** We have developed a biopen which enables the deposition "manually" of living cells and biomaterials. Such a hand-held biopen facilitates the deposition of biomaterials and living cells with potential application on skin tissue regeneration.

24 - Assessment Of A Universal Printing Technology For Biphasic Scaffolds Containing Decellularized Bone And Cartilage Matrix

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***Purpose/Objectives:** Tissue engineered scaffolds should be cytocompatible, bioactive, provide biomimetic architecture, and maintain mechanical stability. Although these requirements are universal, many complex manufacturing techniques - which still fail to address these requirements - are proposed for each tissue type. For example, methods that use thermopolymers such as polycaprolactone (PCL) provide mechanical strength, but no bioactivity. Others that allow bioactivity (often in the form of proteins embedded in gels) lack rigidity. These limitations become particularly important when considering structural or anisotropic tissues that need different properties and protein compositions distributed throughout the constructs. Here, a universal, all-in-one manufacturing technique was utilized that can easily create multiphasic scaffolds. Large osteochondral defects manifest in severe joint pain. Scaffolds for osteochondral injury (OCI) must provide backbones for cartilage and bone layers that are strong enough to withstand in vivo forces and provide positional cues for the surrounding tissue and infiltrating cells. Based on our initial success in using this technique for uniphasic cartilage regeneration,[1,2] we sought to assess constructs for OCI, theorizing that they may be resolved with a platform 3D printing technology capable of producing depth-dependent architectures, mechanical properties, and biological compositions.
***Methodology:** Porcine tissue was acquired post-mortem. Samples were collected from: articular cartilage (AC) and subchondral bone (SB). The samples were decellularized in a series of rinses and washes as previously described.[1] The resultant decellularized extracellular matrices were encapsulated in polylactic acid (PLA) microspheres via emulsion.[1] The microspheres were co-extruded with PCL powder in a 1:4 ratio to make filaments.[2] Biphasic scaffolds of 8 mm diameter which included AC and SB were created using a commercially available 3D printer with 0/60/120^{o} and 0/90^{o} laydown patterns, respectively. The scaffolds were press-fit into surgically created defects on stifle joints of juvenile pigs for 12 weeks.
***Results:** Histological assessment showed ingrowth of the surrounding tissue, interface homogeneity, and mineral deposition in the positive control (intact) and experimental (AC/SB scaffolds) group and minimal cellularity and mineral deposition in the negative control group (empty defects and scaffolds without decellularized matrix microspheres).
***Conclusion/Significance:** The successful fabrication and implantation of these multiphasic, multi-compositional scaffolds shows the flexibility of the proposed system as a platform technology for tissue engineering, specifically for repair of large osteochondral injuries. Ongoing studies are focusing on further characterization of the constructs.

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392 - 3D Bioprinting And Bioreactor-based Approaches For Complex Tissue Engineering

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***Purpose/Objectives:** Fabrication of complex tissues is an important facet of tissue engineering and has enormous therapeutic potential in regenerative medicine. The Center for Engineering Complex Tissues (CECT) is a NIH/NIBIB funded Biotechnology Resource Center (BTRC) engaged in advancing 3D printing and tissue engineering strategies to develop novel, transplantable, and clinically relevant tissue constructs. The center aims to educate and grow the biofabrication community in order to accelerate advances in regenerative medicine and provide transformative medical solutions to hospitals and patients in need. This talk addresses the broad range of printing technologies such as stereolithography, micro-extrusion, and electrospinning, applied by research leaders at CECT along with several academic collaborators to develop novel 3D-printed platforms for improving our understanding of the etiology and treatment of clinical pathologies.
***Methodology:** The center applies a multifaceted approach involving bioreactor design, live cell patterning, and complex scaffold fabrication for *in vitro* tissue generation. 3D-printed bioreactors printed at the University of Maryland provide dynamic culture strategies that promote rapid and controllable exchange of nutrients via fluid flow, simulate physiological effects of shear stress found *in vivo,* and promote vasculature development. Using novel live cell 3D-printing strategies, researchers at the Wake Forest Institute for Regenerative Medicine have successfully fabricated biologically relevant tissue constructs such as ear, nose, muscle, and cardiac tissue. Researchers at Rice University have developed heterogeneous, multi-phasic, and bioactive scaffolds with relevant extracellular composition and a complex microarchitecture for bone tissue repair.
***Results:** Microbioreactor designs capable of varying cell spacing within a dynamic culture were fabricated to systematically probe cell response to external stimuli while controlling the cell signaling modalities. Bioreactors have also been applied for tissue engineering applications such as extracellular vesicle generation, cardiac tissue engineering, and flow-induced vasculature development. Furthermore, novel bioink formulations have been devised that serve as scaffolding for 3D bioprinting to enable the successful patterning of cells and biomolecules. We have investigated the printability of common bioinks as it relates to their intrinsic rheological properties and the effects of extrinsic printing parameters. Bioinks derived from decellularized matrix (dECM) functionalized with peptides, as well as silk-based bioinks have been successfully printed for a broad range of applications. By optimizing printing strategies and biomaterial combinations, we have engineered radially graded porous 3D polycaprolactone (PCL)-based scaffolds with controlled degradation and bioactive components. Composite hydroxyapatite (HA)/polymer and βtricalcium phosphate (β-TCP)/polymer scaffolds have been fabricated for enhanced pore morphology, mechanical integrity, and ceramic composition. This work highlights the fabrication of scaffolds using novel 3D printing strategies for osteochondral repair.
***Conclusion/Significance:** CECT researchers work closely with several collaborators and partners on research directives that complement the center's research and technical capabilities. Collaboration

projects range from 3D-printed knee-joint bioreactors, bioengineered constructs for tooth regeneration, and 3D-printed osteochondral constructs for *in vivo* implantation. Through these efforts, CECT aims to be a national and international resource for the biomedical community and a platform to establish collaborative efforts towards regenerative medicine applications.

26 - Development Of A Universal Bioink Technology For Multi-modality Bioprinting Compatibility And Support Of Multiple Tissue Construct Types

A. Skardal;
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***Purpose/Objectives:** 96 800x600 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:10.0pt; font-family:"Times New Roman",serif;} The need for replacement tissues in human patients continues to increase. Donor organs are limited, and tissue engineering has yet to make an impact. This is due to a lack of standardization of technologies. Our objective is to develop a bioink that is tunable to multiple tissue products and bioprinting hardware types.
Methodology:** 96 800x600 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:10.0pt; font-family:"Times New Roman",serif;} We designed a biomaterial system with 1) material properties that allow dynamic response to mechanical stresses placed on it by any bioprinter, ensuring cross-compatibility, 2) customization of proteins to match biochemical profiles of tissues, and 3) mechanical manipulation to match elastic moduli of tissues. Collagen methacrylate (CollMa) is mixed with thiolated hyaluronic acid (HA) at pH 7.0. Cells are added for cellularized bioprinting. After thiol-methacrylate crosslinking and subsequent printing, remaining unreacted methacrylate groups can be harnessed by photocrosslinking to stabilize and drive increases in ***G' for matching a particular target tissue elastic modulus. Thixotropy was achieved by integrating functionalized catecholamines or gelatin nanoparticles, creating reversible crosslinks in the bioink. Laminin and fibronectin were modified to be coupled covalently into the bioink, tailoring formulations to different tissue types. Rheological tests were performed to validate the thixotropic properties, after which printability was assessed using multiple bioprinters to assess cross-platform compatibility. The influence of cell adhesion protein manipulation was assessed in the context of liver, cardiac, adipose, neural, and pancreatic islet 3D constructs using tissue-specific functional tests.
***Results:** 96 800x600 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:10.0pt; font-family:"Times New Roman",serif;} Controlling ratios of covalent crosslinks to reversible crosslinks modulated thixotropy. Importantly, these bioinks extruded smoothly in multiple bioprinter hardware platforms, forming 3D constructs. LIVE/DEAD and MTS assays verified high cell

viability over multiple time points in the various tissue construct types, once the appropriate HA, CollMa, fibronectin, and laminin rations were reached. Covalent integration of laminin and fibronectin resulted in significant changes in cell morphology and function, showing 1) the clear integration of these proteins into the bioink, and 2) the capability to now use the ratios of these proteins to drive cellular behavior. Bioprinted 3D constructs were functional, supporting tissue-specific functional assays successfully.
***Conclusion/Significance:** 96 800x600 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:10.0pt; font-family:"Times New Roman",serif;} These data show the versatility that a well-designed, modular bioink can have for tissue engineering applications that in the future may support commercial biomanufacturing of various tissue products using a well-defined bioink platform.
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27 - A Comparative Study Of 3d Bioprinting Strategies For Biomedical Applications

J. Park, S. J. Crotts, K. M. Petersen, S. J. Hollister;
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***Purpose/Objectives:** Several 3D bioprinting strategies have been developed and used for biomedical applications. In this study, a comparative study of two different 3D bioprinting strategies was performed to understand each printing characteristic and make the most appropriate choice for creation of reliable biomedical devices.
***Methodology:** We employed two representative 3D bioprinting strategies, laser sintering- and extrusion-based strategies and one material, a poly-ε-caprolactone/hydroxyapatite (PCL/HA) blend material to print the same 3D structures. Both solid and porous structures were printed from PCL/HA blended materials using the laser sintering and extrusion strategies. The printed structures were characterized using scanning electron microscopy (SEM), micro computed tomography (μCT) scanning, mechanical testing, and cell attachment and proliferation *in vitro*. We also performed an *in vitro* accelerated degradation study to analyze the changes in the mechanical properties/behaviors of the 3D structures printed from different 3D bioprinting strategies.
***Results:** Although we employed the same printing material, the 3D structures printed from different 3D bioprinting strategies showed different morphology, geometry, and surface roughness. These characteristics consequently had direct effects on cell attachment and proliferation. In addition, different mechanical properties caused by different void volumes were observed, and the difference was more significant in porous structures than solid structures. The changes in mechanical properties/behavior of the structures over the degradation time showed different trends according to the bioprinting strategies.
***Conclusion/Significance:** In conclusion, each 3D bioprinting strategy has inherent characteristics, and understanding the characteristics is important in choosing the best strategy to create specific and reliable biomedical devices.

28 - Manufacturing Of Functional Tissues In Vitro Using Bioprinting And Bioreactors. Application In Spinal Cord Tissue Regeneration.

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***Purpose/Objectives:** A lot of efforts have been directed to the creation of functional spinal cord tissue in the lab. The lack of tissue regene ration in human beings and the deficiency of allogenic transplants in addition to the increasing of life expectancy make this problem to be considered as one of the most important ones of humanity in the current era. Spinal cord tissue is a connective tissue that lacks vascularization and innervation, and is composed of a specific extracellular matrix. The healing process of spinal cord tissue is slow and results in a fibrous scar-like tissue that lacks the functional properties of the hyaline cartilage leading to further tissue degeneration. However the results obtained are still far away from the desired. For the creation of a living tissue it is crucial the bioprinting process but also the maturation of the construct. Replicating the human being adult conditions *in vivo* in the lab or the stimuli that occur in embryogenesis could improve the results of tissue engineering towards the clinical application of the technology.
***Methodology:** Here we propose a unique approach to create functional spinal cord tissue starting from bioprinted constructs (using bioprinting) and a device that mimic the physiology and apply the right mechanical conditions of the structure to be replaced and through the maturation procedure, applying the right stimuli, creates a functional tissues. For us the best stress distribution is the real one, and other approaches fail as do not mimic the real conditions happening in nature.
***Results:** In this projectwe show a method that helps to create functional spinal cord tissue after bioprinting. For the creation of a living tissue it is crucial the bioprinting process and the ingredients selected to achieve the objective to create a functional specific tissue. But also the maturation procedure applied to the 3D cell laden constructs, that is even more important (second block of the image). If we think about bioprinting as a technology to recreate all the structure in the same form as shown in a living spinal cord tissue, we are going to fail. We have to think on bioprinting as a way of creating cell laden 3D constructs as a precursor of a functional tissue. The maturation and tissue formation process will be as important or even more than the bioprinting one. Considering the strategies of both blocks in the diagram will be crucial to obtain the desired functional spinal cord tissue.
***Conclusion/Significance:** The stress distribution is crucial as stimuli to create the right tissue. Also the scaffold architecture as it will affect the stimuli distribution and other important parameters as the biodegradation time. Selection of the right ingredients and the bioprinting procedure is very important in the success of the creation of functional spinal cord tissue, as well as the maturation procedure applied to the 3D cell laden constructs is even more important. This approach opens a wide research area for tissue engineers to develop protocols with different stimuli to create functional spinal cord tissue after bioprinting.

Session Number: 6
Translating Microphysiological Systems Technology to Preclinical Drug Development and Disease Modeling I
Tuesday, December 3, 2019, 1:00 PM - 2:30 PM

29 - Drug Efficacy And Safety Determination In Drug-dosed Human-on-a-chip Systems

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***Purpose/Objectives:** The current drug development process is inefficient and costly and takes years from compound identification to marketable drug, with costs up to 2.5 billion dollars per drug. One reason for the lack of novel drugs is that preclinical animal models do not simulate multidimensional clinical conditions leading to poor performance in human clinical trials of efficacy and safety. Consequently, human-based in vitro systems capable of measuring "organ" physiology, biomarker generation, and the interactions between organs would provide an improved platform for drug testing for efficacy and off-target toxicity.
***Methodology:** Optimally configured human-on-a-chip (HoaC) systems integrate micro-electro-mechanical systems (bio-MEMS) devices in a housing with recirculating serum-free medium supported long-term survivability. Hesperos has constructed stem cell-based, human-on-a-chip systems demonstrating long-term physiology (>28 days) in configurations of up to five organs [1]. Acute and chronic compound testing in systems has generated drug efficacy and safety responses similar to those seen in clinical data or reports from literature [2].
***Results:** We will describe HoaC systems composed of liver, cardiomyocytes, skeletal muscle myotubes, motoneurons and a functional, rudimentary kidney module in configurations relevant for parent compound and metabolite efficacy and safety testing. The organ modules functioned for 28 days in a recirculating serum-free medium providing efficacy and safety data generated by noninvasive measurements in acute-dose, chronic-dose and control systems [3]. In dosed systems, we have observed clinically-relevant responses to over 20 compounds in all modules indicating the predictive capabilities of this system for drug testing. Application of these systems for ALS, Alzheimer's, rare diseases, diabetes and cardiac and skeletal muscle mechanistic toxicity will be reviewed. The development of in vitro PDPK models that are being used to predict in vivo results will also be presented. Concurrent measurement of both efficacy and toxicity can also be done in the same system for therapeutic index estimation [4].
***Conclusion/Significance:** Scaled accurately, these systems can capture and predict organ physiology, organ-organ interactions, and PKPD in response to drug candidate dosing. Taken together, these data can generate predictive models regarding drug efficacy and safety for clinical testing, reducing the cost of drug development. This talk will also give results of six workshops held at NIH to explore what is needed for validation and qualification of these new systems.
References

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2. Oleaga C, et al. Investigation of the Effect of Hepatic Metabolism on Off-Target Cardiotoxicity in a Multi-Organ Human-on-a-Chip System. Biomaterials 182, 176, 2018.

3. Oleaga C, et al. Long-Term Electrical and Mechanical Function Monitoring of a Human-on-a-Chip System. Advanced Functional Materials 2018, 1805792, 2019.
4. McAleer C, et al "Reconfigurable Multi-Organ System for the Evaluation of Anti-Cancer Therapeutics on Efficacy and Off-Target Toxicity," Science Translational Medicine accepted 2019.

30 - Integrated Human Multi-tissue Platform For Preclinical Modeling Of Drug Toxicity And Disease

K. Ronaldson-Bouchard, K. Yeager, D. Tavakol, G. Vunjak-Novakovic;
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***Purpose/Objectives:** To combat the rising costs and timelines of drug development, human engineered tissues can be used as miniature models of their respective organs to prescreen a drug's safety and efficacy in-vitro, providing human data before the drug is actually at the clinical stages of development. These physiologically relevant organ-on-a-chip platforms require the development of additional methodologies to functionally integrate the tissues, for studies of the human-on-a-chip, in a way that preserves their individual biological fidelity.
***Methodology:** To do this, we designed a series of custom bioreactors to first develop and mature each of the five tissues (heart, liver, bone, skin, vasculature/immune) separately, using a single humaninduced pluripotent stem (iPS) cell line to develop all tissues. This approach enables patient-specific human-on-a-chip studies for personalized medicine approaches and the development of patient disease models. Upon tissue maturation, the engineered tissues were combined into an integrated platform that was designed to connect each tissue via culture above a vascularized transwell insert, while maintaining the homeostatic tissue specific niche in the tissue culture compartment above.
***Results:** We demonstrate that all tissues types (heart, liver, vasculature, bone, skin) and the immune cells in the perfusate maintain their stable phenotype throughout 4 weeks of vascular perfusion in the integrated platform. The expression of molecular markers, tissue morphology and functional readouts were measured for each tissue type under these conditions and compared against isolated cultures and those integrated without vascular separation but instead using a common mixed media. Mixed media conditions resulted in tissue dedifferentiation, whereas integrated and isolated conditions maintained high biological fidelity. We then examined tissue-specific responses to Dox-induced toxicity including decreased vascular barrier function, increased cardiac troponin release in circulation, and morphological degradation of each of our micro-tissues after exposure to drug. In addition, we identified micro-RNA values that were differentially expressed between the Dox-treated and control groups, which mapped very closely to clinically measured cardiotoxicity micro-RNAs in humans.
***Conclusion/Significance:** Overall, we demonstrate the advantages of integrating mature engineered tissues in a way that preserves the homeostasis of each tissue while enabling communication between organ systems. The development of methods to functionally integrate mature organ-on-a-chip systems while maintaining and promoting physiological relevance will enable future studies of the human-on-a-chip. These results detail the importance of maintaining the tissue-specific niche when designing integrated organ-on-a-chip platforms, particularly when integrating functionally mature tissues.By using a single patient iPS cell line to derive all tissue systems, patient-specific studies can be investigated. These methods can be further utilized as tools to mechanistically understand the variations in population specific responses to drugs and disease.

31 - Microfluidic Enabled In Vitro Analysis Of The PK/PD/Efficacy Relationship For Preclinical Testing Of Oncology Compounds

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***Purpose/Objectives:** High failure rates of drugs through clinical development is well documented. Although many complex in vitro models have been developed, thorough characterization of the relationship between pharmacokinetics (PK), pharmacodynamics (PD) and efficacy is critical in the effective discovery and development of new drugs, schedules and combinations. The PK/PD/efficacy relationship has historically been characterized in xenograft models, owing to an absence of viable alternatives. The study of this relationship *in vitro*, has to date been problematic as the generation of varying concentrations over time in multi-well plates has not been possible.
***Methodology:** We have explored an *in vitro* methodology utilizing a microfluidic delivery device capable of precision addition and removal of medium from the wells of a microtiter cell culture plate. Through stepwise addition and removal of medium the device was able to recapitulate PK-like, time varying concentration profiles of one, or more drugs in individual wells. We used this approach to explore the effects of several oncogenic pathway inhibitors, initially we explored the effects of a PI3K/mTOR inhibitor on p-AKT levels, and viability in a number of cancer lines. The PI3K/mTOR pathway is a central oncogenic pathway deregulated in cancer and p-AKT is a marker of PI3K pathway activity. In a following study DNA-PK inhibitors were used to compare the efficacy of a combination of compounds dosed in a PK-like manner. FaDu tumor xenograft studies were performed and results were compared to the microfluidic device.
***Results:** BYL719 and PI-103, are PI3K inhibitors with varying PK profiles and varying target coverage against PI3K isoforms. The compounds were tested on multiple cell lines. Using the microfluidic device, we generated PK-like dosing profiles for the two compounds, (BYL719 - $t_{1/2}$ 6 hours) (PI-103 - $t_{1/2}$ 3 hours) which mimicked their *in vivo* clearance profiles over a 24-hour period. p-AKT levels were initially reduced by both compounds but recovered at 24 hours as the concentration of the compound in the well declined. This replicates findings in xenograft models from literature. The shorter $t_{1/2}$ of PI-103, resulted in a more rapid recovery of p-AKT than observed for BYL719. PK-like profiles of a combination of DNA-PK inhibitors were generated using the microfluidic device. Efficacy was understood *in vitro* through relative cell growth and compared to tumour volume in xenograft models, the same rank order of inhibition was observed.
***Conclusion/Significance:** In conclusion, this study demonstrates the microfluidic addition and removal device can be used to recapitulate PK-like profiles *in vitro* and allow exploration of the PK/PD/efficacy relationship. The availability of an *in vitro* method could enable these important parameters to be determined at an earlier stage of the drug discovery process.

32 - Cardiac Amyloidosis On A Chip

G. Tansik[1,2], A. Alassaf[1], J. Musi[1], G. Sharma[3], V. Mayo[1], R. Prabhakar[3], A. Agarwal[1,2];
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***Purpose/Objectives:** Presence of extracellular amyloid deposition within the heart results in cardiac amyloidosis (CA), leading to progressive heart failure. Initially believed to be a rare phenomenon, an increasing number of end-stage heart failure patients are now being diagnosed with CA. This debilitating disease is poorly understood and has no cure. A clear understanding of the mechanisms that promote this disease pathogenesis is important to identify and develop effective treatments. However, current planar, static cell culture systems or animal models have limitations such as cells cultured *in vitro* do not function like human bodies and animal studies take years to complete. These challenges highlight the urgent need for more physiologically relevant *in vitro* model of CA. Microengineered cell culture models can be used to address these problems due to the many advantages that they offer. Organ-on-Chip models include microfabrication and microfluidics technologies not only to provide a variety of extracellular cues to cultured cells in a physiologically relevant context, but also to control the cellular microenvironment with high spatiotemporal precision. In addition, biomaterial platforms can be modulated to mimic the mechanical extracellular matrix changes occurred during disease conditions. Importantly, gelatin is derived from collagen and does not require any additional extracellular matrix linkage steps to facilitate cell adhesion, and its stiffness can be tuned within physiological range. Therefore, in this study, we seek to combine the information obtained from computational modeling of amyloid peptide-gelatin hydrogel interaction with recent advances in Heart-on-a-Chip technology to develop a CA disease model by maintaining biomimetic cultures of engineered cardiac tissue that represent both healthy and diseased states and collecting electrical and contractility readouts.
***Methodology:** Gelatin hydrogels were prepared by mixing a certain amount of 4% (w/v) microbial transglutaminase (mTG) solution with different concentration of gelatin solutions. Viscoelastic properties of prepared hydrogels were determined via a rheometer. Amyloid fibril formation was performed through acid-mediated denaturation of human Transthyretin protein. Fourier-transform infrared (FTIR) spectroscopy, thioflavin T (Tht) fluorescence assay and circular dichroism (CD) spectroscopy measurements were performed to determine the amyloid fibril formation. All-atom molecular dynamics simulations were used to analyze the integration between gelatin hydrogel and amyloid peptide. Commercially available microelectrode array (MEA) chips were tested for collection of electrical potential readouts.
***Results:** Tht fluorescence assay, FTIR and CD spectroscopy measurements validated the formation of amyloid fibrils. In order to mimic healthy cardiac tissue and disease conditions of CA, gelatin hydrogels with different stiffness values were fabricated through crosslinking with mTG. MEA chips were optimized for recording and analysis of electrophysiological readouts from aligned cardiomyocytes on top of hydrogels. The interaction between the gelatin hydrogel and amyloid peptide was analyzed through computational modeling approach.
***Conclusion/Significance:** In future studies, cardiac monolayers will be engineered on amyloid peptide deposited hydrogel network. Electrical and contractility readouts from engineered cardiac tissues within amyloid peptide deposited hydrogel microchip will be collected and analyzed.

33 - Differentiation And Characterization Of HiPSC-cortical Neurons And Their Application To Drug Evaluation In CNS Disease Models

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***Purpose/Objectives:** The development of an iPSC (induced pluripotent stem cell)-derived cortical neuron-microelectrode array (MEA) microphysiological system (MPS) that can recreate in vivo synaptic architecture and physiology is an ideal drug delivery platform for testing the safety and efficacy of novel compounds for disease treatment.
***Methodology:** Initially, a protocol was developed to differentiate cortical neurons from human iPSCs. Then, cortical neurons were characterized morphologically by phase microscopy and immunocytochemistry and functionally by patch-clamp electrophysiology. Subsequently, neurons were cultured on multi-electrode arrays (MEAs) to determine the effects of chemicals on neural circuit physiology for modeling brain disease phenotypes. Long-term potentiation (LTP) was induced via a high-frequency stimulation (HFS). In this system, we also tested $GABA_A$ receptor antagonists and agonists as chemical convulsants or anti-convulsants, respectively.
***Results:** Specifically, the expression of neuronal markers and neuronal activity increased throughout maturation.On day 0 of maturation, 50 percent of the culture expressed layer V cortical neuron marker ctip2 and neuronal marker beta-III tubulin and displayed spontaneous and repetitive firing through whole-cell patch clamp. By day 28 of maturation, 90 percent of the culture expressed the aforementioned markers and displayed electrical activity of much higher intensity. After cells were cultured on MEAs, the induction of LTP was established and could subsequently be abolished following the dosage with AMPA and NMDA receptor inhibitors. $GABA_A$ receptor antagonist administration enhanced spontaneous activity mimicking an epileptic phenotype that further increased upon electrical stimulation, while $GABA_A$ receptor agonist administration quieted spontaneous activity.
***Conclusion/Significance:** The versatility of this model lies in its ability to investigate an array of brain diseases characterized by functional brain deficits. Chemicals affecting receptor binding can be added to either enhance or inhibit neuronal activity. This serum-free, hiPSC cortical neuron model establishes a platform for the evaluation of neuron activity as well as a platform for drug testing in vitro.

Session Number: 9
Skin, Wound Healing, and Inflammation
Tuesday, December 3, 2019, 3:00 PM - 4:30 PM

34 - Integration Of Bioprinted Skin In Full-thickness Wounds Promotes Epidermal Barrier Formation And Normal Collagen Organization

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***Purpose/Objectives:** 1.1 million burn injuries are treated annually in the United States. Current tissue engineered skin fails to meet the need for full-thickness replacement. Bioprinting technology has allowed fabrication of full-thickness skin with multiple cell types organized into biomimetic layers in vitro. The purpose of this study is to test the effects of bioprinted human skin integration on epidermal barrier formation and normal collagen organization.
***Methodology:** Human keratinocytes, melanocytes, fibroblasts, dermal microvascular endothelial cells, follicle dermal papilla cells, and adipocytes were suspended in a fibrinogen bioink, and bioprinted to form a tri-layer skin structure. The bioprinted skin was then implanted onto 2.5 x 2.5cm full-thickness excisional wounds on mice. Digital planimetry was performed and analyzed with ImageJ to quantify total wound closure, epithelialization, and contraction. Skin samples were taken for histology at 21, 42, and 90 days, and stained with hematoxylin and eosin (H/E), Masson's trichrome, and picrosirius red. H/E results were analyzed with ImageJ to compare skin thickness, vascular density, and blood vessel diameter between treatment groups. Samples stained with picrosirius red were quantified using CurveAlign software to determine collagen fiber orientation for scar formation analysis. Scanning electron microscopy (SEM) was performed to further analyze tissue extracellular matrix (ECM) morphology. Immunohistochemical (IHC) staining with pan-cytokeratin, mel5, vimentin, CD146, and anti-human lamin A+C were performed to confirm epidermal barrier formation, melanin production, dermal maturation, vascularity, and human cell integration, respectively.
***Results:** A highly significant difference in total wound closure was found between bioprinted skin and non-treated wounds at day 7 and day 14 (76% vs. 15%, $p<0.001$; 92% vs. 61%, $p<0.001$), as well as an accelerated time to wound closure (14.8 vs. 24.5 days, $p<0.001$). Epidermal barrier formation progressively increased in the bioprinted skin group at days 7, 14, and 21 (50% vs. 8%, $p<0.001$; 43% vs. 15%, $p<0.001$; 42% vs. 27%, $p<0.05$). Contraction was not significantly different between groups. However, at 90 days we observed increased contraction in the hydrogel only group compared with the bioprinted skin group. H/E and Masson's trichrome staining revealed rete peg formation with bioprinted skin comparable to human skin, while a flat epidermal barrier formed in the hydrogel only group. Quantification of picrosirius red stained samples with CurveAlign software confirmed a normal basket weave orientation in bioprinted skin-treated wounds, and hypertrophic scar-like, parallel collagen fiber alignment in the hydrogel only group (40.5% vs. 82.8% alignment, $p<0.001$). Further ECM analysis with SEM confirmed these findings. IHC staining at day 21 confirmed the presence of human cells in the regenerated skin, the formation of a stratified epidermis, melanin production, dermal maturation and

blood vessel formation.
***Conclusion/Significance:** Bioprinted skin accelerates full-thickness wound closure by forming an epidermal barrier without increasing contraction. This healing process is associated with human cells from the bioprinted skin laying down a healthy, basket-weave collagen network. The remodeled skin is phenotypically similar to human skin and composed of a composite of graft and infiltrating host cells. Ultimately, this skin bioprinting technology could translate into a new treatment for full-thickness wounds in human patients.

35 - A Fibronectin-derived Peptide, Engineered To Resist Endo- And Exo-peptidases, Enhances Cell Survival In Vitro, And Speeds Healing And Reduces Scarring In Vivo

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***Purpose/Objectives:** Failure to heal in chronic wounds and burns is due, in part, to growth factor (GF) depletion that occurs secondary to enzyme digestion. Attempts to counter this have included addition of super-physiological concentrations of recombinant GF to such wounds daily. We have discovered that GF-binding peptides in fibronectin (FN) can markedly enhance platelet-derived growth factor-BB (PDGF-BB) cell growth and survival activity (*J Invest Dermatol* 134:1119-1127, 2014), even when PDGF-BB is at the low concentrations typically found in chronic wounds and burns (*J Invest Dermatol* 134:921-929, 2014). One 14-residue peptide (P12) from the first type III repeat of FN ($FNIII_1$), which enhances PDGF-BB bioactivity, was cyclized to resist amino- and carboxyl exopeptidases (cP12). Peptide cP12, given as a single intravenous infusion 1-4h post-burn, speeds healing and reduces scarring in a porcine burn model (*Wd Repair Regen* 24:501-513, 2016). At present cP12 is in Phase 1 Clinical Trials. Even though cP12 is very effective when given by intravenous infusion, it does not speed healing when applied topically as it is rapidly digested by burn wound fluid (*Wd Repair Regen* in press, 2019). Since neutrophil elastase, an endopeptidase, is known to be responsible for FN digestion in burn wound fluid (*J Invest Dermatol* 103: 155-161, 1994), we posited that neutrophil elastase is responsible for cP12 digestion when applied to burn wounds.
***Methodology:** Therefore, another bioactive FN-peptide from $FNIII_1$ (P46), which is intrinsically more elastase-resistant than P12 (*J Invest Dermatol* 138:2480-2483, 2018*)*, has been engineered by cyclization and amino acid substitution to be both exopeptidase- and elastase-resistant, respectively.
***Results:** Two engineered peptides (cNP7 and cNP8) resisted human neutrophil elastase digestion at 1:100 enzyme:substrate ratio and 37°C for 24h. However, cNP8, but not cNP7, was 40% more effective than cP12 in enhancing PDGF-BB-driven survival and growth of adult human dermal fibroblasts in nutrient-depleted medium, as judged by XTT assay and cell counts. Western blot assay demonstrated that cNP8 increased PDGF-BB-driven AKT phosphorylation, a signal transduction node for cell survival and growth, both in amplitude and duration. By plasmon surface resonance spectroscopy, both cP12 and cNP8 bound PDGF-BB with a KD ~200nM, but cNP8 prolonged k_{off} indicating a longer residence time. Most importantly, cNP8 improved burn-wound healing when administered intravenously once between 8 and 24h post-burn (70% wound closure compared to 20% for buffer control at 14d post-burn) using our porcine vertical injury-progression burn model. Furthermore, when administered topically to acute excisional porcine skin wounds for three days, cNP8 promoted healing (22% granulation tissue formation compared to 7% for delivery-vehicle control at 4d post injury).
***Conclusion/Significance:** Thus, cNP8 had a wider and longer time-window (8 - 24h post-burn)

compared to cP12 (1-4h post-burn) for therapeutic-effective intravenous administration. Furthermore, cNP8 was effective, while cP12 is not effective, for acute wound healing when applied. Currently, IND-enabling studies for cNP8 treatment of burns and wounds are in progress.

36 - Bioactive Silk Dressing For Accelerated Wound Healing

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***Purpose/Objectives:** Slow healing and chronic non-healing wounds cost the US healthcare system over $25 billion a year and are a common complication of diabetes. Compared to non-diabetic wounds, diabetic wounds take longer to heal, which can lead to the development of chronic wounds that can significantly worsen outcomes in patients. Current methods for chronic wound care include topical antibiotics, dressings, growth factor injections, and skin substitutes. However, these methods are often either costly or fail to achieve proper healing. Wound healing typically follows a programmed sequence which involves hemostasis, inflammation, proliferation, and remodeling.
***Methodology:** In this work, we show local delivery of histamine, an immune modulator, in combination with silk dressing (silk fibroin - gold nanorod) film resulting in faster closure of acute and slow healing diabetic wounds when compared to conventional wound dressing (Tegaderm). Immunocompetent *BALB/c* mice served as a model for acute wounds, while genetically diabetic BKS.Cg-*Dock7*^{m} +/+ *Lepr*^{db}/J (*db/db*) mice were utilized as a model for slow healing diabetic wounds. Histamine was applied topically on 5-millimeter mid-dorsal full thickness wounds and covered with either Tegaderm or a silk dressing and irradiated with 800 nm near-infrared laser (NIR) laser.
***Results:** Significant reduction in wound area and improved tissue biomechanical recovery was observed in histamine treated wounds. Silk dressing-histamine treated wounds showed complete wound closure and higher tissue strength compared to Tegaderm-histamine treated wounds at day 7 post-wounding in acute wounds (n=10) and 11 (n=4) days in diabetic wounds. Immunohistological analyses of acute wounds also showed that silk dressing-histamine treatment promoted angiogenesis (CD31+ cells, p-value <0.05, n=4) and myofibroblast-mediated wound contraction (aSMA+, p-value <0.05, n=4). This also resulted in the increased epidermal thickness (pan-cytokeratin, p-value <0.01, n=4) and reduced proliferation (Ki67+ cells; p-value <0.01, n=4) compared to Tegaderm-histamine treated wounds. IL-1beta and IL-6 levels in serum samples showed significant increased at day 1 post wounding and reduced to basal level at day 3 in silk dressing-histamine treated wounds showing a robust early inflammatory response to set the stage for subsequent proliferation and remodeling. We also looked to further accelerate healing by delivering stromal-derived factor 1α (SDF1)-elastin-like polypeptides (ELP) fusion protein nanoparticles, to the wound site in combination with the silk dressing and histamine treatment. SDF1-ELP nanoparticles were added to the wound beds on either the same day as the wounding along with silk dressing-histamine treatment (simultaneous treatment), or on day 3 (sequential treatment) post-wounding and silk dressing-histamine treatment.
***Conclusion/Significance:** We found that adding SDF1-ELP as a simultaneous or sequential treatment further accelerated wound closure and resulted in higher skin strength recovery (n=4 for acute and diabetic wounds). These findings demonstrate that bioactive silk dressings are a promising treatment

option for enhancing wound healing and that they outperform clinically approved polyurethane wound dressing.

37 - Effects Of Covalent Nanosilver Incorporation On Platelet-like Particle Properties

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***Purpose/Objectives:** Platelets hold a crucial role in wound healing but can become depleted in conditions like chronic wounds and traumatic injuries. Platelet depletion results in inhibited wound healing, which can become further exacerbated by bacterial infection. To address platelet depletion, our group has developed platelet-like particles (PLPs), consisting of ultra-low crosslinked (ULC) microgels coupled to a fibrin-targeting antibody. Due to the fibrin affinity and particle deformability of this design, like natural platelets, PLPs can hone to injuries when injected intravenously and can retract clots, increasing clot stiffness. Previous studies by our group indicate PLP-mediated clot retraction, and concurrent increased clot stiffness, enhances wound healing outcomes by durotaxis. However, unlike native platelets, our first-generation design is not antimicrobial. We hypothesized that incorporating nanosilver into the PLP design would impart antimicrobial activity without diminishing the ability of PLPs to augment clotting, induce clot retraction, and enhance healing.
***Methodology:** ULCs were fabricated by precipitation polymerization of NIPAm copolymerized with Acrylic Acid. Nanosilver composite ULCs (Ag-ULCs) were produced by *in situ* reduction of silver ions using sodium borohydride. Particle deformability was characterized by particle spreading via atomic force microscopy (AFM). Nanometal distribution was characterized by transmission electron microscopy (TEM). Clot retraction, following coupling of a fibrin-targeting antibody by EDC/NHS chemistry and incorporating nanosilver composite PLPs (Ag-PLPs) into fibrin clots, was analyzed by confocal microscopy. *In vitro* antimicrobial activity was evaluated by colony forming unit (CFU) assays following culturing *E. coli* and *S. aureus* in the presence of Ag-PLPs for 24 hours. *In vivo* hemostatic ability was evaluated in a murine liver laceration injury model. Next, wound healing outcomes were evaluated in a murine full dermal thickness injury model, where H&E staining was conducted to evaluate epidermal thickness and immunohistochemistry was conducted to analyze angiogenesis and co-localization of PLPs with fibrin deposits.
***Results:** AFM analysis indicates similar deformability between Ag-ULCs and ULCs. TEM confirmed homogenous distribution of nanosilver particles throughout the microgels. Clot retraction data indicated no significant differences between Ag-PLPs and PLPs, while both PLPs groups had significantly higher fiber density than control groups. Significantly lower blood loss from liver laceration injury was observed when comparing PLP groups to saline and ULC controls; no difference in total blood loss was observed between PLP groups. Significant increases in average epidermal thickness and total CD31 area were observed in the presence of Ag-PLPs and PLPs groups compared to controls in the full thickness injury model.
***Conclusion/Significance:** In conclusion, we have created nanosilver composite PLPs and found that nanosilver incorporation imparts significant antimicrobial activity while retaining all properties of the base PLP design. Future work includes investigating efficacy of Ag-PLPs in an infected full dermal thickness injury model.

38 - Microphysiological Body-on-a-chip System To Evaluate Transdermal Drug Delivery And Toxicity

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***Purpose/Objectives:** Novel physiologically relevant in vitro platforms are in high demand due to the need to revitalize the drug development industry. Body-on-a-chip *in vitro* systems are a promising technology for increasing the predictive power of drug efficacy and toxicity in humans compared to traditional animal models. However, there is still a need to increase the complexity of these systems by interconnecting multiple organ tissues in the same recirculating medium as well as expanding the drug delivery methodologies to include systemic, oral, and transdermal applications. Thus, we have developed an integrated heart-liver "body-on-a-chip" system utilizing a skin surrogate and non-invasive monitoring of organ physiology to assess the toxicity of topically administered drugs dosed acutely and chronically.
***Methodology:** To validate the topical delivery system, the moderately permeable drug diclofenac sodium (1.5% and 3% solution), and the low permeable compounds ketoconazole (0.11% solution), hydrocortisone (1% solution), and acetaminophen (1.5% solution) were applied acutely to a synthetic skin surrogate (Strat-M membrane) and toxic effects on liver and cardiac physiology were compared to toxicity data generated from an acute drug exposure applied systemically into the recirculating medium. Medium concentration of each drug after topical application was monitored over time to verify the selective permeability of the Strat-m membrane.
***Results:** As expected, diclofenac had the highest degree of permeability (7-8%) and consequently, affected cardiac function to the greatest degree. Specifically, a 3% topical solution resulted in reductions of 96%+/-4% in cardiac conduction velocity, 44 ± 12% in contractile force, and 90 ± 10% spontaneous beating frequency after 24 hours. Acute, topical applications of acetaminophen, ketoconazole and hydrocortisone caused no significant changes in cardiac or hepatic physiology; this lack of toxicity being attributed to the low permeability across the Strat-M membrane (0.06%, 0.4%, 0.04%, respectively) after 24 hours. Acute dosing of 1.5% diclofenac solution revealed no toxicity. However, chronic, repeat exposure of 1.5% diclofenac solution to the skin surrogate affected cardiac electrical activity and decreased albumin secretion from liver cells.
***Conclusion/Significance:** One important advantage of our heart-liver-skin system is the ability to non-invasively monitor organ physiology which facilitates determination of the effects of chronic drug exposure (over 10 days) mimicking repeat application of transdermal therapies to the skin. In conclusion, we have demonstrated the effectiveness of a heart-liver-skin surrogate, body-on-a-chip system to assess transdermal drug delivery and subsequent toxicity effects on physiological indicators of organ performance.

39 - Improving Third Degree Burn Wound Healing By Using Non-enzymatic Detachment Of Keratinocyte Sheets Cultured On Temperature Responsive Dishes

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***Purpose/Objectives:** Large burn wounds are temporarily covered with cadaver skin, which eventually rejected requiring autologous skin grafting. To overcome this obstacle, cultured autologous keratinocyte sheets (KS) have been proposed to substitute the autologous skin; however, this technology has not been translated to clinical practice because of poor quality of KS, which is suggested to be related to the use of enzyme for their detachment. We hypothesized that non-enzymatic detachment using temperature responsive dishes will preserve extracellular matrix (ECM) of KS and result in better burn wound healing than those cultured in conventional dishes.
***Methodology:** Confluent human keratinocyte sheets were detached using dispase and the protein level of sheet's ECM was compared to physical scraping to the cell sheet using a rubber blade in the lysis buffer. Also, to compare the efficacy of KS that was cultured on temperature-responsive dish (KS-T) detached by temperature reduction to 20°C without using enzyme against KS cultured on conventional dish and detached using enzyme Dispase (KS-D), six full thickness skin burns were induced in sheep dorsum under anesthesia and analgesia. After 24 hrs, burned skin was excised and wounds were grafted with ovine cadaver skin. Autologous keratinocytes were isolated at the same day of cadaver skin grafting mimicking clinical situations. After 3 weeks, when cadaver skin was rejected, wounds were randomly allocated to groups: covered with KS-T or KS-D or wounds without any cover (served as a control). After KS treatment, wounds were monitored for additional 14 days and wound samples were collected postmortem.
***Results:** The levels of the ECM proteins, Collagen IV and laminin 5, were significantly diminished after dispase treatment compared to physical scraping. *In vivo*, the epithelialization rate was significantly greater in wounds treated with KS-T detached by temperature reduction vs. those detached with Dispase at days 7 and 14. The thickness of KS-T before grafting was significantly higher than KS-D; however, the epidermis thickness after grafting the KSs was comparable in both treatment groups. The remarkable difference was pronounced in the dermal-epidermal junction as it was well defined in wounds covered with KS-T at 14th day evidenced by continuous and better-defined lamina densa and significantly higher numbers of hemi-desmosomes. Wounds treated with KS-T sheets had less ulceration, hemorrhage and neutrophil infiltration compared to wounds treated with KS-D. Collagen density and vascularization percentage were significantly higher in the wounds treated with KS-T compared to wounds covered with KS-D and control. Wounds healing was more mature in wounds treated with KS-T evidenced by normal range of KGF that was transiently increased in early wound healing phase similar to the level detected in control and wound treated with KS-D.
***Conclusion/Significance:** The effects of non-enzymatically detached KSs are superior to those of enzymatically detached sheets on ovine grafted burn wounds healing. Ovine grafted burn wound healing model closely mimics the clinical situation. Non-enzymatic detachment of cultured keratinocytes may potentially enable their translation to clinical practice.

40 - Assessment Of UBM Products In A Porcine Third-degree Burn Model

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***Purpose/Objectives:** Third-degree burn wounds can be difficult to treat, resulting in devastating loss of functionality and cosmesis to patients. As such, there is a need for platform technologies that allow effective wound management and subsequent ease in closure or epithelialization, while minimizing/ preventing of scarring. Herein we describe the use of urinary bladder matrix (UBM)-based wound products in a porcine third-degree burn wound model. The primary objective for this study was to evaluate differences in wound closure, wound contracture, re-epithelialization, and histologic wound properties of UBM-based wound products versus untreated wounds or a commercially available product to treat third-degree burn wounds (Integra® Dermal Regeneration Template).
***Methodology:** This study was reviewed and approved by the Wake Forest University Institutional Animal Care and Use Committee (A18-012 ACell Therapy for Burn Wound Healing). A total of sixteen 8.0 x 8.0 cm full thickness third degree burns were created on the dorsal skin of pigs followed by 10.0 x10.0 cm excisional debridement. MatriStem™ UBM product treatment groups included a UBM particulate device that was applied in conjunction with a UBM sheet device comprised of two, three, or six layers of UBM. The UBM treatment groups were compared to a commercially available product for the treatment of third degree burns, Integra DRT. These treatment groups were compared with untreated wound controls. Wounds were followed over the course of 8 weeks (56 days). During the course of the study, the wounds were inspected and measured twice each week for 1) documentation of overall wound size, re-epithelialization, and contracture, and 2) cleaning and re-bandaging. At 56 days post-treatment, the study was terminated and the wound areas harvested. Wounds were grossly evaluated, as well as examined through histology and immunohistochemistry for compositional differences between untreated wounds and the remodeled wound sites following a product application.
***Results:** All UBM-treated wounds appeared to have the most complete wound closure and epithelialization compared to untreated and Integra wounds. Multi-factor wound closure analysis further revealed that UBM products performed better than untreated and Integra treated wounds based on minimal contraction, superior epithelialization, and faster wound closure rates. Histological analysis demonstrated that all wounds other than untreated controls showed a complete epidermis at various levels of maturation. The three layer UBM and Integra treatments had a dermis most similar to healthy skin with mature, thick and organized collagen bundles. Three layer UBM, six layer UBM, and Integra-treated wounds had extracellular matrix composition, organization, and staining intensities similar to healthy skin. Six layer UBM and Integra-treated wounds showed collagen content and organization most similar to healthy skin.
***Conclusion/Significance:** Overall, all UBM treatments were easy to administer to full thickness, debrided third degree burn wounds, and resulted in rapid wound closure rates, driven primarily by new epithelialization.

41 - Lxw7 Functionalized Ecm Scaffolds Loaded With Endothelial Progenitor Cells Potentiate Neovascularization And Promote Diabetic Ischemic Wound Healing

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***Purpose/Objectives: Diabetic ischemic wound treatment** remains a critical clinical challenge. We previously identified a cyclic peptide LXW7 that specifically binds to integrin αvβ3 on endothelial progenitor cells (EPCs) and endothelial cells (ECs), activate VEGF receptors, and promote EC proliferation and maturation. In this study, we propose to create a proangiogenic molecule LXW7-DS-SILY by linking LXW7 with a collagen-binding proteoglycan mimic DS-SILY. This will be used to functionalize small intestinal submucosa (SIS) ECM to generate a **LXW7-DS-SILY-SIS scaffold** that can promote neovascularization and accelerate ischemic wound healing.
***Methodology:** *In vitro*, the effects of LXW7-DS-SILY-SIS on EPC biological functions were assessed by cell attachment, MTS assay and Western blotting. *In vivo*, we have established a Zucker Diabetic Fatty (ZDF) rat ischemic skin flap model and evaluated the function of LXW7-DS-SILY-SIS scaffold seeded with EPCs in promoting neovascularization and wound healing. The percentage of re-epithelialization, granulation tissue area, cellular organization, and amount of visible scar tissue was determined by histology of excisional wounds. The new capillary formation was determined by immunohistochemistry.

***Results:** LXW7-DS-SILY-SIS significantly promoted EPC attachment, spreading and survival in ischemic environment *in vitro*. Treating wounds with LXW7-DS-SILY functionalized SIS loaded with or without-EPCs significantly improved wound healing, enhanced neovascularization and modulated collagen fibrillogenesis.
***Conclusion/Significance:** LXW7-DS-SILY-SIS scaffold seeded with EPCs holds promise as a novel treatment to accelerate healing of ischemic diabetic foot ulcers, thereby reducing limb amputation and mortality rates of diabetic patients.

42 - Primary Human Skin Cells Self-organize To Form Layered, Pigmented, Spherical Organoids

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***Purpose/Objectives:** Tissue equivalent (organoid) technology is a rapidly developing field that enables unique analysis of tissue development and disease. To date, most skin organoids have consisted of multi-layered, multicellular constructs fabricated using hydrogel, and matured at an air liquid interface. However, this fabrication method is time and labor intensive and is not adaptable to high throughput analyses. Furthermore, the transwell culture system required for an air-liquid interface is difficult to incorporate into body-on-a-chip platforms. The purpose of this study is to determine if primary human skin cells will self-organize to form a layered, pigmented skin-like tissue when cultured as a multicellular spheroid.
***Methodology:** Human skin cells including keratinocytes, melanocytes, fibroblasts, dermal microvascular endothelial cells, follicle dermal papilla cells, and adipocytes were induced to form spheroids. Bright-field microscopy was performed every 7 days and analyzed with ImageJ to quantify spheroid size and pigmentation. Samples were taken at days 7, 14, 21, and 42 for histology, live/dead staining, and Scanning electron microscopy (SEM). Histological samples were subsequently stained with hematoxylin and eosin (H/E), Masson's trichrome, and picrosirius red. Immunohistochemical (IHC) staining with pan-cytokeratin, mel5, vimentin, CD146, krt71, and adiponectin were performed to

determine cellular self-organization, maintenance of cell specific markers, and accessory skin structures.
***Results:** Brightfield microscopy confirmed that the human skin cells aggregated to form spheroids by day 7, with spotted pigmentation. From day 7 to day 42, the spheroids did not significantly change in size. However, by day 42, the spheroids became uniformly pigmented. H/E staining revealed a clear bilayer pattern with a spherical dermal core and epidermal surface. Live/Dead staining confirmed high cell viability at 42 days in culture. SEM confirmed both the size and spherical shape of the organoids, as well as the distinct separation between the dermal core and epidermal surface. Masson's Trichrome and picrosirius red staining revealed increased collagen deposition in the spheroid core and keratin deposition on the surface over time. The organoids stained positively for skin cell markers: vimentin and pan-cytokeratin confirmed a separation of the dermal core and epidermal surface; krt71 stained positively for follicle dermal papilla cells in the dermal core; Mel5 demonstrated a higher concentration of melanocytes at the border of the dermal core and epidermal surface, with melanin present in keratinocytes.
***Conclusion/Significance:** Primary human skin cells self-organized to form multicellular pigmented spheroid with a dermal core and epidermal surface. The spheroids were stable long-term and expressed skin specific markers. This novel method for fabrication of multicellular skin organoids is less technically demanding and more reproducible than prior skin organoid models. Ultimately, this technique provides an in vitro model of skin development, and could be used as a platform for investigation of dermatopathologies.

43 - Therapeutic Intradermal Delivery Of Exosome-encapsulated Curcumin Using Dissolvable Microneedle Arrays For Enhanced Treatment Of Inflammatory Skin Diseases

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***Purpose/Objectives:** Exosomes, the smallest cell-derived vesicles, have emerged as ideal drug carriers for treatment of many diseases without off-target effects[1,2]. The therapeutic efficacy of curcumin has been proven against inflammatory diseases[3]. Importantly, complexing curcumin with exosomes resulted in enhanced therapeutic potency[4]. However, the exosome-encapsulated therapeutics are typically administered systemically, requiring high doses to achieve locally effective concentrations[5]. Thus, local delivery of exosomes has yet to be exploited. Here, we evaluate a new therapeutic approach combining the use of biodissolvable microneedle arrays (MNAs) for local application of exosome-encapsulated curcumin towards enhanced treatment of inflammatory skin diseases.
***Methodology:** To this end, exosomes derived from murine macrophages were bioengineered to integrate curcumin. To monitor cellular uptake and skin penetration, exosomes were labelled with a fluorescent dye PKH26. MNAs were created from a combination of biodissolvable materials carboxymethylcellulose and trehalose using a unique micromilling/elastomer molding/spin-casting based micromanufacturing strategy[6,7].
***Results:** *In vitro* cellular uptake studies with both native and MNA-embedded exosomes showed that the cellular uptake was completed in 24 h. Therapeutic efficacy of MNA-integrated exosomes was determined through a raw-blue assay, where effective downregulation NF-κB expression was

demonstrated. Subsequently, successful intradermal delivery of exosomes from MNAs into living rat skin was shown. Specifically, MNAs delivered greater than 80% of their exosome content into rat skin in 20 min. Finally, successful MNA-exosome treatment of lipopolysaccharide-induced local inflammation in living rats was demonstrated.
***Conclusion/Significance:** Taken together, this new therapeutic approach could be used for effective treatment of inflammatory skin diseases.

Session Number: 10
New Approaches to Cardiovascular Repair and Regeneration I
Tuesday, December 3, 2019, 3:00 PM - 4:30 PM

44 - Engineering Arterial Substitutes that Recapitulate Vessel Microstructure

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***Purpose/Objectives:** Vascular disease is an unsolved medical problem. Outcomes after surgical and catheter-based interventions remain compromised with low patency rates. Tissue-engineering strategies have been explored as alternatives; however, no clinically available graft exists. The main limitation is failure to recapitulate the blood vessel microstructure, and, as a consequence, native physiological properties. Considering that the arterial wall is a circumferentially aligned fibrous matrix, the capacity to create oriented structures is critical. Here, we present a solution that leverages molecular crowding and hydrodynamic focusing to fabricate ultrathin, aligned collagen sheets, as well as the methodology for fabricating arterial substitutes that recapitulate the microstructure of native arteries.
***Methodology:** For the scalable production of aligned sheets, monomeric rat-tail tendon collagen was dissolved in acidic solution, and injected into the middle layer of a multilayered, PDMS-based microfluidic device at varying flow rates. As a sheath flow, a basic solution with 10% (w/v) polyethylene glycol (PEG) was used. After extrusion, sheets were seeded with vascular smooth muscle cells (vSMC), rolled around mandrels at various orientations, and cultured for 7 days. Standard *in vitro* biomechanical tests were conducted to assess burst pressure, compliance, and suture retention. Biological characterization was performed before and after *in vivo* implantation as rat infra-renal interposition model.
***Results:** Extruded collagen sheets have thicknesses of 1-4 μm, widths of 15-30 mm, ultimate tensile strengths of 1.25-13MPa, Young's moduli of 1.3-130MPa, and strains to failure of 15-35%. Molecular alignment of the collagen sheets induced preferential alignment of vSMC, maintained cellular expression of phenotypic markers, and guided active film contraction. Efforts involving the controlled assembly of these collagen sheets seeded have yield arterial constructs that recapitulates native blood vessel microstructure. Biomechanically, these constructs exhibit burst pressures of 1800-2600 mmHg, suture retentions of 100-200 grams-Force, and compliances as high as 4%. Preliminary short-term *in vivo* implantations have demonstrated patency and minimal inflammatory response.
***Conclusion/Significance:** We have developed a microfluidic approach for the scalable, continuous formation of collagen sheets with tunable alignment and compaction of collagen fibrils. The combination of molecular crowding and hydrodynamic focusing results in unprecedented ultrathin sheets with sustained collagen fibril alignment in the direction of flow, and density of fibrils consistent throughout the entire sheet width. Ultimately, we believe the presented strategy to be promising in developing large collagen substrates of biologically relevant composition and tunable mechanical properties for tissue engineered vascular grafts.

45 - Fibrin-modulating Nanogels For Treatment Of Disseminated Intravascular Coagulation

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***Purpose/Objectives:** Disseminated intravascular coagulation (DIC) is a pathological process causing systemic coagulopathy through excessive thrombin generation. While over-activation of clotting causes microthrombi throughout the body and multi-organ failure, DIC also contributes to bleeding as clotting factors are consumed. Different clinical events can lead to DIC, including cancer, trauma, and infection. Additionally, patients can present with bleeding or thrombosis, creating therapeutic dilemmas in managing. We hypothesize that simultaneously addressing microthrombi and bleeding associated with DIC can improve treatment. We have developed fibrin-specific nanogels (FSNs) loaded with tissue plasminogen activator (tPA) that can be used to treat the multifaceted complications of DIC.
***Methodology:** Core-shell poly(N-isopropylacrylamide) nanogels with 10% N,N'-Methylenebis(acrylamide) (BIS) cross-linked core and 2% BIS cross-linked shell were synthesized in precipitation polymerization reactions, including acrylic acid copolymer for EDC/NHS coupling of an anti-fibrin fragment E antibody. Release studies were conducted using 70kDa dextran to mimic tPA release. For loading of tPA, core-shell FSN particles were purified and rehydrated in a tPA loading solution followed by subsequent purification. Unloaded and tPA-loaded FSNs were examined for their ability to target clots and modulate clotting dynamics in the presence of fibrin clotting *in vitro* and *in vivo*.
***Results:** Release studies with 70kDa dextran show core-shell particles release a significantly higher percent of their payload at early time points (1-2hrs) compared to single layer 2% BIS cross-linked particles. In a custom-made PDMS fluidics chamber, unloaded FSNs exhibit fibrin-binding at stationary clot boundary sites at wall shear rates of $1sec^{-1}$ and augment the clotting process *in vitro*. Without fibrin-specificity, nanogels do not bind to the clot boundary or induce clot augmentation. Conversely, tPA-loaded FSNs bind to fibrin and facilitate targeted clot dissolution more effectively than bolus tPA administration or tPA delivered with non-binding nanogels. In a lipopolysaccharide-induced DIC rodent model, animals exhibit darkened organ color, hemorrhagic areas, microthrombi, and significantly more fibrin deposition in organs compared to healthy animals, which are diminished in the tPA-FSN treatment group. Elevated fibrin degradation products in DIC-induced animals validate microthrombi formation and fibrin turnover, while platelet counts demonstrate recovery to normal levels only in the tPA-FSN treatment group. In addition, clot structure visualized using cryogenic scanning electron and confocal microscopy show fibrin network structure is severely compromised in DIC as clotting factors are consumed in various organs, and is not restored in animals receiving FSNs alone. However, FSNs loaded with tPA restore clot structure to that seen in healthy animals. Preliminary results show that upon injury, animals with DIC had higher blood loss than healthy animals, and treatment with unloaded FSNs did not reduce blood loss in DIC animals. However, tPA-FSN treatment did somewhat reduce blood loss in DIC animals.
***Conclusion/Significance:** Together, these data demonstrate that fibrinolytic loaded fibrin-targeting microgels can act as a targeted therapeutic strategy in DIC to improve outcomes.

46 - Jagged1 Presenting Cell Surrogate Biomaterials For Notch Signaling

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***Purpose/Objectives:** The Notch signaling pathway plays a critical role in regulating vascular morphogenesis during development and differentiation of vascular smooth muscle cells (VSMCs) during

maturation. In arteries, Notch3 is the predominant receptor on VSMC and signaling is initiated upon binding to a Notch ligand Jagged1 found on vascular endothelial cells (VECs). EC and SMC contact specific signaling of this pathway has been proven necessary to maintain vascular homeostasis in arteries. Recently it has been demonstrated that ligand-receptor binding alone may not be sufficient to induce downstream signaling, and a mechanical tension force may also be necessary for signal transduction. In this study we aim to demonstrate that Jagged1 presenting biomaterial surfaces can act as EC surrogate materials to direct and control differentiation and phenotype regulation of VSMCs. The force transmission and mechanosensitivity of Jagged1-Notch3 signaling pathway will also be investigated.
***Methodology:** Notch ligand Jagged1 was bound to magnetic Protein G beads through affinity immobilization and was used as an endothelial cell surrogate biomaterial to direct transcriptional activation of both iPSC-derived and primary human coronary artery SMC-specific genes. Cells were plated on a 96-well plate and introduced to varying concentrations of Jagged-1 beads (100-400 beads/cell), a magnetic tweezer assay with a terraced magnet configuration was used to provide a tension force to the ligand-receptor complex in the piconewton force range. A Notch inhibitor DAPT was used as a negative control for the system. Mechanotransduction, and response to Jagged-1 immobilization was analyzed through gene expression and protein synthesis.
***Results:** Transcriptional activation of VSMC-specific genes was not induced by soluble jagged-1 but immobilization of 5 μg of Jagged-1 to Protein G beads resulted in an induction of the contractile HCASMC phenotype as demonstrated by increased smooth muscle-α -actin, calponin and myosin heavy chain gene expression. Transcriptional factors of Notch signalling including the hHES-1 gene was up-regulated in the presence of Jagged-1 immobilized beads and furthermore the addition of DAPT a Notch S2 inhibitor successfully downregulated gene and protein expression. These results suggest that activation of the Notch signaling pathway is necessary to induce contractile SMC phenotype. Mechanosensitivity of the Notch signaling pathway has been investigated for Jagged1-Notch3 signaling and has been suggested as a useful tool to analyze force transmission in vascular morphogenesis .
***Conclusion/Significance:** The Notch ligand Jagged-1 immobilized to magnetic nanoparticles was successful in regulating the contractile smooth muscle phenotype in SMCs, and the mechanosensitivity of this pathway has been investigated. These findings may have clinical importance and therapeutic potential for modulating vascular SMC phenotype during various cardiovascular disease states and in tissue engineering. The use of biomaterials as cell surrogate materials suggest high potential in modulating development and maturation of cells within the vasculature.

47 - ECM-associated Il-33: A Mechanism By Which Fibrosis And Tissue Restoration Are Regulated

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***Purpose/Objectives:** Cardiac disease or injury, such as myocardial infarction (MI) or the host response to allograft heart transplantation (HTx), results in fibrosis, myocardial stiffness, loss of function and heart failure (HF). Chronic rejection following heart transplantation is associated with cardiac allograft vasculopathy and myocardial fibrosis, which contribute to failure of more than 50% of transplants within 11 years. An excessive and chronic pro-inflammatory environment has been implicated in adverse cardiac remodeling and progression to HF. Numerous experimental studies have shown that a timely

resolution of inflammation after MI or HTx may prevent development and progression of immune-driven fibrosis. The effects of the pro-inflammatory macrophage and T-cell phenotype can be minimized by anti-inflammatory and immunosuppressive drugs, but these drugs are ineffective in preventing pathogenic remodeling. An alternative immunomodulatory, but not immunosuppressive, approach involves signaling molecules contained within the extracellular matrix (ECM). Matrix bound nanovesicles (MBV) embedded within the extracellular matrix contain biologically active molecules that can rapidly activate macrophages to a pro-remodeling phenotype. MBV are a rich and stable source of extra-nuclear interleukin-33 (IL-33). IL-33 is an IL-1 family cytokine that is a transcription factor that is classically considered as an alarmin but has emerging reparative and immunoregulatory properties.
***Methodology:** MBV were isolated from the intestine of wt or *il33-/-* mice using Liberase enzymatic digestion followed by ultracentrifugation. Primary bone-marrow derived macrophages from wt or ST2-/- mice were exposed to wt MBV, IL33-/- MBV, or recombinant IL-33. Immunolabeling was used to determine the expression of pro-inflammatory (iNOS) and anti-inflammatory (Arginase) markers. IL-33+ Bm12 or IL-33 deficient Bm12 mouse hearts were transplanted heterotopically into the abdomen of wildtype or *il33-/-* C57Bl/6 mice. In some experiments, *il33-/-* donor hearts were coated in a collagen hydrogel containing IL33+ MBV prior to transplantation. At post-operative day (POD) 3, explanted hearts were digested and populations infiltrating cells were identified using flow cytometry. On POD 100, transplants were fixed and stained for Masson's Trichrome or H&E.
***Results:** We have found that the IL-33 encapsulated within the MBV can bypass the canonical IL-33/ST2 receptor signaling pathway and is required to activate macrophages toward a reparative M2-like phenotype via a non-canonical ST2-independent pathway. In mouse model of heart transplant, the absence of IL-33 in grafts isolated from *il33-/-* mice resulted in increased chronic rejection-associated fibrosis, vasculopathy and immune cell infiltration in both wild-type and *il33-/-* recipients. Administration of a collagen hydrogel loaded with IL-33+ MBV to IL-33 deficient grafts immediately following transplantation limited the infiltration of pro-inflammatory immune cells and decreased the extent of fibrosis and vascular occlusion.
***Conclusion/Significance:** These data suggest that IL-33 encapsulated within the MBV promotes a reparative phenotype in infiltrating immune cells and helps to prevent fibrotic diseases. An MBV-based approach may represent an alternative immunomodulatory therapy to protect against immune-driven cardiac fibrosis.

Session Number: 11
Engineering Implants for the Treatment of Endocrine/Metabolic Diseases
Tuesday, December 3, 2019, 3:00 PM - 4:30 PM

48 - Engineering An Oxygen-generating Microbeads Scaffolds For Islet Transplantation Within An Extrahepatic Site

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***Purpose/Objectives:** Hypoxia is a serious issue arising within cell-based implants. While accelerating vascularization of the implant is a promising approach to address this issue, there is inevitably a hypoxic period between implantation and revascularization of the graft. Limiting or eliminating this period should significantly mitigate hypoxia-induced cell death and dysfunction as well as improve the efficacy of these implants, particularly for devices containing cells that are highly sensitive to hypoxia, such as pancreatic beta cells. We have previously published the development of an *in situ* oxygen generating disk, termed OxySite, based on the encapsulation of calcium peroxide within polydimethylsiloxane (PDMS) [1]. This device has been shown to abrogate hypoxia-induced beta cell death and dysfunction over extended culture times [1,2,3]. While highly effective, the planar disk geometry prevents dimensional flexibility, for instance, in combination with macroporous scaffolds. To address these challenges, the geometry of the system was modified to microbeads embedded within a scaffold.
***Methodology:** OxySite microbeads were fabricated via an emulsion method into a size range of 100 to 300 µm. They were then incorporated with PDMS and NaCl to obtain an 85% macroporous scaffold (5 mm diameter; 1.5 mm height), by a particulate leaching and solvent casting method. Oxygen production of the scaffold was tested *in vitro* by a non-invasive method. OxySite microbeads scaffolds were further tested in a Lewis rat syngeneic transplantation model (2000 IEQ/device) and compared to PDMS-only microbeads scaffolds. To validate clinical relevance, variable islet isolation purities (80%, 90%, and 99%) were screened to evaluate transplant efficacy.
***Results:** OxySite microbeads showed an average bead diameter of 224 ± 71 µm. Kinetic oxygen generation profiles indicated that OxySite microbeads scaffolds could generate oxygen at levels between 30 and 65 mmHg per day for over 10 days, with nontoxic levels of reaction intermediates or by-products (e.g. hydrogen peroxide and calcium). Transplantation outcomes showed there was significant accelerated achievement of normoglycemia in the OxySite group, when compared to the PDMS-only group ($p=0.0029$). Furthermore, lower purity of islets led to a greater difference in normoglycemia achievement between the OxySite group and the PDMS-only group.
***Conclusion/Significance:** This device presents a highly translational platform for clinical islet transplantation where islets purity is highly variable. Future studies aim at exploring the potential of this oxygen generating system to improve transplant outcomes in larger diabetic animal models, as well as to other cell-based implants. **References:** [1] Pedraza et al., Proc Natl Acad Sci USA,109:4245-4250, 2012. [2] Coronel et al., Biomaterials, 129:139-151, 2017. [3] Coronel et al., Biomaterials, 210:1-11, 2019.

49 - An Engineered Fail-safe Approach For Pancreatic Cell-Replacement Therapy

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***Purpose/Objectives:** Type 1 diabetes (T1D) is an autoimmune destruction of pancreatic β cells. Currently, islet transplantation is the only therapy for T1D; however, it is facing major hurdles such as poor islet survival, life-long immunosuppressant side effects, shortage of donor islets and variability in islet quality. Researchers have attempted to generate insulin-producing β-like cells for T1D treatment but these immature cells pose substantial risk of tumor formation with unregulated insulin secretion or no glucose response. This study aims to generate a fail-safe (FS) insulin-producing β-like cell line incorporated into an immune-isolating hydrogel system as a potential approach for safe T1D β-cell replacement therapy.
***Methodology:** Using the interruption of β-cell reprogramming in a pancreatic β-cell specific reprogrammable mice, we generated proliferative β-like cells representing most characteristics of the *bona fide* β-cell. Then, to avoid uncontrolled cell proliferation, we established FS β-like cells. We transcriptionally linked Thymidine Kinase (TK) suicide gene into the downstream of the CDK1 gene using CRISPER/Cas9 editing system in such a way that dividing cells, which express CDK1-TK, commit suicide in the presence of the FDA-approved pro-drug Ganciclovir. A microfluidic device resembling blood-tissue cross-interaction was fabricated and optimized in order to validate the functionality (insulin secretion) and safety of the FS β-like cells in a 3D dynamic microenvironment. A mathematical model was also developed to describe the dynamic insulin secretion of the FS β-like cells in the microfluidic device. Finally, the FS β-like cells were encapsulated in immune-isolating alginate-poly-L-lysine-alginate (APLA) microspheres and characterized *in-vitro* for cell viability, size distribution, degradation and insulin secretion, and *in-vivo* for reversing hyperglycemia in diabetic mice.
***Results:** Our developed microfluidic-based assay, which was validated with flow cytometry results, showed that proliferating FS β-like cells committed suicide in response to GCV. The cell viability score 50 (CVS50) significantly (p-value<0.01) decreased with increasing GCV dosage. The GCV treatments at various concentrations led to surviving cell populations (20 - 35%) which were representing non-dividing β-like cells. The insulin expression of the FS- non-dividing β-like cells were confirmed by immunostaining. Also, our microfluidic-based assessment of the insulin secretion kinetics confirmed a regulated insulin secretion of the FS GCV-treated β-like cells in response to different glucose concentrations (2 - 20 mM). The mathematical model, which was experimentally validated, was used to estimate insulin secretion kinetic parameters of the cells. The APLA microcapsules (~400 µm), which were mechanically stable and prevented the β-like cells from protrusion over 30 days *in-vitro*, supported insulin secretion from the FS GCV-treated β-like cells. Our *in-vivo* study has suggested that the ALPA-encapsulated FS β-like cells could restore normoglycemia in diabetic mice.
***Conclusion/Significance:** Implantation of β-like cells present substantial risks of tumor formation and unregulated glucose response. We have successfully engineered a functional β-like safe-cell system and characterized it in a dynamic 3D-hydrogel microenvironment that resembles blood-tissue cross-interaction. The ALPA hydrogel microencapsulated safe-cell system has shown promise to restore normoglycemia in diabetic mice. Findings from this study pave the road for a safe pancreatic cell-replacement therapy.

50 - A Bioengineered Artificial Interstitium Supports High Density Islet Cell Transplantation Without Immunosuppression In Nonhuman Primates

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***Purpose/Objectives:** Widespread clinical application of islet cell transplant is hampered by the limited availability of islets, immunosuppressive risk, and moderate numbers of patients experiencing graft functional decline over time. We developed an artificial interstitium (AI) using a silicone construct for implanted islets by harnessing existing convective flow mechanisms between blood-interstitium-lymph compartments that eliminates the need for immunosuppression, advantages a xenogeneic cell source, and permits non-invasive access for loading, reloading, biopsy and graft recovery.
***Methodology:** The AI is a silicone template composed of two disks incorporating a plurality of obstructive struts. The AI disk is engineered to create a void or open space in the subcutaneous tissue via a pressure gradient, so that interstitial fluid moves from the surrounding tissue into the disc space and chamber containing cells for exchange. Proof-of-concept studies in rats and nonhuman primates (NHPs) were performed to evaluate the suitability of interstitial fluid as a medium to sustain immunoisolated islet viability and function.
***Results:** In both rodents and NHPs long-term continuous accumulation of IF with normal oxygen, pH, and chemistry with a favorable cytokine profile for islet survival and function was achieved. Base characteristics including hematologic, chemistry, electrolyte, and oxygen content of fluid generated by the accumulation disc were evaluated.Interstitial fluid that accumulated within the disc had properties similar to serum with a neutral pH, normal electrolyte composition, but slightly lower protein profile. The tissue surrounding the construct was highly vascularized with only sparse inflammatory cells observed on histologic evaluation. The AI was evaluated in the stringent NHP model to evaluate response to a marginal load of porcine islets. Interstitial fluid was evaluated for porcine C-peptide levels, islets retrieved via biopsy were stained for insulin and evaluated histologically. There was no evidence of cellular infiltrate on histological analysis at all timepoints. Likewise, porcine C-peptide was detectable and positive for >180d in animals indicating the AI is capable of supporting long-term survival, biopsied islets were strongly insulin positive, and the diabetic animal achieved marked glycemic benefit, in reduction of HbA1c.
***Conclusion/Significance:** The interstitial space is a novel implant site for β-cell transplant. The engineered AI uses interstitial fluid for bi-directional exchange of nutrients, waste, and signal factors to isolated islet cells and harnesses existing convective flow. The complete absence of infiltration and strong insulin staining of porcine islets recovered from the device provides evidence of immunoprotective capacity. Interstitial fluid and graft biopsies were successfully obtained via percutaneous needle access at regular intervals, demonstrating the feasibility of non-invasive access to the graft and potential for reload. The AI is a fundamentally new device platform engineered to support islet cell replacement in the absence of immunosuppression with potential to dramatically increase the longevity of therapeutic benefit and accessibility to a larger population of diabetic patients.

51 - A New Multibore Hollow Fiber Device For Macroencapsulation Of Islets Of Langerhans

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***Purpose/Objectives:** Macroencapsulation of pancreatic islets is a promising strategy for extrahepatic transplantation of islets to treat type 1 Diabetes. Hollow fiber membranes are interesting for macroencapsulation because they can offer a large surface-to-volume ratio and can potentially be retrieved or refilled. However, the application of fibers with suboptimal morphology and transport properties often contributed to graft failure, due to a limited exchange of nutrients, oxygen, and insulin. Especially single-bore hollow fibers can suffer from performance instability during long-term use. In this work, we develop multibore hollow fibers consisting of seven bores suitable for encapsulation of a high number of islets.
***Methodology:** Poly (ether sulfone) basedmultibore hollow fibers were fabricated by dry-wet spinning via immersion precipitation using a specially designed spinneret. Membrane porosity was optimized to allow sufficient insulin and glucose transport. Various numbers of human islets were encapsulated within multibore hollow fibers and islet functionality was assessed by glucose induced insulin secretion test. Additionally, the multibore hollow fibers were implanted subcutaneously for 28 days into immunocompetent C57BL/6BrdCrHsd-Tyrc mice and material biocompatibility as well as blood vessel formation were histologically assessed.
***Results:** The developed multibore hollow fibers have high mechanical stability, and low cell adhesion. Human islets encapsulated within the fiber retain their glucose responsiveness, similar to non-encapsulated islets, during 7 days of cell culture. Moreover, insulin secretion upon glucose challenge increases with increasing number of encapsulated islets. Preliminary implantation study shows material biocompatibility with minimal tissue response towards fiber material. Moreover, we observed formation of new blood vessels within 100 µm distance from the implanted device.
***Conclusion/Significance:** The developed fibers are non-degradable, mechanically stable, offer good protection of encapsulated islets and allow the encapsulation of a high number of islets, crucial for device upscaling and the development of a clinically applicable bioartificial pancreas

Session Number: 13
3D Bioprinting in Engineering Tissues and Organs II
Wednesday, December 4, 2019, 10:00 AM - 11:30 AM

52 - In Vivo MRI Of 3d Bioprinted Cartilage Contructs

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***Purpose/Objectives:** Transport of oxygen and nutrients relies on adequate vasculature due to the limited range of extravascular diffusion, which effectively limits the size of 3D bioprinted constructs. Printing of gridded structures may facilitate neovascularization and/or diffusion, and thereby allow increased size of the constructs. 3D bioprinting of cartilage with cell-laden nanocellulose/alginate ink has proven to be successful, but detailed knowledge of the vascularization and transportation of nutrients is lacking. The aim of the present study was to analyze the spatiotemporal properties of perfusion and diffusion in gridded 3D bioprinted cartilage, xenografted to nude mice.
***Methodology:** Human nasal chondrocytes were mixed with cellulose/alginate ink (ratio 80:20) to a concentration of 10^7cells/ml bioink. Cartilage constructs were printed in gridded structures, cross-linked with $CaCl_2$for 5 minutes andimplanted into BALB/c nude mice (n=10/group) within 1 hour after printing. Magnetic resonance imaging (MRI) experiments, including dynamic contrast enhanced (DCE) and diffusion weighted MRI (DWI) were performed on anaesthetized animals (2% isofluran) within 1-3 days post implantation (week 1) and repeated weeks 2 and 4 (preclinical 7T Bruker BioSpec and 30-mm volume coil). In-house developed post-processing scripts (Matlab) were used for calculating the DCE parameters contrast arrival time (AT) to image voxels and initial slope (IS) of the DCE signal after i.v. injection of gadolinium contrast agent. The diffusion coefficient (D) was calculated from DWI data using mono-exponential model fitting to the DWI signal of b-values 200, 400 and 900 s/mm^2, where perfusion effects are minimal.
***Results:** Generally, AT was longer in the printed constructs compared with surrounding tissues, and IS was low, which indicates that oxygen and nutrient supply is limited to diffusion. On week 4, however, IS hot spots and shorter AT could be observed in locations corresponding to the grid holes, which may indicate neovascularization.Maps of D, sensitive to microstructural features such as membrane structures, viscosity and macromolecular content, showed varying degree of diffusion throughout the constructs, possibly due to the grid holes. Median D of the printed cartilage, excluding visible holes, was 2.34 (interquartile range: 2.07-2.65) μm^2/ms, and remained in the same range throughout the observation period.
***Conclusion/Significance:** In summary, this study shows that MRI has the potential to visualize and study the benefits of a gridded structure regarding vascularization and transport of nutrients, including microstructural and functional development over time. It also shows that the 3D bioprinted cartilage, as anticipated, is mainly nourished by diffusion and that the biomaterial used preserves its beneficial diffusion characteristics over 30 days *in vivo.*

151 - 3D Bioprinting Of A Mechanically Reinforced Hybrid Tissue Construct For Advanced Fibrocartilaginous Regeneration

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***Purpose/Objectives:** The demand for bioengineered tissues has risen rapidly because of the limited availability of donor tissues for reconstructive surgery. 3D bioprinting is a platform that produces patient-specific and customizable complex structures mimicking human tissues. Despite the remarkable perspective that this technology offers in the bioengineering of personalized tissue constructs, there are still critical challenges that should be addressed, including the development of novel bioinks with suitable biological properties, printability, and structural integrity. Here we developed a novel hybrid tissue construct by 3D bioprinting of a cell-laden bioink together with silk-based bioink that imparted mechanical stability, thereby overcoming previous limitations on the size, shape, and structural integrity of bioengineered soft tissue constructs.
***Methodology:** A novel bioink formulation of gellan gum (GG) and fibrinogen (FB) was optimized for cell-based bioprinting. A silk methacrylate (Sil-MA) was synthesized and formulated with gelatin and glycerol for microextrusion-based 3D printing. The rheological and mechanical properties of these bioink formulations were examined. The biological properties were also evaluated by measuring the cell viability and proliferation in the bioprinted constructs. In order to fabricate a hybrid tissue construct, multiple cartridges (integrated tissue-organ printing system, ITOP) were used to deliver and pattern cell-laden GG/FB and Sil-MA bioinks, sequentially. The characteristics and function of the bioprinted hybrid tissue constructs were evaluated *in vitro* and *in vivo*.
***Results:** The hybrid tissue constructs revealed to be suitable and exceptional for the 3D bioprinting of fibrocartilaginous tissue. Furthermore, the biological and mechanical properties of these bioinks could be tailored, not only by varying the concentration of their constituents but also by changing the methacrylate degree of Sil-MA bioink. *In vitro* biological evaluations indicated that the 3D bioprinted constructs containing porcine meniscus cells (PMCs) showed good cell adhesion, viability, and proliferation. In addition, *in vivo* studies showed that tissue maturation and the shape and structural integrity of the hybrid constructs were maintained up to 10 weeks of implantation.
***Conclusion/Significance:** We demonstrated that the GG/FB bioink could provide biological properties and printability for the cell-based bioprinting, while the Sil-MA bioink could offer exceptional structural integrity. This 3D hybrid system is believed to offer a versatile and promising alternative, not only for the production of fibrocartilaginous tissue constructs but also to be applied in a variety of soft tissue engineering applications.
Acknowledgments: This study was supported by National Institutes of Health (1P41EB023833-346 01).

54 - Thermal Inkjet Printing Elicits Activation Of The Nf-kb Pathway In Primary And Cancer Cells

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***Purpose/Objectives:** Theheat and shearing experienced by cells that are bioprinted may elicit heat shock protein expression, which is known to be activating the NF-κB pathway. This pathway is implicated in angiogenesis and cancer cell hormesis. We tested this hypothesis in vitro and in vivo.
***Methodology:** Primary Human microvascular endothelial cells and MCF-7 breast cancer cells were

bioprinted using a custom-built inkjet printer. ELISA readings for ANG-2, VEGF-A, FGF-1, HSP70, IL-1, and IL-8 were measured in the supernatant. For Western Blot experiments, the supernatant was discarded and cells were lysed. 46 phosphorylated kinases were measured for four groups: manually pipetted cells, heat shocked cells, and cells suspended in PBS were used for controls; bioprinted cells were used as the treatment group. For implant studies, cells were printed onto 2% alginate 5% gelatin gels, incubated for 12-24 hours and implanted subcutaneously in SCID mice. After 6 weeks, H&E staining was performed on the explants, immunohisto-chemistry staining for Human DNA and CD 31 were performed. The MCF-7 cells were incubated, then treated with tamoxifen at 5µM, 10µM, 50µM, 90µM, 110µM concentrations. Manually seeded cells, positive, negative and solvent controls were also included. Cytotoxicity was measured 24 hours post-treatment with a differential nucleotide assay.
***Results:** ELISA showed a significant increase in FGF (10x), HSP-70 (4x), VGEF-A (8x) and IL-1 (8x), in bioprinted cells and compared to control cells. Results of the Western blots revealed intracellular overexpression of HSP27, HSP60, p53, and p27 and eNOS in bioprinted endothelial cells after 12 hours compared to controls. A visual assessment of the blood vessels present in each tissue section of the H&E stains indicate a significant difference between the tissues where bioprinted endothelial cells were implanted compared to those with pipetted cells ($p=0.009$). A six-fold increase in blood vessels was seen. MCF-7 cell response to treatment showed that when the bioprinted samples were exposed to higher doses of tamoxifen, resulted in higher viability in the bioprinted samples than in the normally seeded cells, ($p=0.002$). The higher survival is attributed to the hormesis effect, which is also seen in vivo.
***Conclusion/Significance:** Thermalinkjetbioprinted cells get activated via the NF-κB pathway which leads to a drastically increase in microvessels in vivo. The bioprinted MCF-7 models showed hormesis, suggesting the bioprinted breast cancer cell model can be a better option for 2D models which don't show this effect.
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55 - A Dual Crosslinking Approach To Improve The Mechanical Properties And Stability Of Cell-laden Printable Collagen-Based Constructs

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***Purpose/Objectives:** Methacrylated collagen (CMA) has immense potential to be used as a bioink for 3D bioprinting of tissue scaffolds while retaining the natural properties of native collagen and allowing for photopolymerization of CMA hydrogels to improve print fidelity and resolution of the 3D printed construct [1, 2]. While photochemical crosslinking is a reliable method for the synthesis of cell-laden CMA hydrogels, these hydrogels are mechanically weak and susceptible to expedited enzymatic degradation *in vivo* [1]. In an effort to overcome these limitations, the goal of the current study was to develop a dual crosslinking scheme for the generation of mechanically viable cell-laden printable constructs for tissue engineering applications. We hypothesized that application of a dual crosslinking strategy will help improve the mechanical and degradation properties of CMA hydrogels while maintaining cell viability and metabolic activity.
***Methodology:** CMA hydrogels were formed by incubating a mixture of neutralized CMA (3 mg/ml) and

VA-086 (1% w/v in ultrapure water) at 37 ^{o}C for 30 min. For photochemical crosslinking, CMA hydrogels were exposed to UV light for 1 min. The remnant amine groups (i.e., not methacrylated) of the photopolymerized CMA hydrogel were then chemically crosslinked with two different concentrations of genipin (i.e., 0.5 mM (low dual) and 1 mM (high dual)) in 50 mM HEPES buffer for 1 h at 37 ^{o}C. Uncrosslinked CMA hydrogels, CMA hydrogels photochemically crosslinked with 1% VA-086 alone, CMA hydrogels crosslinked with 0.5 mM genipin only (low genipin) and 1 mM genipin only (high genipin) were used as controls. The effect of dual crosslinking conditions on gel morphology, compressive modulus and stability was evaluated using scanning electron microscopy (SEM), uniaxial compression tests and *in vitro* collagenase degradation test, respectively. Viability and metabolic activity of human MSCs encapsulated within dual crosslinked CMA hydrogels was assessed via live-dead assay and Alamar blue assay. Finally, the effect of dual crosslinking conditions on print fidelity was evaluated via line width and pore size measurements of 3D bioprinted constructs using Image J analyses.
***Results:** SEM results showed that gel morphology was not altered by crosslinking. Further, dual crosslinking significantly improved the compressive modulus and degradation time of CMA hydrogels. Cell viability results showed that high cell viability (i.e., > 90%) and metabolic activity in uncrosslinked, 1% VA-086, low genipin and low dual crosslniked CMA. On the other hand, cell viability and metabolic activity decreased significantly in high genipin and high dual crosslinked CMA hydrogels. Quantitative fidelity measurements showed the measured parameters (i.e., line widths, pore size) were comparable between photochemically crosslinked and dual crosslinked constructs, suggesting that print fidelity is maintained upon dual crosslinking.
***Conclusion/Significance:** In conclusion, application of low dual crosslinking is a viable strategy to yield mechanically superior, cell compatible and printable CMA hydrogels.
Acknowledgements: Supported by a grant from NIH (1R15AR071102).
References: [1] I. D. Gaudet and D. I. Shreiber, “Characterization of Methacrylated Type-I Collagen as a Dynamic, Photoactive Hydrogel”, 2012.[2] N. Diamantides et al., “Correlating rheological properties and printability of collagen bioinks : the effects of riboflavin photocrosslinking and pH”, 2017.

56 - 3D Bioprinted Renal Tissue Constructs Using A Novel Photo-crosslinkable Kidney ECM-derived Bioink

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***Purpose/Objectives:** End-stage chronic kidney disease is an incurable condition for which dialysis and renal transplantation are the only available treatments. 3D bioprinting strategies in tissue engineering have been proposed to fabricate clinically applicable tissue constructs that can replace damaged or diseased tissues and organs. A tissue-specific bioink is needed to create constructs that provide a biologically competent microenvironment to the encapsulated cells so that they can organize and create the appropriate architecture and functionality of renal tissue. In this study, we hypothesized that kidney ECM materials could provide renal-specific molecules and structural and biomechanical signals to regulate the renal cell behavior and, eventually, tissue maturation and formation. We have developed a photo-crosslinkable kidney ECM-derived bioink (KdECMMA) that could provide a kidney-specific microenvironment for renal tissue bioprinting.
***Methodology:** Porcine whole kidneys were decellularized through a perfusion method, dissolved in an acid solution, and chemically modified by methacrylation. This KdECMMA-based bioink was formulated

and evaluated for rheological properties and printability for the printing process as well as for biological properties *in vitro*. We implanted 3D printed renal constructs containing human primary kidney cells (HKCs) and KdECMMA in the kidney of nude rats to investigate the clinical feasibility.
***Results:** We successfully developed the photo-crosslinkable kidney-specific ECM bioink formulation for renal tissue bioprinting. This bioink formulation composed of gelatin, HA, glycerol, and KdECMMA provided the desired printability and structural integrity. The bioprinted HKCs in the KdECMMA constructs were highly viable and matured with time. Therefore, we implanted the 3D bioprinted renal constructs into a defect created on the outer aspect of the left kidney of nude rats. At the 1- and 2-month time points, it was evident that the implanted region displayed newly formed tubular and glomerular-like structures. Human-specific cell marker showed the presence of the HKCs in the newly formed renal structures. This renal tissue formation was also evident in the bioprinted constructs without cells, suggesting the recruitment of host renal progenitor cells into the implanted region.
***Conclusion/Significance:** The KdECMMA-based bioink formulation provided the kidney-specific microenvironment that supported the human kidney cell maturation and tissue formation. Therefore, the bioprinted renal tissue constructs showed high cell viability and proliferation, and also exhibited the structural and functional characteristics of the native renal tissue. *In vivo* studies using KdECMMA in the rat model showed the clinical feasibility and the interaction between the bioprinted renal construct and host tissue. 3D bioprinting strategy with kidney-specific ECM bioink has great potential to bioengineer a functional renal tissue construct for use in future regenerative medicine applications.
Acknowledgments: This study was supported, in part, by National Institutes of Health (1P41EB023833-346 01).

57 - A Poly(N-isopropylacrylamide)-Based Thermogelling Bioink For Extrusion Bioprinting

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***Purpose/Objectives:** Bioprinting is a developing technology for the fabrication of cell-laden constructs using additive manufacturing techniques. Hydrogels are a common ink material for extrusion bioprinting as they provide a hydrated environment that supports cell viability and proliferation during and after printing. However, they often lack the mechanical properties for appropriate printing resolution. Researchers have therefore investigated a number of ways to dynamically alter the rheological properties of hydrogel bioinks to favor printing resolution and mechanical strength post-printing while minimizing shear forces exerted on the encapsulated cells. Our objective is to design a novel bioink material that addresses these criteria through the use of thermal gelation to facilitate low shear and high printing resolution, as well as a prolonged covalent crosslinking mechanism to add mechanical strength post-printing.
***Methodology:** Here, we present a poly(*N*-isopropylacrylamide)-based bioink that undergoes dual-gelation. Poly(*N*-isopropylacrylamide) provides the ink with thermoresponsive properties, and is copolymerized with glycidyl methacrylate, dimethyl-*γ*-butyrolactone, and acrylic acid which facilitate covalent crosslinking, hydrolysis-dependent resolubilization of the macromer, and a modified lower critical solution temperature, respectively. The hydrogel precursor solution is extruded from a cartridge cooled below the lower critical solution temperature of the macromer and into a poloxamer hydrogel bath heated to 37°C, initiating thermal gelation, which allows the printed structure to form despite the low viscosity of the ink. The poloxamer bath has the additional benefit of maintaining the printed

construct within a hydrated, cytocompatible environment at physiologic temperature for the duration of the print. Next, a diamine crosslinker, pre-mixed within the ink, crosslinks the gel over several hours through an initiator-free epoxy-amine reaction, providing mechanical strength to the printed construct including facilitation of adhesion between intersecting strands. The construct can then be removed from the poloxamer bath by cooling the poloxamer below its gelation point.
***Results:** Through this method, we have fabricated hydrogel scaffolds with multiple distinct, attached layers. The gel precursor solution is readily extruded using printing pressures ranging between 40 and 150 kPa and has a complex viscosity of less than 2 Pa*s. Fully gelled and crosslinked hydrogels possess an elastic modulus on the order of several kPa, with a maximum observed value of 14 kPa. Additionally, the scaffolds possess interconnected pores and can be printed to a centimeter scale. The poloxamer bath successfully holds the printed strands in place and facilitates uniform gelation in all dimensions and is readily removable once crosslinking is complete.
***Conclusion/Significance:** These preliminary results demonstrate the potential for this material as a bioink in extrusion bioprinting. It contributes a novel ink and printing method for cell-laden printing that relies on an initiator-free dual-gelation mechanism, producing structures with adequate resolution and mechanical strength.

58 - A 3d Bioprinted Vascularized Omentum Model For Ovarian Cancer Metastasis

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***Purpose/Objectives:** The omentum is a visceral adipose tissue located in the peritoneal cavity, and is the primary metastasis site for advanced stage ovarian cancer. In order to develop effective treatments for ovarian cancer, we are in critical need of developing a 3D tissue models that include the interactions of cancer cells with the microenvironment niche that supports their growth into tumors as predictive assay models for drug screening. Here, a human 3D bio-printed vascularized tissue model that mimics the ovarian cancer metastatic microenvironment is described.
***Methodology:** In this model, endothelial cells, pericytes, and primary human omentum fibroblast were bioprinted to create a vasculaturized tissue underneath a monolayer of mesothelial cells. Ovarian cancer cell lines including OVCAR5, SKOV3 and HeyA8 were added on top of mesothelial monolayer. Automated high content imaging was used to quantify the ability of cancer cells break the barrier formed by mesothelial cells and migrated to the vascular tissue.
***Results:** Upon addition of cancer cells, cancer cells migrated to the vascularized tissue and formed tumor spheroids that could be quantitated as an assay for ovarian cancer metastasis.
***Conclusion/Significance:** The development of this novel human model allows us to screen for potential drugs that can prevent cancer cells from attachment and invasion into omentum.

59 - Biofabrication Of A Breast Cancer Microenvironment For In Vitro Modelling Cancer Process.

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***Purpose/Objectives:** Cancer is one of the major causes of morbidity and mortality. According to the American Cancer Society, over 1.7 million people were diagnosed with cancer in 2016, and approximately 0.6 million people lost their lives to it in the same year. It is further expected that the annual cancer incidences will rise from 14 million in 2012 to 22 million within the next 20 years, leading to significantly increased healthcare costs and the great need to better understand cancer to improve therapy. The cancer microenvironment is highly complex, and is highly dynamic with distinctive key features (*e.g.* cellular and ECM compositions, matrix stiffness, and degree of vascularization) present at each of the different stages of the disease. In particular, the large scale growth of a tumour ultimately requires a blood supply. To fully grasp the complexity of the tumour microenvironment, as well as screening of various anticancer drugs, it has been increasingly realized that *in vitro* engineered human cancer models are strongly desired. In particular, three- dimensional (3D) cancer models are anticipated to precisely mimic the *in vivo* tumour microenvironment in human patients by recapitulating the proper tumour cell/matrix composition and other key parameters that match both the type and stage of the disease, and therefore provide accurate mechanistic studies as well as a tool for personalized anti-cancer therapeutics studies. In this work, we present a tumour-on-a-chip device for modelling cancer disease.
***Methodology:** The tumour-on-a-chip is composed of two microfluidic channels separated by a porous membrane coated with extracellular matrix (ECM) and human ECs (HUVECs, 2-6 ×106 cells/ml) in a protein based hydrogel (GelMa) with the aim to completely cover the porous scaffold. Breast tumor cells (MCF7) embedded in GelMa were bioprinted in the biochip. Then the GelMa with embedded cells was crosslinked using UV light (385nm) to ensure cell adhesion around the porous membrane structure .After that the cellular construct was incubated for in vitro maturation. Confocal images were obtained to see cancer cells dissemination along the tumour microenvironment.
***Results:** The resulting tumour-on-a-chip device was fabricated using microfluidics, tissue engineering and biomaterials. We have demonstrated the 3D bioprintability of endothelial and tumour cells inside the microfluidics biochip for recreating a tumour microenvironment.
***Conclusion/Significance:** The resulting tumor-on-a-chip can be used for i) facilitating the study of the intrinsic biology of the tumor to analyze the origin, evolution and metastasis; ii) perform screening of new drugs or therapies; iii) reduce the use of experimental animals and above all iv) generate models of effective personalized therapy, generating a high-performance platform with a small biopsy of a patient for the analysis of drugs that provide the patient with the most effective treatment.

60 - Bioprinting Methods To Automate Biofabrication And Improve Regenerative Capacity For Implantable Tissue Engineered Muscle Repair Constructs

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***Purpose/Objectives:** Volumetric muscle loss (VML) injuries are defined as the loss of skeletal muscle tissue which exceeds the endogenous mechanisms for repair and results in a permanent reduction of volume and function. These injuries are prominent in both combat environments and civilian accidents. With a high occurrence of VML injuries and a lack of treatment options that adequately address the incurred functional and cosmetic deficits, there is a clinical need for improved therapies. Our lab has

developed an implantable tissue engineered muscle repair construct (TEMR) designed as a therapy for VML. The TEMR consists of a porcine acellular bladder (BAM) seeded with skeletal muscle progenitor cells (MPCs) and preconditioned in a cyclic stretch bioreactor, which promotes unidirectional orientation and phenotypic characteristics that improve functional outcomes. The efficacy of TEMR has been tested via implantation in surgically-created, biologically relevant, VML injuries in two rodent models: 1) tibialis anterior muscle, and 2) latissimus dorsi (LD) muscle. Functional tests indicate significant recovery in both models of between 70-90% within 3 months of implantation (Corona et al., 2013; Passipieri et al., 2019).
***Methodology:** With such a promising engineered technology, we are developing methods to automate the TEMR biofabrication process to reduce costs and production timelines. Additionally, we plan to incorporate vascular cell types such as endothelial cells and pericytes to "pre-vascularize" the TEMR and more adequately recapitulate the native microenvironment required for skeletal muscle repair. 3D bioprinting can be employed to not only address manufacturing challenges, but seed multiple cell types at discrete locations in the TEMR.
***Results:** Preliminary results indicate that primary rat and human muscle progenitor cells can be bioprinted at high (>90%) viabilities. Furthermore, these cells can be bioprinted to achieve high densities of cells on the BAM after just 24 hours. Primary vascular cell types such as mouse and rat endothelial cells, and rat adipose-derived stem cells as a source of pericytes, have been printed at high (>90%) viability after 24 hours.
***Conclusion/Significance:** These results indicate the potential for applying these methods to bioprint TEMR constructs for in vivo implantation. Future work will focus on additional characterization of the bioprinted TEMR construct, as well as analyzing the formation of vascular networks.

61 - 3D Printed Composite Scaffold Enhances Bone Healing In Rat Critical-sized Calvarial Defect

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***Purpose/Objectives:** Bone nonunion resulting from trauma, congenital abnormalities, or cancer resection are major medical concerns. A plethora of studies have reported considerable shortcomings of current clinical treatments using autografts, allografts and xenografts. Tissue engineering (TE) has emerged as a highly promising alternative to conventional treatment strategies for the repair or replacement of damaged bone. TE applications commonly encompass the use of three-dimensional (3D) scaffolds for the incorporation of cells or biomolecules. Various techniques have been used for the fabrication of 3D scaffolds. Generally, conventional fabrication techniques do not enable the fabrication of complex architectures. However, 3D bioprinting enables the process of a broad range of materials and the fabrication of scaffolds with improved design and complicated 3D microstructures.The purpose of the present study is to design a nontoxic and osteoconductive composite scaffold using 3D bioprinting and to evaluate bone healing in rat critical-sized calvarial defect.
***Methodology:** Polycaprolactone (PCL) was combined with poly(lactic-co-glycolic acid) (PLGA) and hydroxyapatite nanoparticles at a ratio of 40:40:20, fully melted at 110°C and then stirred until all components were homogeneously mixed. Scaffolds were manufactured using a Regemat V1 3D bioprinter (REGEMAT 3D, Spain) equipped with a fused-deposition modeling (FDM) system. The

composite material or PCL alone were loaded into the nozzle chamber, heated to a semi-liquid state and extruded. Printing parameter such as temperature, pressure, and extrusion head speed were optimized in order to maximize print quality. The morphology of the scaffolds was studied using a scanning electron microscope equipped with an energy dispersive detector. Porosity and pore size were determined by microcomputed tomography (microCT). Printed scaffolds (10x10x2 mm), were sterilized and subsequently loaded with $5x10^5$ human bone marrow MSCs (BMSCs) and cultured in osteogenic medium for various periods. Following each culture period, the proliferation rate, ALP activity and tissue mineralization were analyzed and transcripts of osteogenic markers were quantified using real-time PCR. Male Sprague Dawley rats aged 12-weeks were used as animal model of critical-size calvarial defects. Briefly, full-thickness defects measuring 9x9 mm were created in the parietal bones with attention paid to preserving the dura mater. The 3D printed scaffolds were then inserted into the defect. After 12 weeks, animals were euthanized, and the bone healing was analyzed using microCT and histological observations.

***Results:** The resulting PCL/PLGA/HA scaffold displayed a high uniform porosity and highly interconnected pores. Surface analyses revealed the presence of HA particles on the surface of the scaffold. Proliferation assay, microscopic observations, and gene analysis showed that BMSCs were able to attach, proliferate, and differentiate into osteoblasts. MicroCT and H&E analysis revealed that the composite scaffolds showed a significant increase in new bone formation compared to the PCL scaffold.

***Conclusion/Significance:** We produced a bioactive and osteoconductive scaffold using 3D bioprinting technology with high osteogenic potential for bone repair. This scaffold may be useful as patient-specific implant for guided bone regeneration in clinical sitting.

Session Number: 14
Translating Microphysiological Systems Technology to Preclinical Drug Development and Disease Modeling II
Wednesday, December 4, 2019, 10:00 AM - 11:30 AM

62 - A Multi-tissue Chip For The Modeling Of Osteoarthritis Pain

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***Purpose/Objectives:** Osteoarthritis (OA) is a debilitating disorder that afflicts more than 30 million people in the US alone. Pain is the most prominent symptom of OA, and pain caused by lower extremity OA is the leading reason for impaired mobility in older adults. Therefore, pain relief remains the primary focus of current treatments. Unfortunately, there are no consistent, effective approaches to retard or reverse disease progression or treat OA pain. Thus, there exists a critical need for understanding the mechanisms of OA pain in human and screening/developing more efficacious pain relievers. Given the limitations of current *in vitro* and animal models in simulating human physiology and OA pathology, we propose the development of a novel three dimensional (3D), human cell-based, multi-tissue chip that contains major joint elements as a whole. In particular, a neural component is incorporated to model OA pain and test the efficacy and toxicity of potential pain medications.
***Methodology:** Our previous study has reported the establishment of a human stem cell-based microphysiological joint (microJoint) chip. Briefly, 3D osteochondral complex, synovium, and adipose tissue were engineered from stem cell-laden hydrogels and subsequently integrated into a 3D printed bioreactor. The communication and interactions among the microtissues were enabled through microfluidic flow. The phenotype and function of the microJoint have been validated using different analytical methods. We have also generated "OA" microJoints by challenging synovium with the pro-inflammatory cytokine interleukin (IL)-1β. To generate the pain-enabled microJoint (Neu-microJoint) chip, a new chamber was 3D printed and incorporated into the microJoint chip, in which human sensory neurons were cultured. Neural activities in Neu-microJoint chips under normal and "OA" conditions were assessed with electrophysiology and imaging. To validate the capacity and efficacy of this novel chip in modeling OA, we have also introduced pain management drugs such as opioids in the culture and examined their effects on pain level and tissue health.
***Results:** The real-time PCR, histology and immunohistochemistry results confirmed the phenotypes of normal and "OA" Neu-microJoint. In particular, IL-1β treatment of synovium induced OA-like degeneration of joint tissues. For example, the cartilage tissue showed significantly increased expression levels of major catabolic genes such as matrix metallopeptidase 13 (*MMP13*) and a disintegrin and metalloproteinase with thrombospondin motifs 4 (*ADAMTS4*) as well as obviously downregulated expression of chondrogenic genes such as aggrecan and type II collagen. Under this OA-mimicking condition, the exposure of neurons to "synovial" eluate markedly changed their excitability, suggesting the generation of pain-associated changes in the tissue chip. Interestingly, we also found that the

introduction of opioids may deteriorate cartilage degeneration in Neu-microJoint.
***Conclusion/Significance:** The pain-enabled microphysiological joint chip developed in this study promises to serve as a robust system to study the mechanisms of OA pain and develop effective pharmaceutic interventions.

63 - An In Vitro Functional Assay To Predict And Study In Vivo Skeletal Muscle Stem Cell Engraftment Outcomes

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***Purpose/Objectives:** Functional validation of promising stem cell endogenous repair drugs or biomolecules constitutes a major translational bottleneck. As a consequence, following a compound screen, candidate selection is often biased to minimize risk and prioritize hits most likely to produce dramatic *in vivo* phenotypes. To address this gap and compress the drug discovery pipeline, we developed an *in vitro* stem cell mediated skeletal **m**uscle **end**ogenous **r**epair platform (MEndR) that predicts *in vivo* transplantation outcomes in mice. With this approach, we demonstrate we can stratify drug candidates in parallel and of uncover species specific responses.
***Methodology:** Human primary myoblast lines (hpMBs) were established from human biopsy tissues obtained with patient consent and under REB approval. hpMBs were encapsulated within a fibrin/Geltrex hydrogel and seeded into a thin cellulose scaffold (3D). Muscle stem cells (MuSCs) were FACS enriched from hindlimb muscles of mice expressing GFP under the control of β-actin. For the drug screen, freshly isolated MuSCs were seeded and treated across a titration curve with 360 kinase inhibitors for 1-week. An ATPLite luminescence assay was used to identify hits. NSG mice used for *in vivo* validation assays were hindlimb irradiated and $BaCl_2$ injured prior to intramuscular cell transplants. After harvesting and fixing *in vivo* or *in vitro* tissues, immunostaining was used to label specific proteins before imaging. Images of donor-derived fibers were captured using CellSens and Fluoview softwares, and adjusted and analyzed consistently using open source ImageJ software.
***Results:** We generated thin sheets of mature human skeletal muscle fibers by infiltrating a thin cellulose scaffold with primary patient-derived myoblasts encapsulated in an extracellular matrix hydrogel. In the study validation phase, sheets were engrafted with freshly isolated MuSCs, injured by myotoxin exposure, to create a regenerative microenvironment, and then muscle 'repair in a dish' was assessed over 10 days. Notably, the spatiotemporal dynamics of the *in vitro* repair process matched those observed *in vivo*, but only when both stem cells and injury were present. Repair outcomes were modulated using 9 drugs: one drug which was previously reported to promote ex-vivo expansion of MuSCs, and 8 small molecule inhibitors which were newly identified from our MuSC inhibitor screen. In vitro outcomes in MEndR were compared to outcomes of the 'gold standard' *in vivo* transplantation studies. *In vivo* outcomes, both positive and negative, were predicted in MEndR. Side-by-side comparisons accurately stratified a range of repair phenotypes. By incorporating human iPSC derived $Pax7^+$ cells into the regenerative environment of the microtissue, we uncovered species specific responses to drug treatments.
***Conclusion/Significance:** MEndR is a system to study muscle stem cell mediated repair in a dish by way of deconstructing and reconstructing the *in vivo* niche. To our knowledge, this is the first *in vitro* model to mimic *in vivo* progression post injury. Moreover, MEndR faithfully predicts donor fiber engraftment outcomes while enabling side-by-side stratification of donor fiber features. Our platform represents a

powerful opportunity to speed up the discovery pipeline by compressing drug discovery and *in vivo* validation into a single, simple and cheap human cell assay.

64 - Modular Tissue Engineering Of Bone Microenvironment As An Extravasation Model

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***Purpose/Objectives:** Metastasis is the central event in cancer and accounts for 90% of cancer related mortality. Extravasation and colonization are the central events of metastasis. Breast cancer circulating tumor cells (CTC) establish metastatic tumors in the bone due to a close interaction with local cells including endothelial cells, perivascular mesenchymal stem cells (hMSC), and osteoblasts. An *in vitro* recapitulation of the bone microenvironment could provide new insight into the metastatic cascade and aid in developing new therapeutic strategies.
***Methodology:** A microfluidic device containing cell populations relevant to the extravasation process was fabricated in-house to recapitulate the extravasation site. The device was fabricated from clear acrylic using CNC micromilling and laser engraving. The device consists of three main components: (1) an engineered bone compartment housing functional osteoblasts derived from human mesenchymal stem cells (hMSC) from healthy donors; (2) a vascular membrane fabricated by seeding endothelial and perivascular cells on either side of a porous membrane; and (3) a channel in which human primary breast cancer tumor cells (3384T) are circulated. The osteoblast compartment is upstream of the vascular membrane, allowing cytokines to filter though the membrane and downstream into the channel containing 3384T cells. The device was reversibly sealed and perfused for 5 days. 3384T cells were then analyzed using RT-qPCR and western blot.
***Results:** The microfluidic device was successfully seeded with the relevant primary human cells and continuously perfused for 5 days without leaks and allowed for the imaging and visualization of the channel and compartment during culture. When cultured in the presence of osteoblasts, 3384T cells increased expression of CD9, linked to cell activation, growth and motility; Cadherin11, linked to tumor progression; FGF13, linked to tumor and cell growth; and CXCR4, linked to invasion and metastasis. This suggests that 3384T cells are responding to the engineered metastatic niche similarly to how they respond *in vivo*.
***Conclusion/Significance:** The device presented two key components of the bone marrow niche in a modular fashion: osteoblasts and a vascular wall. The system was compatible with cancer cell culture, inducing upregulation of metastatic markers, and could serve as an extravasation model once optimized to promote migration of 3384T through the vascular membrane into the engineered bone. Given that the device was populated with human primary cells as opposed to cell lines, it will serve as a personalized platform that will allow the mechanistic interrogation of 3384T during the extravasation and colonization process of individual patients, and ultimately, serve as a high throughput platform for testing therapies that may arrest this process.

65 - Characterization Study Of Fabricated Alginate-pectin Composite Foams By Control Of Pectin Content

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***Purpose/Objectives:** Alginate and pectin have been widely applied in various industrial and biomedical applications due to their synergistic interaction properties. Although the alginate and pectin were used as composite materials such as films, gels and particles, studies on the characterization of foam types are scarce.
***Methodology:** Thus, In the present study, we prepared a composite foams composed of alginate-pectin blended for fabricating of optimal wound dressing. The alginate and pectin blended ratio for foam fabrication was 10:0, 9:1, 7:3 and 5:5 (A10P0, A9P1, A7P3 and A5P5). Then, the foams were cross-linked polymeric network by calcium ion. After fabrication of the alginate-pectin foams, we carried out SEM, FT-IR, rheometer, gel fraction, swelling test and drug release ability for characterization of alginate-pectin foams. In addition, we conducted *in vitro* experimentation in terms of cytotoxicity by MTT assay and Hoechst.
***Results:** Fabricated all foams have an excellent three-dimensional network structure. Among the rehydrated foams, the G' values of rehydrated A9P1 foam were higher than the other rehydrated foams at all frequencies. In the swelling ability of foams, increasing of pectin content in composite foams lead to an induced more water to be absorbed, while increasing of alginate content in composite foams lead to an induce more PBS to be absorbed. In case of the indirect and direct cytotoxicity, all foams did not show the cell cytotoxicity on NHDF-neo fibroblast. In addition, increasing of pectin content in composite foams induced the BSA release ability.
***Conclusion/Significance:** These results suggested that the control of pectin content in the alginate-pectin foams can be induce the mechanical property, water absorption and drug release ability. In addition, alginate-pectin composite foams can be good candidates as wound dressing application.

Session Number: 15
Biomaterials and Regeneration I
Wednesday, December 4, 2019, 10:00 AM - 11:30 AM

66 - Development Of Zinc And Its Alloys As Biodegradable Metals

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***Purpose/Objectives:** The development of zinc and its alloys as degradable metallic biomaterials is just starting. Till now over 50 articles can be searched from the Web of Science, on the topic of zinc and its alloys as biodegradable metals, by authors from 10 countries including USA, Australia, Germany, Sweden, Japan and China.
***Methodology:** In the present talk, we will give a comprehensive review on the research progress, based on our recent works, and propose the future research direction in this research area.
***Results:** Firstly our recent works on the in vivo degradation of pure zinc stent within the blood vessel microenvironment will be presented; secondly several kinds of Zn-based biocomposites (Zn+Mg, Zn+HA, Zn+ZnO) aiming for orthopaedic application usage were fabricated, and their in vivo degradation behavior within bone microenvironment will be reported, with pure Zn as control group; thirdly we will show you our recent works on the development of binary Zn alloys, with over 10 kinds of alloying elements were added into Zn to enhance its mechanical properties and bioactivities; finally we will demonstrate how to use surface modification techniques to adjust the degradation rate and enhance the biocompatibility of pure Zn. All these preliminary in vitro and in vivo studies showed acceptable biodegradability and reasonable biocompatibility in the bone and blood micro-environments for the experimental Zn-based biodegradable metals, and for some alloy systems superior mechanical performance than Mg-based biodegradable metals.
***Conclusion/Significance:** Future continuous works are waiting to be done to further optimize the chemical composition of multi-component Zn alloys and their process technology to obtain well balance among mechanical properties, degradation behaviour (biocorrosion rate and mode) and long-term biological effects of the degradation products to the host.

67 - **Sema3C: A Novel Coupling Factor In Bone Remodeling Mediated By Microstructured Titanium Surfaces**

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***Purpose/Objectives:** A critical stage of osseointegration is primary bone remodeling, which involves replacing primary bone with mature bone through biological interaction between bone cells and the implant surface. At the bone remodeling stage, bone formation is coupled to bone resorption by coupling factors. Understanding the coupling mechanisms and investigating the coupling factors will allow us to discover potential therapeutic targets to improve osseointegration. A new class of molecules has been recently reported to affect remodeling and the coupling process-the semaphorins. No research

has been conducted on the role of sema3C (class 3 semaphorin) in bone biology, however, previous research from our lab showed that the regulation of sema3C gene expression in hMSCs is surface-dependent, with expression on the most osteogenic substrate. Therefore, the purpose of this project was to determine if sema3C can be a potential therapeutic target to improve osseointegration.
***Methodology:** Institut Straumann AG (Basel, Switzerland) provided 15mm diameter grade 2 Ti disks modified to be smooth/hydrophobic (PT), or rough/hydrophilic (modSLA). hMSCs and normal human osteoblasts (NHOsts) were cultured on PT or modSLA with tissue culture polystyrene (TCPS) as an optical control. Fresh media were added on day 7 and conditioned media were collected at day 8 for testing sema3C production by ELISA. DNA was quantified in cell layer lysates. Temporal production of Sema3C was determined at day 3, day 4, day 6 and day 8. To assess the indirect effect of the surfaces on sema3C expression from osteoclasts, hMSCs and NHOsts were cultured for 7 days on PT and modSLA. Conditioned media were collected and added to osteoclasts cultured for 7 days on an osteolyse kit plate. After two days treatment, osteoclast activity was measured and sema3C gene expression was determined by RT-PCR.
***Results:** The production of sema3C from hMSCs at day 8 was inverse to the surface-roughness (TCPS>PT>modSLA), suggesting that sema3C has inhibitory effects on osteoblastic differentiation induced by Ti, particularly hydrophilic microrough substates. Surface-dependent decreases in sema3C production were observed in hMSCs on all tested days, with the highest amount of sema3C produced at day 3, suggesting that sema3C may be important at the early stage of hMSC osteogenesis. Conditioned media decreased osteoclast activity in a surface-roughness dependent manner (TCPS>PT>modSLA). When cultured with hMSCs conditioned media, osteoclast expression of sema3C expression increased with surface roughness but the opposite was seen when condition media from NHOsts was used.
***Conclusion/Significance:** This study demonstrates for the first time that sema3C may be important in early stage of osteogenesis and involved in the crosstalk between osteoblasts and osteoclasts that is mediated by surface properties.

226 - Functional Polyesters That Harness An Anti-inflammatory And Inflection Fighting Powerhouse Of Innate Immunity

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***Purpose/Objectives:** Although successes are notable, the use of synthetic biomaterials have been limited by poor integration, fibrosis, bacterial colonization of implants and limited biocompatibility. Through a biomimetic approach, we have developed a library of polyester materials that incorporate a key molecule in innate mammalian cell immunity, itaconic acid (ITA), into polymer backbones for establishment of inherent antibacterial and anti-inflammatory characteristics. An aspect of host macrophage defence, small molecule ITA inhibits bacterial metabolism and regulates further innate inflammation. Considered as a powerful potential therapeutic, ITA is limited by a short half-life in systemic circulation. This motivated us to approach the biomaterial microenvironment differentially, considering the hydrolytic degradation of polyester materials as a slow release drug reservoir of ITA into the local cell microenvironment.
***Methodology:** We incorporated ITA into linear polyester materials using a one pot polycondensation reaction (120°C, 6 hr at 1atm, 12hr at vacuum pressure). The release of ITA was quantified through hydrolytic degradation mass spectroscopy assessment. We considered the cellular activity of materials

under multiple conditions to understand specific efficacies in vitro when compared to degradable poly(lactic acid) (PLLA) or poly(lactic-co-glycolic acid) (PLGA). Material incubation with Escherichia coli with an acetate carbon source was used to consider ITA specific inhibition in complex growth conditions and compared additionally to commercially available silver nanoparticles. Transwell culture of materials with activated bone marrow derived macrophages (BMDM) determined immunoregulation, assessed by cytokine secretion (ELISA), expression of nitric oxide synthase (NOS2) and nitrile production. Specificity of cellular activity was assessed with human dermal fibroblasts. In vivo translation was determined with peritoneal material injection, comparing single immune cell infiltrate and infection fighting properties to liquid silicone.
***Results:** Using a one pot polycondensation reaction, we optimized an adaptable synthesis method to combine ITA with different length alcohol monomers (gel: 1 yielding materials of high purity (1HNMR, FTIR) in gel (1,6-hexanediol, 1,8-octanediol), and solid form (1,10-decanediol). These constructs demonstrated quantifiable ITA release in a hydrolytic environment. Multiple ITA containing material combinations demonstrated a decrease in Escherichia coli growth ($p < 0.05$) when compared to a polystyrene and PLLA groups, with comparable inhibition to commonly utilized silver nanoparticles. Pre-treatment of murine BMDMs with ITA containing materials prior to pro-inflammatory stimulation (LPS, LPS/IFNγ) presented a significant down-regulation in a number of pro-inflammatory cytokines (IL-1β, IL-6, IL-10, IL-12p70, CCL2, IFNβ) and phenotypic nitric oxide production (NOS2 expression, nitrile secretion) when compared to PLGA, suggesting release mediated anti-inflammatory characteristics. Specificity of material cell functionality was verified by demonstrated non-toxic behaviour with human dermal fibroblasts. Upon peritoneal material injection, ITA containing gel material presented reduced biomaterial associated inflammation (neutrophils, monocytes, eosinophils; $p<0.05$) when compared to silicone ten days post-implant.
***Conclusion/Significance:** We have demonstrated a novel biomimetic approach where we harness the advantages of the innate immunity, using a biomaterial design to incorporate bioactivity into polymer backbones, achieving localized antibacterial and anti-inflammatory material properties. These outcomes indicate the potential of ITA based material design as a platform of active material microenvironments with dual functionality for improvement of material adoption.

69 - Kidney Regeneration With Biomimetic Vascular Scaffolds Based On Vascular Corrosion Casts

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***Purpose/Objectives:** Vascularization is one of the major hurdles affecting the survival and integration of implanted three-dimensional tissue constructs *in vivo*. We have developed a biomimetic renal vascular scaffold based on a vascular corrosion casting technique. This study evaluated the feasibility of using this novel biomimetic scaffold for kidney regeneration in a rat kidney cortical defect model.
***Methodology:** Vascular corrosion casts were prepared from normal rat kidneys by perfusion with 10% polycaprolactone (PCL) solution, followed by tissue digestion. The corrosion PCL cast was coated with collagen, and PCL was removed from within the collagen coating, leaving only a hollow collagen-based biomimetic vascular scaffold. The fabricated scaffolds were pre-vascularized with MS1 endothelial cell coating, incorporated into 3D renal constructs, and subsequently implanted either with or without human renal cells in the renal cortex of nude rats.
***Results:** The implanted collagen-based vascular scaffold was easily identified and integrated into native

kidney tissue. The biomimetic vascular scaffold coated with endothelial cells (MS1) showed significantly enhanced vascularization, as compared to the uncoated scaffold and hydrogel only groups ($P<0.001$). Along with the improved vascularization effects, the MS1-coated scaffolds showed a significant renal cell infiltration from the neighboring host tissue, as compared to the other groups ($P<0.05$). Moreover, addition of human renal cells to the MS1-coated scaffold resulted in further enhancement of vascularization and tubular structure regeneration within the implanted constructs. The biomimetic collagen vascular scaffolds coated with endothelial cells are able to enhance vascularization and facilitate the formation of renal tubules after 14 days when combined with human renal cells.
***Conclusion/Significance:** This study shows the feasibility of bioengineering vascularized functional renal tissues for kidney regeneration. The use of this scaffold system could address the challenges associated with vascularization, and may be an ideal treatment strategy for partial augmentation in patients with chronic kidney disease.

70 - Cellular Micromechanical Environment In 3d-printed Scaffolds

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***Purpose/Objectives:** A prevalent approach to fibrocartilage engineering utilizes 3D-printed, cell-laden, bioresorbable scaffolds which can play an acute mechanical role in fibrocartilage engineering to: (1) control the overall engineered tissue mechanics and (2) provide the requisite micromechanical environment within the cell-laden matrix (eg. bioink/hydrogel) to drive regeneration as well as maintenance of the healthy mature tissue. However, this critical three-dimensional micromechanical environment within the matrix is difficult to characterize experimentally and, thus, cell fate is difficult to predict. Therefore, in this study, a parametric finite element model was developed to predict the three-dimensional, micromechanical environment within a hydrogel matrix of an engineered annulus fibrosus scaffold.
***Methodology:** A representative geometry of a polycaprolactone (PCL) angle ply laminate scaffold (fibre angle = 42.5°, fiber diameter = 0.3 mm, fiber spacing = 1 mm) with a fibrin hydrogel infill was created in ABAQUS. Within the geometry, a refined mesh was generated for a subunit cell region of interest (ROI). The fibrous PCL was assumed to be linear elastic ($E = 265$ MPa, $\nu = 0.3$). Cylindrical specimens of fibrin hydrogel were tested in confined compression (to 33% maximum strain and 1.7 %/s strain rate) and fitted to a hyperelastic constitutive model. Biaxial tensile stress, which mimics the dominant *in vivo* annulus fibrosus loads, was simulated on the geometry followed by a secondary custom finite element algorithm to reproduce 3D stresses in representative cell volumes within the ROI and predict the corresponding cell fates using previously published 3D stress criteria [1].
***Results:** Unconfined compression of fibrin hydrogel yielded elastic responses best characterized by a second-order polynomial hyperelastic model (with coefficients: $C_{10} = 3230$, $C_{01} = -2510$, $C_{11} = -11800$, $C_{20} = 9940$, and $C_{02} = 3980$). The base model predicted a micromechanical environment conducive to fibrochondrogenesis for 0.6% of cell units (2442 of 382,504 cell units). Of the 5.7% of cell volumes with at least one node attached to PCL fibers, 10.4% (2252 of 21,692 cell units) predicted a stress that would generate fibrocartilage. Conversely, the 94% of cells with no attachment to the PCL fibers predicted 0.05% fibrochondrogenesis.
***Conclusion/Significance:** The results of this study predicted that effective cell attachment to the load-bearing scaffold may be critical for tissue engineering of annulus fibrosus and other fibrocartilage

tissues. Further, increasing the available surface area for cell attachment (e.g., with smaller diameter fibers via perhaps melt electrowriting) may be beneficial for driving desirable cell fates. The finite element model presented in this study will be validated against histological results from a companion *in vitro* study of engineered scaffolds cultured under equivalent biaxial loading conditions. In future work, the model will predict the influence on cellular micromechanical environment from: (1) different multiaxial loading conditions, (2) different material properties (eg. inherent manufacturing variations, alternative materials, temporal degradation, etc.), (3) different scaffold architectures (eg. fiber angle, spacing, size, etc), and (4) stresses within implantable geometries.

224 - Ti6al4v Lattice Structure Via Extrusion Based 3d Printing For Bone Substitute

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***Purpose/Objectives:** Additive Manufacturing (AM) is the most common technique for developing complex dense/porous implant with control porosity and pore size distribution by combining computer-aided design (CAD) with computer-aided manufacturing (CAM). This technique has been widely explored towards developing customized implants via manipulation of geometrical distribution, modification of mechanical properties and creating porous structures for tissue *ingrowth* as well as better mechanical fixation. Amongst all other biomaterials, metals have demonstrated great potential for long-term load-bearing applications due to its excellent mechanical properties. Titanium and its alloys exhibit better performance in this prospect. In this study, we have proposed an alternative method towards fabricating 3D printed lattice architecture in a cost-effective way.
***Methodology:** The CAD files were generated using SolidWorks software package and changed into an STL file to feed into the machine for final printing. Ti6Al4V loose powder (Good Fellow, U.K.) was mixed with 4 wt% Chitosan solution with 2% acetic acid into a homogeneous dispersion suitable for printing. An FDM (Fused deposition modeling) based customized 3D printer was used to print optimized Ti6Al4V slurry into 3D Ti6Al4V scaffolds and finally sintered at the 1400 ^{O}C in inert atmosphere. The sintered samples were further carried out for *in vitro* and *in vivo* characterization for its biocompatibility.
***Results:** The titanium lattice structure was successfully sintered under controlled atmosphere and the lattice structure was maintained after sintering. The sintered scaffolds showed good compressive strength and elastic modulus which is close to natural bone. The resultant lattice structure had highly open porous structure, and most suitable for tissue integration at the interface via tissue in-growth. It also showed promising results in *in vitro* study for 3 days, 5 days and 7 days and bio-compatibility in vivo.
***Conclusion/Significance:** The scaffolds showed significantly cytocomapatibilty after 3 days, 5 days and 7 days cell culture study. And it also showed significant bone in growth for 30 days and 60 days after implantation.

72 - Engineering Pulmonary Valve Tissue Sheets From Human Umbilical Cord Perivascular Cells And Electrospun Polyurethane

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***Purpose/Objectives:** Existing materials used to repair the hypoplastic pulmonary valve in Tetralogy of Fallot are non-viable structures that fibrose and degrade, requiring repeated intervention. Bioengineered valve tissue synthesized from mesenchymal stromal cells (MSCs) on a degradable biomaterial scaffold can overcome this limitation through the production of living valve repair materials with the potential to grow and remodel within the heart. A promising but underused autologous cell source for heart valve tissue engineering (HVTE) are human umbilical cord perivascular cells (hUCPVCs), which are readily isolated from the Wharton's jelly at birth and have excellent regenerative capacity in vivo. Translation of the extracellular matrix (ECM) producing capacity of hUCPVCs to a viable valvular repair material requires a temporary biomechanical structure to support the cells until sufficient neo-tissue has been synthesized. Degradable electrospun nanofibrous polycarbonate polyurethane (PU) scaffolds are a favourable biomaterial for HVTE as fibre alignment of electrospun scaffolds can be varied to confer the mechanical anisotropy seen in the native pulmonary leaflet. Furthermore, these scaffolds have been shown to support fibroblast attachment and biodegrade without producing any cytotoxic by-products.
***Methodology:** To assess the in vitro performance of hUCPVCs for HVTE applications, we examined their growth kinetics, differentiation capacity, and ECM producing capacity under clinically advantageous xeno-free and chemically-defined (XF-CD) conditions. The compatibility of hUCPVCs and electrospun PU was evaluated by assessing cell attachment, morphology and viability under XF-CD conditions.
***Results:** hUCPVCs proliferated more rapidly ($p<0.05$) and retained a significantly larger population of self-renewing colony forming unit-fibroblasts ($p<0.05$) than conventionally used bone marrow-derived MSCs (bm-MSCs) in XF-CD conditions. Further, hUCPVCs synthesized significantly more collagen ($p<0.05$) and sulphated glycoasminoglycans ($p<0.05$) than bm-MSCs. Supplementation with 50 µM ascorbic acid significantly increased collagen ($p<0.05$) while depressing elastin ($p<0.05$) synthesis by bm-MSCs, but did not affect ECM deposition by hUCPVCs. After induction with osteogenic or adipogenic media, bm-MSCs differentiated accordingly, while hUCPVCs retained their initial phenotype, suggestive of a synthetic cellular phenotype without maturation to non-valvular cell lineages. hUCPVCs demonstrated excellent attachment (97 ± 11%) to PU scaffolds as measured by the Hoechst 33258 dye-binding assay. Further, pre-coating scaffolds with fibronectin promoted spreading and alignment of attached hUCPVCs. After 1 week in culture, calcein AM/ethidium homodimer staining revealed complete coverage of the scaffolds by viable hUCPVCs.
***Conclusion/Significance:** hUCPVCs cultured on electrospun polyurethane offer a promising platform for in vitro engineering of pulmonary leaflet tissue sheets. On-going studies are assessing tissue synthesis and functionality to further evaluate the potential of this novel combination of hUCPVCs and anisotropic PU scaffolds as a platform for HVTE.

428 - Injectable ECM Hydrogel As A Therapy For Pelvic Floor Muscle Atrophy Following Birth Injury

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***Purpose/Objectives:** Pelvic floor disorders, which include urinary and fecal incontinence and pelvic organ prolapse, affect almost a quarter of women in the U.S., with an estimated increase to 44 million by 2050. Childbirth injury to the pelvic floor muscles (PFMs) is a key risk factor for pelvic floor disorders. Currently, preventative measures for PFM dysfunction do not exist and treatments do not target the pathways responsible for PFM pathology. We previously developed an injectable decellularized extracellular matrix hydrogel derived from porcine skeletal muscle (SKM), which displays pro-regenerative properties and prevents muscle atrophy in hindlimb ischemia model. Our objectives were to evaluate the efficacy of SKM 1) as a preventative and 2) treatment modality for PFM atrophy consequent to birth injury in the rat model.
***Methodology:** Three-month-old Sprague-Dawley rats underwent simulated birth injury (SBI) via vaginal distention, which replicates the circumferential and downward strains during vaginal delivery. Animals were randomly divided into 2 groups: SBI + saline; and SBI + SKM. SKM or saline (10 µl) were injected into the pubocaudalis (PCa) muscle, which experiences the highest parturition-related strains analogous to the human pubococcygeus, immediately after SBI as a prevention model (n=6/group) or 4 weeks post-SBI as a treatment model (n=5/group). Injections were done using a system of coordinates derived from magnetic resonance images of the rat PFMs. The muscles were harvested 4 weeks post-injection and prepared for analysis. Anti-laminin antibody was used to determine fiber cross-sectional area as a marker of atrophy. Data were compared between groups using Mann-Whitney test (median (range)), with significance set to $P<0.05$.
***Results:** SKM injection immediately after SBI resulted in a notable shift towards a greater proportion of larger fibers in the hydrogel group. Fiber cross-sectional area was significantly increased in SKM (1909 μm^2 (63-4147 μm^2)) compared to saline (1718 μm^2 (58-4147 μm^2) group, $P<0.0001$. As a treatment for SBI, SKM reversed PFM atrophy resulting in increased fiber area (2071 μm^2 (63-4147 μm^2)) compared to saline (1810 μm^2 (62-4146 μm^2), $P<0.0001$.
***Conclusion/Significance:** A skeletal muscle derived ECM hydrogel offers a promising minimally invasive therapy for the prevention and treatment of PFM atrophy after birth injury.

225 - Biocompatability Of $Pt_{57.5}Cu_{14.7}Ni_{5.3}P_{22.5}$ Bulk Metallic Glass In Orthopaedic Applications.

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***Purpose/Objectives:** Nanopatterning of biomaterials is rapidly emerging as a tool to engineer cell function. Bulk metallic glasses (BMGs), a class of biocompatible materials, are uniquely suited to study nanopattern-cell interactions as they allow for versatile fabrication of nanopatterns through thermoplastic forming (TPF). Platinum-based BMGs are a unique class of amorphous metals with high strength, elasticity, corrosion resistance, and an unusual plastic-like processability. Previously, we showed that $Pt_{57.5}Cu_{14.7}Ni_{5.3}P_{22.5}$ bulk metallic glass (Pt-BMG) could induce the differentiation of mesenchymal stem cells (MSC) towards either the osteogenic or adipogenic lineage based on surface nanopatterning. The investigation of bone cell responses is necessary for the creation of synthetic, load-

bearing implants. At the implant interface, dynamic interactions between bone forming cells (osteoblasts) and bone resorbing cells (osteoclasts) occur. Therefore, in the present study, we investigated osteoclast formation on Pt-BMGs in comparison to titanium and tissue culture plastic.
***Methodology:** The amorphous nature of BMGs allows for the facile creation of nanotopography. BMGs disks were pressed into a commercially made alumina oxide mold using an Instron to apply pressure at 270 degrees Celsius. Flat (pressed without mold) and nanopatterned BMGs with nanorod diameters of either 20, 80, or 200 nm were prepared. Titanium disks and tissue culture plastic served as controls. Osteoclast formation was induced in mouse bone marrow derived precursor cells with RANK-L treatment. Analyses included morphological evaluation of cells, quantification of multinucleation, and gene expression (TRAP). Signaling responses were evaluated by western blot for phospo-Akt.
***Results:** We observed that treatment with RANKL induced distinct morphological changes of OC precursors including spreading and overall size and these parameters were altered on nanopatterned BMGs with either 80nm or 200nm nanorods. OC formation was not altered on the 20 nm nanopattern. Osteoclast formation was associated with reduced tartrate-resistant acid phosphatase (TRAP) expression and changes in cytoskeletal remodeling. Phospho-Akt levels were similar in all groups. Currently, we are evaluating the biocompatibility of Pt-BMGs rod-shaped implants in a mouse bone implantation model.
***Conclusion/Significance:** Collectively, the processability and highly tunable nature of Pt-BMGs coupled with their ability to differentially influence cell function suggest that they could enable the creation of a wide range of surface topographies that can be produced and studied in a highly reproducible manner. This could allow the development of implants capable of engineering MSCs and osteoclast functions and leading to improved bone implants.

Session Number: 17
Biomaterial, Scaffold And Cellular Strategies To Control Tissue Elasticity
Wednesday, December 4, 2019, 1:00 PM - 2:30 PM

75 - Multifunctional Gene Silencing Nanotherapeutics For Elastic Matrix Regenerative Repair

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***Purpose/Objectives:** Breakdown of the extracellular matrix in proteolytic disorders is catalyzed by the overexpression of matrix metalloproteinases (MMPs) which specifically disrupt elastic fibers. Due to the inability of adult smooth muscle cells to generate sufficient elastin precursors to combat this disruption, a simultaneous pro-elastogenic and anti-proteolytic intervention is mandated. Previously, we've shown through use of the MMP-inhibiting drug doxycycline in rat aortic smooth muscle cells that increased downstream elastogenesis and cross-linking of elastic fibers resulted from doxycycline-induced inhibition of the regulatory protein c-Jun N-terminal kinase (JNK-2) in addition to the drug's anti-proteolytic effects. Due to the non-specificity of the drug, we've presently investigated JNK-2 gene silencing through the use of anti-JNK2 siRNA complexed with transfection vector polyethylenimine (PEI) and amphipathic cell-penetrating peptide, MPG8, for optimal intracellular delivery and gene knockdown.
***Methodology:** PEI-siRNA complexes in varying N:P molar ratios (2.5:1-30:1), polymer molecular weights (25kDa, 40kDa), and linearity were systematically evaluated to eliminate cytotoxicity and selectively cultivate non-specific pro-elastogenic effects of JNK-2 gene knockdown. A LIVE/DEAD assay (ThermoFisher) showed PEI-siRNA coupling using 40kDa linear PEI to exhibit <80% viability at N:P molar ratios 10:1 and greater. Gel electrophoresis run to determine presence of secondary bonding between PEI and siRNA showed lack of coupling between 25kDa branched PEI and 12.5nM siRNA. RT-PCR results showed most effective JNK-2 gene knockdown using 12.5nM siRNA in combination with 25kDa linear PEI and 40kDa linear PEI at N:P molar ratios of 5:1 and 2.5:1, respectively. Separately, MPG8-siRNA complexes in varying N:P molar ratios (2.5:1-20:1) were evaluated to elucidate cytotoxicity along with quantification of non-specific elastogenesis and anti-proteolytic effects of JNK-2 gene knockdown. Confocal microscopy was used to visualize MPG8-siRNA complexes with respect to the smooth muscle cell membrane and revealed complex localization inside the membrane but outside the nucleus, enabling cytoplasmic gene silencing. LIVE/DEAD assays (ThermoFisher) revealed viability >80% at all molar ratios studied. RT-PCR was conducted using the MPG8-siRNA complex for JNK-2 and downstream pro-elastogenic and proteolytic genes.
***Results:** Cumulative evaluation of results proved the most effective JNK-2 gene knockdown using PEI and separately, MPG8-siRNA complexes of 12.5nM siRNA at N:P molar rations of 5:1 and 10:1. Reduced MMP-2 and -9 expression was observed with upregulation of elastin and elastin precursor genes (TGF-B1, LOX) in peptide-treated cultures pending results from gel zymography and western blots. Increases were also noted in elastic matrix deposition, expression of proteins involved in elastic matrix homeostasis, elastic fiber density and maturation, and desmosine crosslinking of the elastic matrix.
***Conclusion/Significance:** Our studies indicate that treatment of aneurysmal SMCs with complexes of JNK2 siRNA with linear PEI and MPG8 peptide under certain optimized conditions significantly enhances elastic fiber assembly and crosslinking and inhibits elastic matrix proteolysis, with vital implications to a

future regenerative therapy to arrest or slow AAA growth. Future work will seek to deliver these complexes from surface-functionalized PLGA-based nanoparticles that we have previously developed to provide pro-elastogenic and anti-proteolytic effects to synergistically enhance the outcomes described here.

76 - The Maturation And 3D Organization Of The Novel D-HuSk-hCPC-based Cardiac Bioconstruct Is Improved In Vitro By Mechanical Stimulation

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***Purpose/Objectives:** Myocardial damage caused by ischemic necrosis affects both cellular and extracellular compartment. The extracellular matrix (ECM) makes up most of the non-cellular compartment of living tissue that provides unique combination of biological and mechanical stimuli responsible for cellular behavior. Therefore, any attempt to morphologically and functionally restore the myocardium cannot disregard the significant function of the ECM and can only be accomplished by replacing both compartments. Although the ECM features and composition are site-specific, due to organ donor shortage and to the high incidence of ischemic heart disease the search for alternate biological scaffolds to mimic the myocardial environment is currently a top priority in cardiac tissue engineering. We have recently developed a fully biological scaffold of decellularized human skin capable to support the engraftment, survival and differentiation of human resident cardiac progenitor cells (hCPCs) in static condition [1]. We hypothesize that the intrinsic elasticity of the dermal matrix might be exploited in a cyclic stretch bioreactor to further stimulate the maturation of cardiac bioconstruct *in vitro*.
***Methodology:** To test our hypothesis, we prepared three-dimensional biological scaffolds of decellularized human abdominal skin (d-HuSk), repopulated them with hCPCs and cultured the cellularized scaffold in static conditions for one week to allow cell engraftment and adaptation to the new environment. Then, we transferred bioconstructs to a bioreactor applying a cyclic stretch of 10% strain at a frequency of 1Hz for seven days, and then evaluated the effects of the mechanical stimulation on hCPC engraftment, alignment and differentiation by SEM, histochemistry, immunohistochemistry and gene expression analyses. Bioconstructs cultured in static conditions for two weeks were used as a reference.
***Results:** The histological analysis revealed that hCPCs organized into a structured multilayered tissue on the surface of d-HuSk in both static and dynamic conditions. Intriguingly though, when compared to static controls, bioconstructs cultured in dynamic conditions showed that cyclic stretch greatly promoted hCPC migration towards the inner layers of the dermal matrix. Furthermore, in the cyclically stretched bioconstructs a well-ordered hCPC alignment, mainly along the direction of stretch, resulted apparent. Finally, gene expression profile including genes typical of main cardiac cell lineages, like MEF2C, ACTC1, CX43, TBX-3 and -5, GATA6, ACTA2, ETS1, CD90 and CD105, showed the up-regulation of transcripts for cardiac myocytes, smooth muscle, endothelial and mesenchymal cells in hCPCs cultured on d-HuSk in dynamic conditions, thus providing evidence of further maturation of stretched cardiac

bioconstructs.
***Conclusion/Significance:** Evidence collected thus far supports the hypothesis that d-HuSk might be successfully used as a substitute for cardiac matrix and that the development of a d-HuSk-hCPC-based cardiac bioconstruct is significantly improved under a physiological mechanical stimulation that fosters its maturation *in vitro*.
1. Castaldo C, et al. TISSUE ENGINEERING: Part A, Vol. 23 (Supplement 1): S-67, 2017

77 - Enzymatic Crosslinking Of Dynamic Thiol-norbornene Click Hydrogels.

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***Purpose/Objectives:** Horseradish peroxidase (HRP) has been used extensively for *in situ* crosslinking of macromers containing hydroxyl-phenol groups. The use of HRP on initiating thiol-allylether polymerization has also been reported, yet no prior study has demonstrated enzymatic initiation of thiol-norbornene gelation. The objectives of this study were to exploit HRP on crosslinking of modular thiol-norbornene hydrogels and to demonstrate the usefulness of this system in dynamic 3D cell culture.
***Methodology:** To fabricate HRP-mediated thiol-norbornene hydrogel, macromer poly(ethylene glycol)-octa-norbornene (PEG8NB) was crosslinked with either dithiothreitol (DTT) or bis-cysteine-bearing peptides at a 1:1 thiol-to-norbornene ratio. Low concentrations of HRP (1 U/ml) and H_2O_2 (0.5 mM) were added to the solution, followed by vortexing for ~5 seconds to initiate gelation. Gelation was completed within 5 minutes at room temperature. To stiffen hydrogels using mushroom tyrosinase (MT), PEG8NB hydrogels were crosslinked by tyrosine-containing peptide. Hydrogels were submerged in 1 kU/ml MT for 6 hrs to induce dynamic stiffening. Afterwards, MT was removed via swelling hydrogels in PBS for 24 hrs, followed by rheological measurements of hydrogel shear modulus. Cytocompatibility of the enzymatically crosslinked thiol-norbornene hydrogel was evaluated using murine NIH/3T3 fibroblasts.
***Results:** In this study, we discovered that HRP can generate thiyl radicals needed for initiating thiol-norbornene hydrogelation, which has only been demonstrated previously using photopolymerization. Enzymatic thiol-norbornene gelation not only overcomes light attenuation issue commonly observed in photopolymerized hydrogels, but also preserves modularity of the crosslinking. In particular, we prepared modular hydrogels from two sets of norbornene-modified macromers, including synthetic PEG8NB and biological gelatin-norbornene (GelNB). Bis-cysteine-containing peptides or PEG-tetra-thiol (PEG4SH) were used as crosslinkers for forming enzymatically and orthogonally polymerized hydrogels. For HRP-initiated PEG-peptide hydrogel crosslinking, gelation efficiency was significantly improved via adding tyrosine residues on the peptide crosslinkers. Interestingly, these additional tyrosine residues did not form permanent dityrosine crosslinks following HRP-induced gelation. As a result, they remained available for tyrosinase-mediated secondary crosslinking, which dynamically increases hydrogel stiffness. In addition to material characterizations, we also found that both PEG- and gelatin-based hydrogels provide excellent cytocompatibility for dynamic 3D cell culture.
***Conclusion/Significance:** In summary, we have developed the first orthogonal enzymatic thiol-norbornene click reaction suitable for forming modularly crosslinked hydrogels under ambient conditions. Furthermore, we discovered that HRP can be used to initiate gelation of macromers other than those containing hydroxyl-phenyl groups. Most importantly, the hydrogels can be dynamically stiffened by means of tyrosinase-mediated crosslinking owing to the preservation of tyrosine residues

following the initial thiol-norbornene click gel reaction. The modular and dynamic hydrogels described in this contribution offer researchers an attractive alternative to form modularly crosslink and dynamic hydrogels without the concerns of light attenuation in thick samples or potential cell damage caused by UV light irradiation.

78 - Stem Cell-Derived Extracellular Nanovesicles For Vascular Elastic Matrix Regenerative Repair

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***Purpose/Objectives:** Small abdominal aortic aneurysms (AAA) are characterized by slow growing, localized expansions (<5.5 cm maximal diameter) of the abdominal aorta due to chronic proteolytic disruption of the structural extracellular matrix (ECM; collagen, elastin) within the aortic wall by matrix metalloproteases (MMPs). There are no non-surgical treatments to reverse AAA pathophysiology including enhancing regenerative repair of proteolytically-disrupted elastic fibers which does not occur naturally, but is critical to slow or arrest AAA growth. Bone marrow derived mesenchymal stem cells (BM-MSCs) have emerged as a promising stem cell (SC) type in regenerative therapeutics and their reparative effects are mediated largely through paracrine signaling factors. Non-cell based treatment utilizing the paracrine signaling pathway appears to be more clinically relevant and extracellular vesicles (EVs) seems to be a critical component in facilitating paracrine signaling associated with SC therapy. Our study seeks to understand the role of these EVs on elastic matrix regenerative repair in AAAs.
***Methodology:** EVs were isolated from pooled adult human BM-MSCs, cultured in exosome-free FBS, by successive differential centrifugation technique. The EV pellet reconstituted in PBS was further purified using size exclusion chromatography using qEV size exclusion columns and stored at -20ºC, until further use. EV sizing was performed using nanoparticle tracking analysis (NTA) and dynamic light scattering., and their structure was characterized using TEM. Western blot analysis on the EVs were performed using mouse monoclonal primary antibodies against typical exosomal surface markers TSG101, tetraspanins, (CD63, CD9). Rat aneurysmal SMCs were isolated from elastase-infusion induced AAAs in adult male Sprague-Dawley rats. The cells (P2-5) were activated by IL1β and TNF-α (10 ng/ml each; 24 h) to stimulate the diseased phenotype and treated with the EVs (10-50 ng/ml), were untreated (control) or treated with MSC-conditioned medium (CM). Cells were analyzed by RT-PCR for gene expression of elastolytic MMPs2, 9, TGF-β, key proteins involved in elastic fiber homeostasis, westerns for expression of the corresponding proteins, and gel zymography for MMP activities. A protein array (Raybiotech) was used to detect differential expression of key biologic factors and cytokines in treated and control cultures. Longer term (21 day) cultures were assayed for elastic matrix (Fastin assay), desmosine crosslinks (ELISA), and imaged for elastic fiber formation/components (IF) and ultrastructure (TEM).
***Results:** BM-MSC-derived EVs, but not CCM significantly increased gene expression for proteins involved in elastic matrix homeostasis including fibrillin-1, fibulin 4, LOX/LOXL1 (elastin crosslinking enzymes) and attenuated gene expression for elastolytic MMP2 versus treatment controls. The EVs significantly attenuated synthesis and enzyme activity of MMP-2, increased the protein synthesis of their naturally occurring inhibitors, TIMPs 2 and 4, and stimulated LOX expression and activity. The EVs, but not CCM also suppressed inflammatory cytokines and biological factors involved in TNF-α-mediated pathological signaling in aneurysmal SMCs. Results from ongoing experiments indicate significant increases in crosslinked elastic matrix assembly and mature fiber formation in EV-treated cultures vs. treatment controls and CCM-treated cultures.

***Conclusion/Significance:** Our results suggest that EVs derived from BM-MSCs have significant potential as treatments to augment elastic matrix regenerative repair towards arresting or slowing AAA growth.

358 - Interactome-Based Biomaterial Accelerant Substrate For Tissue Repair

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Introduction: Tissue engineering scaffolds can be created from various sources that influence how they interact within their implanted environment. We sought to develop scaffolds including tissue proteins that are non-permanent, space preserving, and enhance cellular incorporation. **Methods:** Scaffolds were synthesized from musculoskeletal tissue. Tissue was dissociated by non-micronizing crushing, or physical shearing to separate cellular and intercellular interfaces. Particulate sizes were $5mm^3$-$1cm^3$. Dissociated tissue was combined with biocompatible solution in a range of concentrations and volume/volume ratios and incubated. The resulting compositions were centrifuged, and remaining tissues were removed. The compositions were transferred to open face silicone-ready release-coated containers to obtain scaffolds of desired shapes and sizes. 3D-geometry, mechanical properties, solubility, and biodegradation products and kinetics of substrates were assessed. Scaffold biocompatibility was tested *in vitro* with adherent cell lines, such as dermal fibroblasts, C2C12 myoblasts, MG-63 cells and non-adherent cells such as HEK293. **Results:** Scaffolds persisted and released non-toxic proteins for up to a month, which could be modified as desired. They were conformable to complex 3D-geometry and had architecture and porosity resembling native extracellular matrices. Scaffolds possessed low immunogenicity, high biocompatibility and cytocompatibility and induced high cell adhesion and proliferation. **Conclusions:** Novel scaffolds were developed from multi-cellular tissues and interactomes. Substrates were customizable to achieve various physical forms and functions and are biocompatible with pro-adhesion, migratory, and proliferative effects in vitro. Additional *in vivo* testing is planned to understand how these substrates may enhance tissue regeneration.

80 - Development Of A New Biomaterial Through The Coaxial Eletctrospinning Technique For Use As A Skin Substitute

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***Purpose/Objectives: Purpose**: The replacement for extensive areas of skin loss such as those observed in major trauma or burns is a major challenge for tissue engineering because the currently available treatments are unable to prevent scar formation or promote skin regeneration. Currently available options are very expensive, which restricts their use to a small group of patients. Objectives: Develop scaffolds made of PDLLA functionalized with EGF and collagen type 1 and to analyze their physicochemical properties and compatibility with immortalized keratinocytes with view to developing a cutaneous substitute.
***Methodology:** Methods: For this proposal, scaffolds were constructed by the coaxial electrospinning technique and divided into 3 groups: 1) PDLLA, 2) PDLLA/EGF (coaxial fiber with EGF/albumin solution core) and 3) PDLLA/Collagen (a PDLLA/EGF scaffold with type 1 collagen coating). The matrices developed were submitted to physical-chemical and biological analyses with the culture of immortalized

keratinocytes in order to test their properties.
***Results:** Results: The scaffolds were constructed by fibers without beads, randomly distributed and with a mean diameter for group 1 of 1.39 ± 0.38 µm, 0.97 ± 0.430 µm for group 2 and 1.12 ± 0.49 µm for group 3. The mathematical determination of pore diameter based on fiber diameter was 8.34 µm, 5.82 µm and 6.72 µm for groups 1, 2 and 3 respectively. Confocal microscopy was able to demonstrate the formation of core-shell fibers and the FTIR technique confirmed the presence of type 1 collagen in the group 3 fibers. Due to this, the fibers showed an increase in their hidrophilicity, as evidenced by the contact angle analysis. The matrices had a slow degradation rate throughout the study and the release of EGF incorporated into the fiber core presented an initial burst followed by continuous release over 14 days. Group 3 presented higher cell adhesion in relation to the other groups and cells which were metabolically more active in relation to the other groups after 1 and 7 days after seeding. The LDH dosage showed low toxicity in all the analyzed groups.
***Conclusion/Significance: Conclusion**: The developed scaffolds presented good properties for use in studies of cutaneous substitutes, presenting similar fiber and pore diameters with those already described in the literature. Biological analyses via cell adhesion and viability have suggested increased cell affinity for scaffolds functionalized with EGF and type 1 collagen while cytotoxicity assays suggest them to be safe for supporting cell culture. Finally, the developed core-shell scaffolds proved to be effective in in vitro studies. New studies are currently being performed to evaluate the full potential of this new biomaterial, which could become an option for skin substitute studies.Financial support: FINEP, CNPqand Stem Cell Research Institute.

Session Number: 18
Respiratory, Urologic and Gastrointestinal Engineering
Wednesday, December 4, 2019, 1:00 PM - 2:30 PM

82 - Reinforced Electrospun Trachea Patch Containing Cell Adhesion Or Antimicrobial Compounds For In Vivo Trachea Repair

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***Purpose/Objectives:** A 12-week *in vivo* study with 24 rabbits was successfully completed to compare 4 different formulations to repair an induced defect in the trachea. Difficulty breathing due to tracheal stenosis (i.e., narrowing of the airway) diminishes the quality of life and can be potentially life-threatening. Tracheal stenosis can be caused by congenital anomalies, external trauma, infection, intubation-related injury, and tumors. Common treatment methods for tracheal stenosis requiring surgical intervention include end-to-end anastomosis, slide tracheoplasty and/or laryngotracheal reconstruction. Although the current methods have demonstrated promise for treatment of tracheal stenosis, a clear need exists for a treatment that can hold the trachea open after the stenosed region has been surgically removed and that can support healing without the need to harvest autologous tissue from the patient. Following up on an encouraging previous study in a larger animal model (sheep), we identified key challenges that led to comparing potential solutions in the rabbit model.
***Methodology:** The current study evaluated the use of electrospun nanofiber scaffolds encapsulating 3D-printed polycaprolactone (PCL) rings to patch induced defects in rabbit tracheas. The nanofibers were composed of a blend of PCL and polylactide-co-caprolactone (PLCL). The cell adhesion peptide, RGD, or antimicrobial compound, ceragenin-131 (CSA) was added to the electrospinning solution producing fibers with cell adhesion or antimicrobial properties. Blank PCL/PLCL and PCL were employed as control groups. Electrospun patches were evaluated in a rabbit tracheal defect model after 12 weeks using micro-computed tomography and histology.
***Results:** All Trachea patches re-epithelialized on the luminal side of the defect after 12 weeks. No significant difference in lumen volume was observed for any of the PCL/PLCL patches compared to the uninjured positive control. Only the RGD group (14.2 mm^2) did not lead to a significant decrease in the minimum cross-sectional area compared to the uninjured positive control (21.3 mm^2).
***Conclusion/Significance:** We are pleased to report adequate tissue in-growth into the patches and minimal tissue overgrowth was observed inside the patch material. Areas of future investigation include tuning the material degradation time to balance cell adhesion and structural integrity.

83 - Purification Of Anti-fibrotic Compounds From Ecklonia Cava And Application Of Pcl/phlorotannin Endotracheal Tube For Anti-stenosis In Rabbit Model

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***Purpose/Objectives:** Prolonged endotracheal intubation is the most common cause of tracheal stenosis, which may lead to serious airway obstruction. Development of an endotracheal tube coated with biomaterials that exhibit anti-inflammatory or anti-fibrogenic effects may prevent tracheal stenosis. This study demonstrates that an endotracheal tube coated with phlorotannin, which is present in extracts of the brown alga *Ecklonia cava*, can prevent tracheal stenosis in a rabbit model.
***Methodology:** Phlorotannins were isolated active compound from *E. cava* by organic solvent fractionation, open column system and liquid chromatography system. Post consecutive purification, a potent bioactive compound was identified phlorofucofuroeckol and this compound was determined anti-fibrotic activities and signaling pathways by MTT and western blotting. After fabrication of endotracheal tube coated with phlorotannins, we evaluated mechanical properties and in vivo experiment in a rabbit disease model.
***Results:** An *in vitro* study shows that phlrofucofuroeckol from phlorotannin inhibits the proliferation of human tracheal fibroblasts treated with transforming growth factor β1. Phlorotannin-coated endotracheal tubes show a steady release of phlorotannin for up to 7 days, and removal of the tube 1 week after insertion reveals a reduction in both fibrogenesis and thickening of tracheal submucosa. Western blot analysis of tracheal tissues after removal of the phlorotannin-coated tube shows decreased protein expression levels of phenotypic markers of fibrosis such as collagen type I and α-smooth muscle actin.
***Conclusion/Significance:** The ability of phlorotannin-coated endotracheal tube to prevent tracheal stenosis caused by endotracheal intubation indicates that phlorotannin may be considered as a candidate biomaterial for coating the cuff of endotracheal tubes to prevent tracheal stenosis.

84 - A Simple Strategy To Develop Compliant Collagen-derived Materials For Urinary Tissue Engineering Applications

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***Purpose/Objectives:** Collagen-based materials, including decellularized extracellular matrices are one of the most suitable natural biomaterials for constructing tissue-engineering scaffolds. Despite their biocompositional similarities to physiological tissues, collagen-based scaffolds lack host specific and matching mechanical properties. While it is possible to enhance their stiffness by crosslinking, it often compromises their abilities to expand or strain under minimal stress, i.e. compliance (inverse of stiffness). As a result, the success of these scaffolds remains elusive in preclinical and clinical settings. Being an important design criterion in soft tissue engineering, e.g. in urinary bladder reconstruction, development of a compliant yet surgically durable collagen-based scaffold is long sought. Since, urinary bladder stretches more than 100% of its initial dimensions, we aimed to create collagen-based scaffolds that can match the compliance of urinary bladder. Here, we report our findings on development of a simple, inexpensive, elastin-free material composition that results in elastomeric scaffolds that are hyperextensible, soft yet tough and suturable even without external crosslinking.

***Methodology:** Our approach is based on a simple, and inexpensive strategy of modifying collagen-based or -rich materials with linear aliphatic chains (size: C9-C18). The percentage conversion was determined by ^{1}H-NMR, and the percentages of free amine groups in collagen type-I scaffolds were determined using an assay of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Microstructure was studied by transmission electron microscopy (TEM). To study the mechanical properties, in terms of tensile strength, elongation, suture retention strength, and hysteresis, samples were tested with a 5N load cell in the MTS Criterion™ Model 43. Rabbit urinary bladder and ureters were taken as controls. All in vivo experiments were performed in accordance to an approved JHU ACUC protocol, in a rat bladder augmentation model. After 4 weeks grafts were harvested and immunohistostaining (IHC) was performed for urothelium formation (cytokeratin 20), smooth muscle bundles (Vimentin), blood vessels (CD31+). Statistical analysis with one-way ANOVA method was performed to compare samples for their properties and performance.
***Results:** On modification with dodecenylsuccinic anhydride (size: C12, DDSA) collagen type-I scaffold stretched up to 450% of its initial length compared to only ~20% for collagen-control within the applied tensile stress of 0.2 MPa while having a suture retention strength value more than 50 gm-force. The resultant material was non-cytotoxic and supported human bladder smooth muscle cell growth. On DDSA-modification, collagen rich-decellularized pericardium could elongate up to 130% from its initial limit of 30%. DDSA-modified scaffolds further supported the regeneration and formation of urinary bladder urothelium, lamina propria, muscle layers, and blood vessels when implanted in a rat urinary bladder augmentation model.
***Conclusion/Significance:** We successfully developed a simple strategy to modify collagen-based scaffolds with DDSA (C-12 chains), which resulted in compliant and rubbery-like materials with high elongation at break and suture strength values. The resultant materials were non-cytotoxic and allowed cell growth. DDSA-modified decellularized pericardium, when evaluated in a rat bladder augmentation model, supported the regeneration and formation of muscle layers, lamina propria, hyperplastic urothelium and blood vessels, possibly due to the matching mechanical and biological properties of the graft and host urinary tissue.

85 - Remodeling Of The Tumor Microenvironment Architecture And Its Effect On Cancer Progression In 3d Organoids

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***Purpose/Objectives:** A tumor mass does not consist of merely cancerous cells but also the tightly surrounding tumor microenvironment (TME), made up of both the non-cellular extracellular matrix (ECM) and various types of stromal cells. Though chemotherapy resistance is often attributed to tumor heterogeneity, cancer progression and malignancy is also profoundly influenced by its interaction with the surrounding TME. Residing hepatic stellate cells (HSC) in the liver are considered a major component of a liver TME because they transdifferentiate into highly proliferative and motile myofibroblasts that are associated with desmoplasia and tumor growth. HSC activation can occur in response to various cytokines, such as TGF-ß, from either a neighboring cell in response to an injury or from cancerous cells within the tissue. Myofibroblastic HSCs are responsible for the major changes that occur in the liver ECM associated with an aggressive TME via two mechanisms, the deposition of new ECM components and the physical remodeling of the preexisting ECM. A healthy, balanced TME is thought to result in a less

aggressive, more vulnerable cancer while a more remodeled, unbalanced TME that consists of activated HSCs result in a more aggressive and less responsive cancer to chemotherapy.
***Methodology:** In order to test this relationship between the TME architecture and cancer cell malignancy as well as chemoresistance, we have developed a 3-dimensional organoid model that can assess the effect of a remodeled TME on a cancer spheroid. Our lab has published the general organoid system that we utilize here; in short, we embedded a colorectal cancer cell line (HCT116) spheroid into an organoid consisting of HSCs (LX2) and collagen type 1. We then activated the LX2s to become myofibroblastic with exogenous TGF-ß causing excessive deposition and remodeling of collagen around the cancer spheroid. The remodeled TME that resulted from TGF-ß-mediated activation was quantified by analyzing the stiffness of the organoid that surrounds the spheroid as well as the in-depth, computational image analysis of the collagen fibers via CT-FIRE.
***Results:** Our compression data, measured rheologically, revealed TGF-ß-mediated activation of the HSCs resulted in a stiffening of the organoid around the tumor spheroid. Results from the Picrosirus Red staining revealed the more remodeled organoid, containing activated HSCs, produced longer, wider, and more aligned characteristics around the spheroid. Our immunohistochemistry data indicated that the remodeled TME inhibits colon cancer cell growth and migration within the organoid.
***Conclusion/Significance:** The inhibited proliferation, in combination with the physical collagen barrier in the activated TME, is thought to have a large effect on the ability for chemotherapy to successfully penetrate and induce cytotoxic effects on the cancer cells. Collectively, the ability to manipulate the tumor organoids' TME towards fibrosis enables us to predict chemotherapy response based on TME properties and not solely cancer cells in isolation.

Session Number: 19
Translating TERM to the Market
Wednesday, December 4, 2019, 1:00 PM - 2:30 PM

86 - Production Assistance For Cellular Therapies (PACT) Program: Scope And Services For The Academic And Industry Applicants

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***Purpose/Objectives:** Successful cell therapies for clinical administration require transformation of laboratory-based manufacturing and analytical techniques into current Good Manufacturing Practice (cGMP) cell production processing to fulfill federal regulatory cell product production. The Production Assistance for Cellular Therapies (PACT) program, now in its third 5-year cycle (PACT 3), is a federally funded program of The National Heart, Lung, and Blood Institute (NHLBI) to provide support of translational and clinical manufacture of cellular therapy products, and regulatory expertise to address all aspects of the translational, pre-clinical and early phase clinical development process through five Cell Processing Facilities and a Coordinating Center. The main objective of the program is to promote the advancement of research in the use of cellular therapy in the regeneration of damaged/diseased tissues, organs, and biologic systems, and targeted treatments for serious diseases without effective therapies in support of the NHLBI's mission.
***Methodology:** PACT reviews requests for services to support translational (Pre-IND) and (IND) clinical cell production with cell therapy manufacturing through scale up, validation and clinical manufacturing. Furthermore, the program offers regulatory consulting services for meritorious cell therapy applications providing gap analysis reports for pre-clinical and clinical drug development phases, INTERACT, pre-IND and IND meeting support and communication assistance with the FDA.
***Results:** PACT reviews requests for services to support translational (Pre-IND) and (IND) clinical cell production with cell therapy manufacturing through scale up, validation and clinical manufacturing. Furthermore, the program offers regulatory consulting services for meritorious cell therapy applications providing gap analysis reports for pre-clinical and clinical drug development phases, INTERACT, pre-IND and IND meeting support and communication assistance with the FDA.
***Conclusion/Significance:** Early interaction with FDA ensures shared expectations for adequacy of manufacturing, non-clinical, clinical information and regulatory submission approach in support of proposed investigational studies. This approach minimizes the risk of an untoward regulatory decision that would impact the study timeline. Investigators can apply for services by completing a Request for Services Application (RSA). This presentation highlights the nature, scope, objectives and portfolio of services this unique federal government support program offers to the academic and industry community in the cell therapy space.

87 - Commercializing Cellular Therapy

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***Purpose/Objectives:** Cell therapy manufacturing performed offsite from the collection location requires strict control in current Good Manufacturing Practice (cGMP) facilities. Designing a facility or finding a contract manufacturer to meet the manufacturing needs is essential for the success of a cellular therapy product.

***Methodology:**

The facility includes the manufacturing space, the storage warehouse for raw and finished product and laboratory areas. Cellular therapy manufacturing facilities must be designed for aseptic processing. Development of fully enclosed manufacturing equipment and built-in controls for in-process testing is optimal for cellular processing. Process flow needs to be considered from clinical to commercial manufacturing using a Quality by Design model to validate the cGMP manufacturing process. This involves defining the critical process parameters, process parameters and in-process tests. This information should originate from development studies and be used to define a Design Space for the validation of the process. These parameters and tests should be continuously monitored as part of an on-going process validation. This can lead to regulatory flexibility to operate in the defined Design Space and to reduced testing as a result of quality being built into the design of the manufacturing process.
A disposable manufacturing model is ideal cellular therapy manufacturing activities. This reducing cleaning verification and validation activities. Cleaning validations can be optimized by using a matrix model for products and equipment. Other important validation and verification activities include: qualifying the manufacturing facilities and equipment, developing facility cleaning validations, developing analytical testing and method validations, developing environmental monitoring programs, developing batch records and developing an on-going process validation plan. These validation and verification cGMP requirements should be met with the incorporation of Data Integrity best practices for manufacturing and the laboratory using Attributable, Legible, Contemporaneous, Original, Accurate (ALCOA) documentation, sterile processing best practices and chain of custody best practices.

***Results:** Incorporating cGMP into the manufacturing environment is required by regulatory authorities to ensure safety and efficacy of cellular therapy products.

***Conclusion/Significance:** The process by which to take early concept discovery ideas through to pre-clinical, clinical and commercial manufacturing including the application process for Initial Targeted Engagement for Regulatory Advice on CBER products (INTERACT), Investigational New Drug (IND) and Biological License Application (BLA) will be discussed.

Session Number: 21
Immunomodulatory Strategies in Tissue Engineering and Regenerative Medicine
Wednesday, December 4, 2019, 3:00 PM - 4:30 PM

238 - Localized Immune Modulation Of Pancreatic Islet Allografts Via Synthetic Biomaterials

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***Purpose/Objectives:** Type 1 diabetes (T1D) is a chronic condition in which patients produce little to no insulin due to the autoimmune progressive loss of beta cells. Whereas the disease can be managed via external insulin delivery, patients still experience a high incidence of mortality and chronic debilitating comorbidities. Islet transplantation has emerged as a potential strategy to restore a patient's glycemic control, yet long-term rejection and the need for chronic immunosuppression present a major barrier. Herein, we engineered a synthetic material to provide biological cues locally and in a controlled manner to enhance vascularization and promote long-term islet graft survival. Engineered platform was tested in a chemically induced murine diabetic model, and in an autoimmune model of diabetes.
***Methodology:** Hydrogel particles (microgels, 200 µm diameter) were fabricated as previously described [1], and functionalized with a chimeric streptavidin PD-L1 (SA-PD-L1) for delivery with allogeneic islets from Balb/c mice into chemically induced diabetic C57BL/6 mice. Animals were monitored for blood glucose, intraperitoneal glucose tolerance test (IPGTT), and immune profiling.
***Results:** Transplantation of islets from Balb/c mice into chemically induced diabetic C57BL/6 mice using VEGF-delivering hydrogels under the cover of low dose (0.2 mg/kg) rapamycin (15 days) promoted short-term restoration of normoglycemia after transplantation. Notably, the presence of our engineered immunomodulatory platform extended graft survival for treatment group with 52% of the animals remaining euglycemic for > 100 days. Conversely, 90% of control animals rejected the graft during this period. Immune profiling of grafts containing immunomodulatory biomaterial demonstrated an increase in mRNA of regulatory markers FOXP3 and GATA3, compare to unconjugated microgels alone. This suggests this platform has the ability to increase the recruitment of potential T regulatory cells to the transplant site.
***Conclusion/Significance:** These studies establish synthetic materials to deliver immunomodulatory cues to islet transplants without hindering engraftment. As such, this approach has the potential to not only improve current strategies for islet transplantation, but also mitigate the need for chronic immunosuppression with a significant impact on clinical islet transplantation.The authors acknowledge JDRF and NIH for support. **References:** [1] Headen, D*, et al., Local immunomodulation with SA-FasL-engineered biomaterials achieves allogeneic islet graft acceptance. *Nature materials*. 2018; 17 pp. 732-739.

88 - Immunoengineered Hydrogel Platform To Induce Tolerance In Type 1 Diabetes

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***Purpose/Objectives:** Autoimmune, type I diabetes (T1D) is characterized by T cell-mediated destruction of pancreatic beta cells resulting in insulin deficiency and hyperglycemia. Defects in the regulation of tolerance lead to escape of beta-cell autoreactive T cells that migrate to the pancreas and destroy the beta cells. In cancer, tumors escape the destruction by the immune system by secreting chemokine CCL21 and establishing tolerance to tumor antigens by formation of tertiary lymphoid organs (TLOs) that mimic the tolerogenic lymph node microenvironment. Recently, we showed in a transgenic non-obese diabetic (*Ins2-CCL21 NOD*) mouse model for T1D that secretion of CCL21 by beta cells promoted formation of TLOs and protected from T1D. Moreover, transplantation of Ins2-CCL21 islets in immunocompromised mice delayed disease onset after adoptive transfer of diabetogenic splenocytes. Additionally, prevention was associated with regulatory TLO formation. Hence, local CCL21 induces TLOs and has a systemic, protective effect against T1D. Here, we aim to design an immunoengineered platform for local delivery of CCL21 and beta-cell autoantigens to form TLOs and induce systemic, antigen-specific tolerance in T1D.
***Methodology:** To tether CCL21 protein and beta-cell antigenic peptides to fibrin gels, recombinant Tg-CCL21, Tg-INSB15-23, and Tg-BDC2.5 were engineered to contain a fibrin-binding site (transglutaminase substrate of factor XIII, Tg) and a plasmin cleavable linker upstream their protein/peptide sequence. Tg-CCL21 and peptides were characterized through ELISA and Western Blot. Fibrin gels (8mg/ml fibrinogen, 2U/ml thrombin, 8U/ml factor XIII, 2.5mM HEPES, 17ug/ml aprotinin) containing CCL21 or Tg-CCL21 (500nM), or empty gels were implanted subcutaneously in the backs of pre-diabetic NOD mice. Implants were harvested 21days after treatment, fixed in formalin and stained with stromal cell (gp38, α-SMA, Lyve-1) or lymphocyte (CD3, B220) antibodies to assess TLO formation and determine functionality of Tg-CCL21. Release kinetics and functionality of Tg-INS and Tg-BDC2.5 were assessed *in vitro* by transgenic proliferation assays. Briefly, fibrin gels (containing 80ug/ml Tg-INS or 10ug/ml Tg-BDC2.5) were degraded using 0.01U/ml plasmin-enriched media for 1, 3, 7 days. Gel supernatants were collected and used to stimulate CellTrace-labeled splenocytes. Stimulation and proliferation was determined by flow cytometry.
***Results:** Fibrin gels degraded by day 21, while mice implanted with CCL21-gels revealed formation of fibroblastic reticular cell-like ($gp38^{+}Lyve\text{-}1^{-}$) stromal network in the skin. Hence, CCL21 delivery through hydrogels, induced TLO formation near the implant. Fibrin-tethered, Tg-BDC2.5 was released from gels within 3 days and caused robust stimulation/proliferation of BDC2.5 splenocytes. Release of Tg-INS did not yield significant proliferation, suggesting that the insulin peptide did not get cleaved from the gels or that the released peptide was not functional.
***Conclusion/Significance:** Local release of CCL21 by implanted fibrin gels in prediabetic NOD mice, promoted formation of TLOs subcutaneously, reminiscent of CCL21-induced TLOs in tumors and pancreata of Ins2-CCL21 NOD mice. Future studies will correlate CCL21 and antigen release to TLO formation in fibrin, and synthetic PEG gels and will investigate their ability to regulate autoreactive T cells in NOD mice.

91 - Matrix Bound Nanovesicles As A Novel Therapeutic Option For Treating Rheumatoid Arthritis

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***Purpose/Objectives:** Rheumatoid Arthritis (RA) is an autoimmune disease characterized by chronic inflammation and progressive destruction of synovial joints that affects approximately 1.3 million

Americans and 1% of the world population. Synovial infiltration of pro-inflammatory immune cells such as macrophages and lymphocytes release cytokines, autoantibodies, and matrix metalloproteinases that result in bone and cartilage destruction. Specifically, macrophage phenotype polarization is implicated as a causative factor in RA. An M1-like, pro-inflammatory macrophage phenotype is found in RA disease flare-ups and the M2-like, anti-inflammatory phenotype is found in RA disease remission. Macrophage phenotype switching, specifically an appropriately timed M1-like to M2-like macrophage transition, can be induced with acellular biologic scaffold materials composed of mammalian extracellular matrix (ECM). Furthermore, the effects of ECM bioscaffolds can be largely recapitulated by matrix bound nanovesicles (MBV) embedded within the ECM bioscaffold. MBV are enriched in the known anti-inflammatory lipids Lipoxin A4 and Resolvin D1 as well as anti-inflammatory miRNA cargo. It is hypothesized that MBV pharmacotherapy mitigates RA disease through a combination of anti-inflammatory lipids and miRNA that promotes a transition from the pro-inflammatory, M1-like activation state of macrophages to an anti-inflammatory, M2-like activation state.
***Methodology:** To examine the efficacy of MBV therapy for the treatment of RA, MBV were delivered intravenously (i.v.) or peri-articularly (p.a.) to 8-week-old Sprague Dawley rats stimulated with pristane (2,6,10,14-Tetramethylpentadecane) to induce inflammatory arthritis. MBV administration was compared to intra-peritoneal (i.p.) MTX to determine the relative efficacy to the gold standard of care.
***Results:** Disease severity peaked 17 days following the administration of pristane with a mean arthritis score of 21 in the positive disease control group and a mean arthritis score of 2.5 in the negative no disease control group (18.5 difference; $p<0.05$). Relative to the positive disease control group, i.p. MTX reduced arthritis score by 12.5 ($p<0.05$), i.v. MBV reduced arthritis score by 13.5 ($p<0.05$), and p.a. MBV reduced arthritis score by 16.33 ($p<0.05$). There was no significant difference observed among i.p. MTX, i.v. MBV, and p.a. MBV ($p>0.05$).
***Conclusion/Significance:** These results suggest that systemic and periarticular administration of MBV are equally efficacious to MTX for the resolution of an acute RA flare-up and could function as a novel, non-toxic pharmacotherapy option for the treatment of RA. The polarization of pro-inflammatory synovial to an anti-inflammatory environment might not only address acute inflammatory mechanisms responsible for disease flare-up but may also promote long-term resolution of disease symptoms by modulation the function of the joint and synovial parenchyma. Unlike current therapeutics which address aberrant inflammation by systemic immunosuppression, MBV provides a novel therapeutic option directed at modulation and remodeling of the host immune system without compromising immunocompetency.

90 - Tissue-engineered Stromal Reticulum To Study Lymph Node Fibroblastic Reticular Cell Networks In Autoimmune Diabetes

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***Purpose/Objectives:** Fibroblastic Reticular Cells (FRCs) build tensile interconnected networks in the lymph node (LN). FRCs control expansion/contraction of the LN and express molecules that are critical for recruitment of T cells to LNs. Importantly, FRCs maintain peripheral tolerance towards self-antigens to prevent autoimmune diseases like Type 1 Diabetes (T1D) by presenting self-antigens in a tolerogenic

manner that result in the deletion of autoreactive T cells. In preclinical models of T1D and in human T1D there is evidence of decreased expression of T1D autoantigens in LNs. Whether decreased FRC-T cell interactions leads to defective deletion of autoreactive T cells in LNs is not known. Using collagen scaffolds and murine LN-derived FRCs, we developed a tissue-engineered FRC reticula where FRC-T cell interactions and FRC abundance can be adjusted to determine whether FRC reticula properties affect FRC-T cell interactions. This work will identify novel targets for T1D treatment.
***Methodology:** LNs were obtained from low-T1D risk 4-wk-old and high-T1D-risk 12-wk-old non-obese diabetic (NOD) mice and age-matched B6 mice. Formalin-fixed, paraffin-embedded LN sections were stained for FRC markers (αSMA and gp38). LNs were enzymatically digested and FRCs were obtained by FACS sorting live $CD45^-CD31^-gp38^+$ cells. After expansion in tissue culture plates, FRCs were harvested and seeded on collagen-based scaffolds - commercially available sponges or 2% collagen gels - to determine their ability of forming *in vivo*-like reticular networks. Gp38 expression on FRCs was assessed before and after culture in 3D scaffolds by flow cytometry. 24hr after seeding, scaffolds were fixed and stained with Phalloidin (F-actin) and DAPI. Z-stack confocal images of LN sections and engineered FRC reticula were analyzed using ImageJ for FRC reticular pore diameter quantification. FRC contractility was measured by quantifying the diameter reduction of FRC-seeded collagen disks 48 hours after fabrication. FRC-T cell interactions was quantified by measuring proliferation of antigen-specific T cells by flow cytometry (dilution of cell proliferation dye) and by real-time confocal imaging using GFP^+ FRC reticula and violet-labeled T cells.
***Results:** Average pore size of LN FRC reticula of 4-wk-old B6 mice (5.42 ± 1.99 μm) was smaller than age-matched NOD mice (7.03 ± 2.58 μm, $p < 0.0001$). FRCs from B6 mice displayed higher contractility (40%) than FRCs from age-matched NOD mice (10%). Isolated FRCs created reticular networks on both collagen scaffolds analyzed, but the reticula pore sizes were larger (collagen sponges: 42.7 ± 16.3; collagen gels: 22.6 ± 13.9) than LN reticula. FRC culture in collagen scaffolds increased their gp38 expression (MFI, 2D: 121720 ± 6432 ; 3D: 271299 ± 8282).
***Conclusion/Significance:** FRCs from high T1D-risk mice form looser networks in LNs and concordantly display lower contractility than healthy B6 mice, which could decrease FRC interaction with autoreactive T cells. We were able to engineer FRC reticula that recapitulate FRC organization in LNs and preserve their phenotype. Importantly, we show that we can use our engineered FRC reticula to study FRC-T cell interactions, which is critical to determine how FRC properties affect peripheral tolerance in T1D to prevent the disease.

92 - In Vivo Tissue Engineered Diagnostic Sites For Prognosis And Treatment Monitoring In Autoimmunity

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***Purpose/Objectives:** Autoimmune diseases are caused by a breakdown in self-tolerance and are often characterized by relapses and remissions which has made the ability to monitor disease progression and treatment responsiveness using biomarkers an elusive goal of the field. We hypothesized that a site could be engineered and molecularly analyzed to determine disease progression.
***Methodology:** In this study we investigated poly(ε-caprolactone) (PCL) scaffolds as an implantable diagnostic site for autoimmunity, using a mouse model of MS (experimental autoimmune encephalomyelitis [EAE]) and type 1 diabetes (T1D). PCL scaffolds were implanted into mice before

induction of disease. Scaffolds were excised and gene expression analyzed via a TaqMan OpenArray Panel of 632 genes focused on mouse inflammation. Machine learning and dimensionality reduction approaches, including random forest (RF) ensemble classification and singular value decomposition (SVD), were applied to develop a signature of genes to create a multivariate signature capable of determining disease status. Experiments were conducted in both EAE and T1D to develop separate signatures for each disease.
***Results:** Data indicate many genes with differential expression in health versus disease (&gt130 genes with a significant change in EAE, 86 in T1D). For example, Ptgs2, is expressed in scaffolds from control animals at approximately three times the levels of asymptomatic EAE animals (pre-symptomatic and remission), and at 16 times the levels in symptomatic animals (peak disease and relapse). These results suggest that the scaffolds may serve as a diagnostic site that may provide prognostic information that is otherwise unattainable, and importantly, that this information can be obtained prior to the onset of clinical disease. To date, we have demonstrated that the implantable niche can predict disease onset (EAE and T1D), relapse, and response to therapy (EAE).
***Conclusion/Significance:** Synthetic diagnostic sites add to the toolbox of techniques for investigating MS and T1D because they provide an *in vivo* microphysiological model of an inflammatory site that can be molecularly dissected, which is simply not possible in the CNS or pancreas. Furthermore, this tool permits longitudinal sampling of an inflammatory site from a single animal (i.e. prior to and after therapeutic administration) that does not require a terminal procedure. This technology could provide a platform for predictive disease and treatment modeling in humans and could potentially be extended to monitor and prevent autoimmune disease onset in at-risk populations.

93 - Treatment Of Systemic Immune Dysregulation Following Severe Trauma With Synthetic Nanoparticle Antibodies

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***Purpose/Objectives:** Despite advancements in trauma care, severe musculoskeletal trauma remains a clinical challenge with 5-10% of fracture patients experiencing complications with healing. Previous work to improve bone regeneration has largely focused on the *local* environment; however, more recently, the *systemic* environment has been implicated in poor patient outcomes. In particular, systemic immune dysregulation and immunosuppression has been associated with inadequate responses to regenerative treatment strategies, and it is characterized by increased levels of immunosuppressive cells and cytokines, such as myeloid-derived suppressor cells (MDSCs), and decreased levels of immune effector cells, such as T cells. Therefore, in this work, we aim to evaluate a systemic immunomodulatory strategy with the objective of restoring immune homeostasis as a promising new method to improve patient responses and outcomes following trauma. In particular, we will evaluate synthetic nanoparticle antibodies (SNAbs) that represent a fully synthetic multivalent version of a monoclonal antibody to target and deplete MDSCs, a hallmark of systemic immune dysregulation, via antibody-dependent immune responses.
***Methodology:** Solid-phase chemistry was used to fabricate the SNAbs resulting in 30nm gold Janus nanoparticles with a streptavidin-displaying surface and a thiol-displaying surface that are then conjugated with biotinylated MDSC-targeting peptides and maleimide-terminated Fc-mimicking peptides. For *in vivo* studies, 6 13-week old SD rats received an 8mm femoral defect with an 8mm-

diameter volumetric muscle loss that were left untreated. Defects were treated at 8 weeks post-surgery with 2.5ug of BMP-2 delivered in an alginate system. Half of the animals also received systemic injections of the SNAbs with naïve animals as controls. Circulating immune cells and bone regeneration were quantified longitudinally via flow cytometry and uCT. All procedures were approved by the Georgia Tech Institutional Animal Care and Use Committee.

***Results:** Prior to treatment at 8 weeks, there were significantly increased levels of MDSCs and significantly decreased levels of T cells compared to baseline and naïve animals, indicating the presence of systemic immune dysregulation. First, SNAbs were evaluated *in vitro* and were shown to significantly deplete MDSCs in a coculture of MDSCs and macrophages. Additionally, removal of MDSCs from PBMCs isolated from the blood of trauma rats resulted in recovery of T cell proliferation to naïve levels in response to CD3/CD28 co-stimulation when compared to PBMCs from trauma rats still containing MDSCs. This shows that MDSC depletion can positively impact T cells, which will be essential for restoration of immune homeostasis. Preliminary uCT results 4 weeks after treatment *in vivo* show less variability in response to BMP-2 treatment when animals were also treated with SNAbs, suggesting SNAbs may be able to improve the treatment response rate. Additional *in vivo* results are forthcoming.

***Conclusion/Significance:** Results of this study highlight the role of systemic immune dysregulation and immunosuppression for regeneration following trauma. Further, our SNAb approach can successfully deplete MDSCs *in vitro*, and it offers a complimentary method to increase T cell number and function. Ultimately, this systemic immunomodulatory strategy could be important for restoring immune homeostasis and providing a favorable immune environment to enhance bone regeneration following severe musculoskeletal trauma.

Session Number: 23
Biomaterials for Central Nervous System Diseases and Regeneration with Focus on Imaging and Bio-Inspired Strategies
Wednesday, December 4, 2019, 3:00 PM - 4:30 PM

94 - Neural Regenerative Rehabilitation For Traumatic Brain Injury

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***Purpose/Objectives:** Approximately 1.7 million Americans sustain a traumatic brain injury (TBI) annually, leading to a $7 billion economic burden within the US. Current options for therapy are limited to supportive care that primarily serve to alleviate the symptoms of the initial, mechanical injury. However, following mechanical injury there is an expansive biochemical, secondary injury that is largely responsible for many of the long-term deficits associated with TBI. Efforts to curb the deleterious effects of the secondary injury using preclinical stem cell transplantation have had moderate success; yet, their mechanisms of benefit are yet to be fully understood. We have explored two approaches to address stem cell transplant survival and enhanced functional outcomes. First, we developed a hyaluronic acid-based hydrogel that significantly enhanced neural stem cell transplantation retention and migration following intracortical injections compared to bolus injection. Secondly, we explored coupling neural progenitor/stem cell (NPSC) transplants and motor rehabilitation therapy to provide greater functional benefit than either therapy individually.
***Methodology:** Adult male Long-Evans rats (n=9-10) were trained on a skilled forelimb reaching task for 5 weeks and randomly assigned treatment groups such that baseline average reaching scores were the same across groups. Rats were then subjected to either sham surgery or a moderate controlled cortical impact injury. Two days after injury, reaching success rate was assessed prior to administering of saline vehicle or NPSCs (1.8×10^5 cells). Five days after injury (three days post-transplant), reaching success rate was assessed and rats receiving rehabilitation began the rehab regimen; rats that were not receiving rehabilitation were assessed once weekly.
***Results:** We observed the largest functional gains in skilled reaching among the rats receiving both NPSC transplants and motor rehabilitation compared to all other conditions. Rats receiving NPSC transplants and rehab demonstrated a nearly 12-fold increase in reaching success rate over their baseline impairment by week 3 of rehab and significantly greater functional gains than rats receiving only NPSC transplants ($p=0.0233$ at week 3) or rehabilitation ($p \leq 0.03$ at weeks 2,3).
***Conclusion/Significance:** These data implicate a role for motor rehabilitation in improving the efficacy of NPSC transplants in promoting functional recovery after TBI and vice versa. Given that NPSC transplants often suffer low rates of survival in the injury microenvironment we put forth that the behavioral cues provided by motor rehabilitation may create a more permissive environment for transplants and/or direct NPSC transplant-mediated neuroplasticity within the injury environment. The following grants are acknowledged for support: ABRC ADHS18-198843 (SS, JK) and NIH DP2HD084067 (SS). CP Addington is acknowledged for technical support.

95 - Local Delivery Of Flavopiridol Repairs Rat Spinal Cord Injury By Regulation Of Astrocytes And Inflammation

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***Purpose/Objectives:** The repair of spinal cord injury (SCI) is closely related to inflammatory cytokines, among which quite a few have been demonstrated detrimental or beneficial to repair. The sequential changes of local inflammatory cytokine protein levels after rat SCI are still not clear. Flavopiridol has been reported to significantly improve motor recovery and decrease reactivity of astrocytes which are the important source of inflammatory cytokines. But its high systemic dose may cause strong side-effects. The mini-osmotic pump used for intrathecal flavopiridol delivery is costly and may cause problems with histocompatibility. Biodegradable and injectable Poly (lactic-co-glycolic acid) (PLGA)-based methylprednisolone nanoparticles (NP) have been used in SCI repair, and the NP-enabled local delivery is significantly more effective than systemic delivery. So we studied local sequential changes of inflammatory cytokines and developed flavopiridol NP to promote functional recovery from SCI.
***Methodology:** Multiplex immunoassay was used for the study of sequential changes of inflammatory cytokines. Rat astrocyte culture, scratch-wound model and real-time PCR were used for the in-vitro study of flavopiridol. Rat right-hemisection SCI model was used to assess flavopiridol NP. Corticospinal tract tracing and transparent spinal cord technique were applied for the study of neuronal survival and regeneration.
***Results:** So ①we studied the sequential changes by multiplex immunoassay and found 4 cytokines that might be beneficial to repair decreased after SCI, and 9 cytokines that might be detrimental to repair increased. ②We found that flavopiridol inhibited proliferation, scratch-wound healing, and inflammatory factor synthesis in astrocytes, while permitting the survival of neurons. ③We fabricated flavopiridol NP and found that they improved the functional recovery of injured rats. They also increased the integrity of spinal cord gross tissue structure, inhibited the glial scarring and cavitation, and facilitated neuronal survival and regeneration. Flavopiridol NP decreased the cell-cycle related protein expressions of astrocytes, neurons and macrophages in vivo. Multiplex immunoassay showed that flavopiridol NP affected local inflammatory cytokine profile. They increased GM-CSF while decreased IP-10. We confirmed in vitro that they indeed significantly decreased the pro-inflammatory factor synthesis by astrocytes, while the IL-10 expression was elevated.
***Conclusion/Significance:** These findings demonstrated that local delivery of flavopiridol in PLGA NP improves recovery from SCI by regulation of astrocytes and inflammation.

191 - Hipsc-derived Cortical Neuron Human-on-a-chip System To Investigate aβ- And Tau-induced Neurotoxicities And Model Alzheimer'S Disease In Vitro

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***Purpose/Objectives:** Alzheimer's disease (AD) is the most common cause of cognitive deficit in the ageing population, affected an estimated 50 million people worldwide. Presently, there is no preventive medicaments for AD, only a few approved palliative treatment options. These shortcomings can be partly attributed to less than 10% success rate for drugs in clinical trials due to a lack of effective

translational models. In this study, we seek to develop a more efficient and relevant AD model using hiPSC-derived cortical neurons integrated with human-on-a-chip systems to accelerate the evaluation process of novel therapeutic agents.

***Methodology:** hiPSCs-derived cortical neurons cultured on micro-electrode arrays (MEAs) and coverslips were dosed with Aβ1-42 oligomers, tau oligomers, or brain extracts from AD transgenic mice and analyzed using patch-clamp electrophysiology and MEAs recording techniques.

***Results:** Our study indicated that treatment with Aβ1-42 oligomers, tau oligomers, or brain extracts from AD transgenic mice induced significant changes in cortical neuron function. In particular, we observed marked deficits in the physiological functions of the cells (i.e., lack or reduction of action potentials). Futher, treatment with Aβ1-42 oligomers, tau oligomers, or brain extracts abolished increases previously observed in spontaneous action potential generation after an LTP induction protocol.

***Conclusion/Significance:** These results are consistent with other findings in the literature regarding the deleterious effects of Aβ and tau on neuronal cells physiology and MEA recordings (without the invasive aspect of patch-clamp electrophysiology) as a more effective approach to study long-term neuronal physiological activity. In summary, the results to be presented indicate this hybrid system (hiPSC - MEA human-on-a-chip system) is an appropriate and efficient human-based AD model, which can be used to study AD pathology and potentially for drug screening for new therapeutic compounds.

Session Number: 24
Biomaterials and Regeneration II
Wednesday, December 4, 2019, 3:00 PM - 4:30 PM

97 - Controlled Delivery Of Magnesium Ions Enables In-situ Bone Regeneration

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***Purpose/Objectives:** Bone regeneration is usually achieved by growth factors, bone morphogenetic proteins and stem cells. Interestingly, our previous study evidenced that the magnesium ions at the range of 50-200 ppm in tissue microenvironment can significantly trigger new bone formation. Hence, we propose to realize this concept by designing a novel delivery system to precisely control the magnesium ions delivered at the particular concentration *in vivo* in order to effectively stimulate in-situ bone regeneration. To achieve this objective, the sponge-like monodisperse PLGA/nMgO-alginate core-shell microsphere delivery system has been designed by using a customized microfluidic capillary device.
***Methodology:** The PLGA/MgO-alginate core-shell microspheres were prepared by the oil/water/oil double emulsion method via the modified microfluidic capillary device. The TMSPM-treated MgO nano-particles were suspended in 10%(w/v) PLGA solution (dissolved in DCM), named inner phase (oil phase). 10%(w/v) Span 80 in toluene was defined as the outer phase (oil phase), while 3%(w/v) PVA aqueous solution containing 1.5% (w/v) alginate was defined as the middle phase (water phase). The PLGA/nMgO inner phase flowed through the injected capillary and the alginate middle phase connected to the channel of square capillary. Then, the outer phase flowed through the channel between the collected capillary and square capillary and therefore the PLGA/MgO-alginate core-shell droplets can be yielded. The flow rates of inner phase, middle phase and outer phase had been accurately controlled at 500 µl/h, 800 µl/h and 2000 µl/h, respectively. The PLGA/nMgO- alginate core-shell droplets were then cross-linked with alginate in a petri dish containing 0.1%(w/v) PVA calcium chloride aqueous solution. When DCM solvent evaporated, the PLGA/nMgO- alginate core-shell microspheres remained. Lastly, the core-shell microspheres were rinsed with deionized water and lyophilized for 48h.
***Results:** The PLGA/nMgO-alginate core-shell microspheres measured in the diameter of 115.41±3.84µm were able to maintain the release of Mg^{2+}at ~50ppm per day for first 2 weeks and then ~100ppm per day until 28 days. With the aid of sustained and constant release of magnesium ions, the viability and proliferation as well as the osteogenic differentiation capability (ALP, Col I, Runx2 and OPN gene expression) of MC3T3-E1 pre-osteoblasts were significantly up-regulated, when cultured with PLGA/nMgO-alginate core-shell microsphere delivery system as compared with the control. Moreover, the new magnesium ion delivery system could effectively stimulate in-situ bone formation *in vivo*in terms of bone volume and trabecular thickness. Interestingly, higher bone mineral density (BMD) and increased Young's modulus of newly formed bone were found in the PLGA/nMgO-alginate core-shell microsphere group after post-surgery eight weeks. The mechanical property of newly formed bone induced by this new delivery device was about 96% of that of the surrounding mature bone, whereas the control could only achieve 65%.
***Conclusion/Significance:** These results indicate that sustained and precisely controlled release of magnesium ions leads to enhanced osteoblastic bioactivity in vitro and apparent in-situ bone regeneration *in vivo*. Therefore, it is believed that this monodisperse PLGA/MgO-alginate core-shell

microsphere system enabling precise control delivery of magnesium ions may provide a simple and cost-effective approach for local bone regeneration and healing clinically.

98 - Cd86$^+$ And/or Cd206$^+$ Macrophages Are Determinants Of Implant Outcome Across Species

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***Purpose/Objectives:** Several studies have attempted to tease out the divergent immune response to synthetic vs biologic materials. Given the distinct and critical role played by macrophages in tissue remodeling or fibrosis following surgical implantation, a thorough understanding of macrophage phenotype will serve as a predictor of implant outcome. However, the extremely high plasticity exhibited by macrophages has been a roadblock in determining the specific markers of macrophages that correlate with implant outcomes. Therefore, this work was set out to determine if we can objectively correlate implant outcome of synthetic vs biologic material to macrophage phenotype across many species.
***Methodology:** Biologic materials or synthetic surgical mesh materials, either coated with extracellular matrix (ECM) or uncoated were implanted in abdominal wall of rats or mice with or without abdominal wall injury. The host response was evaluated at different timepoints such as 3, 7, 14 or 35 days post-implantation. For the primate study, sacrocolpopexy was performed following hysterectomy in rhesus macaques and sham-operated animals served as controls. At 12 weeks post-surgery, the vagina-mesh complex was excised, and the host inflammatory response was evaluated. For the human patients, twenty-seven mesh-vagina complexes that were removed for the primary complaint of a mesh exposure (n = 15) vs pain in the absence of an exposure (n =12) were compared with 30 full-thickness vaginal biopsy specimens from women who underwent benign gynecologic surgery without mesh.
***Results:** Despite several significant differences, we had a very promising and consistent finding across a range of species including mice, rats, rabbits, primates, and human patients. Investigation of 14 different commercially available surgical mesh materials in a rodent abdominal wall model demonstrated a strong correlation between the macrophage response to implanted materials and downstream histologic outcomes. Increased numbers of CD206$^+$ macrophages and higher ratios of CD206$^+$:CD86$^+$ macrophages at 14 days were associated with more positive remodeling outcomes. Interestingly, upon coating polypropylene mesh with extracellular matrix hydrogels, the host response was skewed with significantly reduced CD86$^+$ macrophages and thus leading to increased CD206$^+$:CD86$^+$ macrophages. In primates, the results to-date demonstrate that the host response to polypropylene mesh used for pelvic floor repair consists predominantly of CD86$^+$ macrophages with increase in pro-inflammatory markers at three months post-implantation. Assessment of the host response in implants retrieved from human patients with complications related to tissue degradation demonstrated that the activated, pro-inflammatory response with CD86$^+$ macrophages can persist in the long term (8+ years in one case) and the outcome correlated with CD206$^+$:CD86$^+$ macrophages. Consistent with these observations, the next generation RNA sequencing data in mice confirmed increased CD86 expression in mice with polypropylene implantation while the mice with urinary bladder matrix implantation showed increased expression of CD206 at day 7 post-implantation.
***Conclusion/Significance:** While additional work is required to establish a causal relationship, these results strongly suggest a link between CD86$^+$/CD206$^+$ macrophages and downstream outcomes in small and large animal studies as well as human clinical cases.

99 - MicroRNA-200c Incorporated 3D-Printed Bio-Scaffolds Enhance Bone Regeneration

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***Purpose/Objectives:** MicroRNAs (miRs) are essential biologic factors and have recently been explored for their therapeutic potential in enhancing bone healing and regeneration. Specifically, *miR-200c* has been found to increase the osteogenic differentiation of MSCs, thus demonstrating its potential as a biologic agent for the treatment of bone defects. Critical-sized defects are challenging to treat using grafting methods; however, recent attention has been given to the design of 3D-printed synthetic bone grafts using hybrid strategies that include polymeric and biological materials into the 3D-printed construct. Bone regeneration in these scaffolds can be enhanced through the inclusion of osteo-inductive materials, such as *miR-200c*. Therefore, we propose that the incorporation of *miR-200c* into 3D-printed β-TCP scaffolds will create an enhanced hybrid scaffold structure that significantly improves new bone formation in critical-sized calvarial defects.
***Methodology:** β-TCP scaffolds were fabricated using suspension-enclosing projection-stereolithography (SEPS). The printing slurry was composed of 40/60 w/v% β-TCP particles and clear photopolymer resin. 3D-printed scaffolds were coated with collagen (3 mg/mL) and *miR-200c* (10 μg/mL) and then freeze-dried. For the *in vitro* study, scaffolds were seeded with human bone marrow MSCs (hBMSCs) and cultured for up to 14 days in osteogenic medium. We evaluated surface topography, collagen distribution, and dispersion of hBMSCs within the constructs using scanning electron microscopy (SEM) and DAPI staining. Biomarkers for osteogenic differentiation, OCN and Runx-2, were quantified to determine the osteogenic differentiation *in vitro*. For the *in vivo* studies, the *miR-200c* incorporated collagen-coated TCP scaffolds were implanted into critical-sized calvarial defects in male SD rat (8.5-mm defect) and canine (10-mm defect) models. Bone formation was measured after four weeks using micro-CT (μCT; SkyScan) and histology.
***Results:** The 3D-printed β-TCP scaffolds had an average pore size of 350 μm. Collagen was evenly dispersed throughout the scaffolds and hBMSCs were able to attach to the surface and collagen coating with a regular distribution. The expression of *miR-200c* in hBMSCs seeded on collagen-200c co-treated scaffolds is significantly higher than single-treated scaffolds. Moreover, the expressions of OCN and Runx2 were more up-regulated in cells seeded on 200c-Coll-TCP than Coll-TCP or control samples. μCT images found more bone formation in 200c-Coll-TCP than other treated samples. Quantitatively the BMD and BV/TV measured by μCT were statistically significantly higher in 200c-Coll-TCP treatment than that found in other treatments. H&E staining further confirmed increased bone formation in scaffolds incorporated with both collagen and *miR-200c*.
***Conclusion/Significance:** β-TCP scaffolds coated with *miR-200c* incorporated collagen increased the transfection efficiency of *miR-200c* and osteogenic differentiation of hBMSCs *in vitro* and bone formation *in vivo*, attesting to the therapeutic potential of miRs for bone regeneration. This 3D printing, hybrid approach may lead to superior bone substitutes for the treatment of critical-sized bone defects.

100 - Bioengineering An Extra-hepatic Prevascularized Pouch For Subsequent Islet Transplantation Using Vegf-loaded Polylactide Capsules

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***Purpose/Objectives:** The transplantation of pancreatic islets into a bioengineered prevascularized bed is one of studied approaches alternative to conventionally used islet transplantation into hepatic portal vein. As islets are highly oxygen-demanding tissue the critical point is to reach a prevascularized cavity before islets transplantation. Such a cavity can be formed using a polymeric scaffold with channeled-shaped porosity and with a surface coating delivering proangiogenic growth factors (GFs). Once reaching a highly vascularized scaffold, islets can be transplanted into the scaffold cavity and reconnect to the host vascular network, resulting in rapid initiation of perfusion, and thereby, improving the subsequent islet graft survival and function. Objectives of our work were to process the scaffolds, to select surface coatings loaded with VEGF and FGF-2, to evaluate the time for an effective capsule prevascularization *in vivo* and, finally, to verify feasibility of such pouch for functional transplantation of islets.
***Methodology:** *Microporous polylactide/polycaprolactone (PLCL) capsules* were prepared by a thermally-induced phase separation according to [1]. *The surface coating* of PLCL capsules was performed by a layer-by-layer (LbL) deposition of polyelectrolytes albumin (alb) and heparin (hep) [2] Than, PLCL/$(alb/hep)_3$ capsules were loaded with 20 µg of both VEGF and FGF-2. The model *in vitro* FGF-2 release was evaluated for 15 days using an ELISA method. *Implantation:* PLCL/$(alb/hep)_3$ capsules without or with VEGF/FGF-2 were implanted into greater omentum of Lewis rats using a previously described technique[3]. *Evaluation of tissue ingrowth and formation of new vessels:* After two- and three-weeks implantation, each scaffold was processed and sections were stained with H&E, Masson's trichrome, and anti-CD31 antibody. *Islet transplantation:* Three weeks after the implantation of the PLCL/(alb/hep)3 capsule, a PTFE inlay forming tissue ingrowth to the cavity was removed, islets were than inserted according to [3] and islets survival was monitored**.**
***Results:** PLCL capsular scaffolds possess interconnected channeled-shaped pores perpendicularly oriented to a central cavity. FTIR and confocal analysis proved the presence of $(alb/hep)_3$ coatings on the surface of 3D PLCL/$(alb/hep\text{-}FITC)_3$ capsules. After a 6-h burst release, the FGF-2 release slowed down and became almost sustained for 2 weeks with the release of 160-300 $pg.ml^{-1}$/mg of the scaffold. Immunohistological analysis of the implanted capsules showed that PLCL/$(alb/hep)_3$ scaffolds are fully biocompatible. The loading with VEGF/FGF-2 stimulated microvascular network infiltration through pores of the capsule wall towards the internal cavity. After three weeks, dense fibrous tissue accompanied by vessels of small caliber completely infiltrated the scaffolds, reached the central cavity and created granulation tissue containing new capillaries. A preliminary study showed islets survival for at least 6 weeks.
***Conclusion/Significance:** We created a cavity surface rich in vessels immediately before the islets transplantation. This provided to successful islets engraftment in rats. The proposed concept represents an approach promising for clinical cell transplantation.
References: 1. Kasoju N. et al, PloS ONE 9, e108792, 1993 2. Kumorek M. et al, Applied Surface Science 411, 240, 20173. Kriz J. et al, American Journal of Surgery 203, 793, 2012, **Acknowledgment:** This work was supported by MH CR (16-28254A) and by MEYS CR (BIOCEV-FAR LQ1604).

101 - Comparative Analysis Of The Regenerative Capacity Of Osteogenic Platforms In The Treatment Of A Rodent Mandibular Defect

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***Purpose/Objectives:** The development of effective bone graft substitute biomaterials has been a critical pursuit in addressing the demand for alternatives to autogenous bone that are both cost effective and readily synthesized. This challenge is further complicated in treatments associated with oral/maxillofacial injuries due to the complex anatomical nature of flat bone. As a result, a wide assortment of biomaterial designs has been implemented with varying effect characteristics and magnitudes, stemming from differences in physical and chemical properties of the matrix. Therefore, it is essential to develop assessment techniques to objectively compare new osteogenic platforms with existing medical technologies that have well-documented effects. This study examines a previously reported layer-by-layer synthesized nanocomposite, comprised of polyurethane (PU)/nano-hydroxyapatite (nHA) films interspersed with bovine-derived decellularized bone particles, as well as a variation employing a modified synthesis method.
***Methodology:** Utilizing a 5mm mandibular defect model in rats, these two scaffold iterations were tested *in vivo* for their osseo-integrative capacity and ability to facilitate new bone formation within the defect site as compared to multiple predicate devices. BioOss Collagen® (Geistlich), a xenograft comprised of decellularized bovine bone particles and porcine collagen, and Syntoss® (Dental Solutions Isreal), a synthetic graft comprised of β-tricalcium phosphate (β-TCP) and hydroxyapatite (HA), were used as predicate groups. Rats were randomly divided into 4 treatment groups and the mandibular defects in each group were treated with one of the four composite materials. Rats were sacrificed and subjected to computed tomography (CT) at 30- and 60-days post-treatment. Ultimately, the flat bones were harvested and subjected to histomorphometric analyses. Histological sections were stained with H&E and Masson's trichrome for tissue analysis. Unstained sections were used for immunohistochemical evaluation.
***Results:** Quantitative and qualitative CT data provided by a certified radiologist indicated that the two nanocomposites were not significantly different from the predicate devices. Histological assessment utilizing ImageJ software for the Masson's trichrome stain indicated a significantly enhanced level of collagen and early bone formation in defects treated with the modified synthesis method nanocomposite. Additionally, observational analysis of stained sections appeared to demonstrate effective osseo-integrative capacities of both the nanocomposites. Immunohistochemistry (IHC) of samples furthermore established the presence of the hematopoietic stem cell marker, CD34, within the defect region of specimens treated with the experimental materials.
***Conclusion/Significance:** These findings suggest that, though both examined nano-composites display biocompatible and osseo-integrative characteristics comparable to common predicate devices, the iteration utilizing a modified synthesis method may offer an effective and superior design for an osteogenic platform.

102 - Efficient Differentiation Of Human ES And IPS Cells Into Cardiomyocytes On Biomaterials Under Xeno-free Conditions

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***Purpose/Objectives:** Current xeno-free and chemically defined methods for the differentiation of hPSCs (human pluripotent stem cells) into cardiomyocytes are not efficient and are sometimes not reproducible. Therefore, it is necessary to develop reliable and efficient methods for the differentiation of hPSCs into cardiomyocytes for future usage in the cardiovascular investigation of drug discovery, cardiotoxicity screening, and disease modeling.
***Methodology:** We evaluated two representative differentiation methods that were reported previously and further developed original and more efficient methods for the differentiation of hPSCs into cardiomyocytes under xeno-free and chemically defined conditions. In protocol 4, on day -4, hPSCs were seeded into ECM-coated dishes and cultured in E8 medium until Day 0 by changing E8 culture medium every day. On day 0, hPSCs were found to show approximately 85% confluence. The E8 culture medium was replaced with CDM3 medium supplemented 6 µM CHIR99021, and hPSCs were cultured for two days. On day 2, the medium was replaced with CDM3 medium, and cells were cultured for two days. On day 4, the medium was replaced with CDM3 medium supplemented with 5 µM IWR-1, and hPSCs were cultured for two days. On day 6, the medium was replaced with CDM3 medium, and hPSCs were cultured until day 14, where the medium was changed every other day.
***Results:** The developed protocol could successively differentiate hPSCs into cardiomyocytes expressing approximately 90-97% of the cardiac marker of cTnT with beating speeds and sarcomere lengths that were similar to those of the healthy human adult heart. The optimal cell culture biomaterials for the cardiac differentiation of hPSCs were also evaluated using several extracellular matrix protein-coated dishes. Synthemax II-coated and Laminin-521-coated dishes were found to be the most preferable and efficient biomaterials for the cardiac differentiation of hESCs from the evaluation of hPSC-derived cardiomyocytes, with high survival ratios, high beating colony numbers, a similar beating frequency to healthy human hearts, high purity levels (high cTnT expression) and longer sarcomere lengths similar to healthy human hearts.
***Conclusion/Significance:** Protocol 4 was developed and was designed from the combined differentiation method between the protocols developed by Bhattacharya and colleagues (Protocol 2) and Burridge and colleagues (Protocol 3). It is the most efficient and reproducible differentiation method found for promoting the differentiation of hPSCs into cardiomyocytes. The two-day resting period seems to be important for the differentiation of hPSCs in conditions where the culture medium does not contain any inhibitors between differentiation period of the cells into mesenchymal cells (day 0-2) and cardiomyocytes (day 4-6). Synthemax-coated and LN-521-coated dishes are found to be the preferable biomaterials for the differentiation of hPSCs into cardiomyocytes. Indeed, in these conditions, the hPSC-derived cardiomyocytes showed a high survival ratio, high beating colony numbers, a similar beating frequency to healthy human heart cells, high purity (high cTnT expression) and a longer sarcomere length that was similar to that of healthy human heart tissue in xeno-free conditions.

103 - Effects Of Surface Pretreatments On 3D Polycaprolactone/nanopolyglycolic Acid Scaffolds For Tissue Engineering Of Human Auricular Cartilage

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***Purpose/Objectives:** Auricle reconstruction is challenging in craniofacial plastic surgery. Use of surface-modified 3D-printed polycaprolactone (PCL)/nanopolyglycolic acid (nPGA) scaffolds seeded with tissue-cultured chondrocytes for engineering an auricle is an alternative to auricle fabrication. The present study examines modification of the hydrophobic surfaces of PCL scaffolds with ethanol (EtOH) or sodium hydroxide (NaOH) to enhance cell adhesion and promote neocartilage formation during the engineering process.
***Methodology:** Human ear-shaped PCL (KLS Martin, Germany)/Neoveil nano (D15, Gunze, Ltd., Japan) scaffolds were divided into four Groups: scaffolds immersed in serial EtOH solutions (100% to 50%, 15 min each) at room temperature (RT) prior to cell seeding (Group 1, N = 2), PCL treated with EtOH (70%, 5 min, RT; Group 2, N = 3) or briefly dipped in 2M NaOH solution (RT; Group 3, N = 3) for pre-scaffold fabrication, and an untreated scaffold serving as control (Group 4, N = 1). Scaffolds in all four Groups were seeded with 250 x 10^6 human microtic auricular chondrocytes obtained from surgical remnant cartilage and enzymatically digested to isolate constituent cells. Seeded constructs were implanted subcutaneously in the dorsum of athymic mice for 10 weeks. Harvested specimens were fixed 7 days in 10% neutral buffered formalin (RT). Samples were rinsed with 70% ethanol (3X, 15 min each) and immersed in 100% fluorinert (RT, FC-40; 3M, Saint Paul, MN) to enhance contrast during MR scanning and neocartilage detection. Ear scaffolds were imaged with a Bruker ICON 1 Tesla small animal MRI in a custom-built chamber using standard acquisition protocols (T1 relaxation times). MR images were analyzed and reconstructed using ImageJ followed by histological analysis for comparison. Presence of proteoglycan and elastin as markers for regenerated cartilage in each implanted ear scaffold was evaluated by Safranin-O (Saf-O) and Verhoeff staining, respectively.
***Results:** Neocartilage was detected with MR and histology in varying degrees in all harvested construct layers (exterior and middle layer nPGA sheets). Cartilage formation was determined by cartilage volume content (C_V, percentage of cartilage voxel number/construct voxel number) and area content (C_A, percentage of stained proteoglycan pixel number/section pixel number) of each construct obtained from reconstructed MR and Saf-O results. Optimal cartilage formation was detected in scaffolds in Groups 2 (C_V = 36.1 ± 4.2%, C_A = 23.74 ± 5.3%) and 3 (C_V = 40.6 ± 3.9%, C_A = 26.1 ± 6.8%). The least amount of neocartilage regeneration was observed in Groups 1 (C_V = 33.3 ± 4.4%, C_A = 17.8 ± 1.9%) and 4 (C_V = 23.6%, C_A = 15.6%).
***Conclusion/Significance:** A cartilage detection methodology has been established utilizing MR and confirmed by histological staining to define differences in surface pretreatment of 3D polymeric scaffolds for human auricular cartilage tissue engineering. 2M NaOH- and 70 % EtOH-modified scaffolds showed optimal cartilage regeneration after 10-week implantation times. These solutions may alter the scaffold microenvironment, resulting in a more hydrophilic surface for chondrocyte attachment and subsequent proliferation and deposition of extracellular matrix. Such procedures may be applied to create “off-the-shelf” scaffolds advancing clinical tissue engineering of human organs.

104 - Development Of Topographical Microstructures Onto Fish Scale Collagen Scaffold To Manufacture A Tissue-engineered Oral Mucosa Equivalent

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***Purpose/Objectives:** We have been using fish scale collagen as a scaffold to construct a tissue-engineered oral mucosa equivalent (TEOME) for future clinical use.[1)] Similar to skin, the topographical microstructure between an epithelial layer and the underlying connective tissue, so called "dermal-epidermal junction (DEJ)", plays a critical role in maintaining the functions and mechanical properties of oral mucosa.[2)] Recently, using semiconductor technology, we were able to develop four prototypes of micropattern (MP) mimicking to oral DEJ on the scaffold. However, during manufacturing TEOME, shrinkage of the original shape of the MP on the collagen scaffold has been a critical issue left unsolved. In this study, we aimed to examine the effect of 1-Ethyl-3-(3-Dimethylaminopropyl) Carbodiimide (EDC) crosslinking on the MP shrinkage by histologic examinations of TEOME.
***Methodology:** We designed four prototypes of MP, constituted of two shapes of grid and pillar, with rectangular or truncated configuration. Using a negative mold made of either polydimethylsiloxane or silicon, four MP prototypes were fabricated onto 1% of fish scale collagen scaffolds. For crosslinking, γ-ray or additional EDC was applied to the scaffolds. TEOMEs were manufactured by seeding primary oral keratinocytes onto the scaffolds having four different MP prototype at a density of 2.6×10^5 cells/cm^2, with EpiLife® medium containing 1.2mM Ca^{++} according to our human clinical protocol. TEOMEs were photographed to measure its diameter over time, and finally stained with hematoxylin and eosin staining for histologic examinations.
***Results:** The shape of four different MPs were successfully transferred onto the collagen scaffold. The macroscopic observation during manufacturing TEOMEs showed decrease in contraction of the scaffold when crosslinked by use of EDC in addition to γ-ray. Histologic examinations revealed development of a continuous, fully-differentiated epithelial layer in all of the TEOMs. The original shape of four prototypes of MP was poorly maintained when crosslinked by γ-ray only, resulting in deformation or flattening of the underlying MPs. In contrast, the shrinkage of the underlying MPs was not remarkable when using additional crosslinking of EDC. In particular, the original shape was well-maintained in the grid type MPs.
***Conclusion/Significance:** These findings demonstrated EDC crosslinking prevented TEOME contraction and the shrinkage of four prototypes of MP. Our platform technique based on semiconductor technology has potential to fabricate various shapes of MP onto fish collagen scaffolds. Taken together, more biomimetic TEOMEs having oral mucosa specific DEJ can be manufactured, and the standardization of the oral specific MP on the fish collagen scaffold will be required for future clinical applications.
References: 1) Terada M, Izumi K, et al. Construction and characterization of a tissue-engineered oral mucosa equivalent based on a chitosan fish scale collagen composite. J Biomed Mater Res B Appl Biomater 2012;100(7):1792-1802.2) Clement AL, et al. Micropatterned dermal-epidermal regeneration matrices create functional niches that enhance epidermal morphogenesis. Acta Biomater 2013;9(12):9474-9484
I have no financial relationship to disclose.

105 - Apoptosis-mediated Decellularization Approaches For Lung Tissue Engineering

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***Purpose/Objectives:** Thousands of chronic obstructive pulmonary disease (COPD) patients receive lung transplant as their only therapeutic option, and yet graft failure and acute rejection are the leading causes of death after the transplant. Further, current *in vitro* models of COPD fail to accurately recapitulate the disease microenvironment as they lack tissue dimensionality and/or lung-specific extracellular matrix (ECM) components. To improve patient outcome, there exists a grave need for creating physiologically relevant *in vitro* culture models to enhance our understanding of COPD and develop novel, effective therapies that may not require transplant. To this end, we developed a lung decellularization protocol based on apoptosis-induced cell death and removal while maintaining extracellular matrices. The significance of apoptosis is that the majority of tissue decellularization relies on detergents for cell removal, which results in necrosis of tissue-resident cells that necessitate harsh washing steps to remove intracellular components and remaining detergents. This often results in loss of tissue architecture and ECM proteins. Here, we propose to decellularize tissues by inducing apoptosis of cells that result in apoptotic bodies that can be removed with gentle washing steps. The goal of this project is to harness the power of apoptosis to obtain acellular lung scaffolds that can then be digested to create *in vitro* models.
***Methodology:** Sprague Dawley rat lungs were procured after systemic heparinization and phosphate buffered saline (PBS) perfusion. Subsequently, lung pieces were generated with sterile biopsy punches inside a laminar flow hood. The pieces were then subjected to camptothecin to induce apoptosis. The lung pieces were subsequently washed in hypertonic PBS, zwitterionic detergent sulfobetaine-10, and isotonic PBS. Lungs were then treated with deoxyribonuclease to remove nuclear debris, and washed again with isotonic PBS. After each step, lung pieces were fixed, sectioned, stained and imaged to assess apoptosis, cell removal, and ECM preservation. For quantitative molecular and biological assays, lung pieces were snap frozen and lyophilized beforehand. For hydrogel creation, pregel solutions were created by digesting decellularized lung pieces in pepsin-HCl. Hydrogels were created by pH neutralization and incubation at 37C. Lung epithelial and endothelial cells were encapsulated in the hydrogels and viability was assessed.
***Results:** Camptothecin-treated lung pieces exhibited apoptosis, as evidenced by the presence of DNA fragments. Nuclear removal was observed as demonstrated by both H&E and DAPI stainings. Employing light sulfobetaine-10 wash improved cellular component removal. All the approaches retained ECM protein, as evidenced by collagen I, IV and laminin staining. Molecular assays further confirmed preservation of collagen, glycosaminoglycan, and removal of nuclear and other intracellular proteins. Lung ECM hydrogels enabled excellent viability of lung epithelial and endothelial cells in vitro.
***Conclusion/Significance:** Lung pieces were successfully decellularized via apoptosis of tissue-resident cells using camptothecin. We confirmed cell removal, ECM preservation, and cell viability in lung ECM hydrogels. Future directions include scale-up of the decellularization process to create transplantable whole lungs via apoptosis-assisted decellularization, as well as 3D cell culture to mimick COPD lungs.

Session Number: 25
New Approaches to Cardiovascular Repair and Regeneration II
Thursday, December 5, 2019, 10:00 AM - 11:30 AM

106 - Treatment Of Abdominal Aortic Aneurysm Using Biomimetic Scaffolds Composed Of Human Smooth Muscle Progenitor Cells

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***Purpose/Objectives:** Abdominal aortic aneurysm (AAA) is characterized by potentially fatal dilatation of the aorta. AAA results in part from the proteolytic loss of elastin structural matrix and death of vascular smooth muscle cells (SMCs) responsible for vessel integrity and contractility. Since the aneurysmal wall is thin and mechanically weak, it is not amenable for direct injection of therapeutic cells. Accordingly, we hypothesized that the transplantation of human induced pluripotent stem cell-derived smooth muscle progenitors (iPSCs-SMPs) seeded within a collagen scaffold will localize the cells to the site of AAA and abrogate wall dilatation in a murine model.
***Methodology:** Human iPSC-SMPs were characterized using qPCR and carbachol contractility assay to confirm SMC phenotype and function, respectively. The viability of the cell-seeded scaffold after 10 days was assessed by Live/Dead Cytotoxicity assay. To test the therapeutic efficacy of iPSC-SMP-seeded-collagen scaffolds, a murine model of AAA was induced in C57BL/6 mice by local infusion of porcine pancreatic elastase (PPE) into the infrarenal region of the aorta, resulting in aneurysm formation. Seven days afterwards, collagen scaffolds seeded with either iPSC-SMPs or primary human smooth muscle cells (pSMCs) were surgically implanting onto the adventitia of the AAA. To keep the scaffold in place, a thin film of polycaprolactone was placed on top of the cell-seeded scaffold and secured using sutures. To obviate immune rejection of human cells, the animals received daily cyclosporine A immunosuppressive treatment. Over the course of 28 days, wall diameters were tracked by ultrasound, and survival of the transplanted cells was tracked by bioluminescence imaging.
***Results:** The iPSC-SMPs demonstrated positive expression of the phenotypic SMC markers smoothelin, smooth muscle α-actin, transgelin, and calponin. Furthermore, the cells expressed the cell cycle marker, Ki67, suggesting that the cells were proliferative, which is consistent with a progenitor cell phenotype. The iPSC-SMPs were further differentiated into terminal SMCs and stimulated with carbachol, leading to contraction levels similar to that of pSMCs. Over the course of 10 days, the survival of both iPSC-SMCs and primary SMCs were maintained within collagen scaffolds, based on confocal microscopy of Live/Dead cytotoxicity assay. After scaffold transplantation on day 7 to the site of AAA, bioluminescence tracking of cell survival demonstrated strong signal for both pSMCs and iPSC-SMPs initially, followed by a gradual decline to ~1-10% survival by 28 days. Ultrasound quantification of abdominal aortic diameter revealed that the control group, which was PPE-induced but did not receive any treatment other than immunosuppressive therapy, showed progressive expansion of the vessel diameter. In contrast, both iPSC-SMP- and pSMC-seeded scaffolds showed a significantly abrogated expansion of the vessel diameter by days 21 and 28. This data suggested that both cell types were capable of reducing the expansion of the aorta by 28 days, compared to the control group.
***Conclusion/Significance:** These findings suggest that the delivery of iPSC-SMPs can abrogate

aneurysmal thinning of the abdominal aortic wall when delivered within a collagen scaffold for treatment of AAA. This work has important implications in the design of cell-based treatments for AAA.

107 - Ready-made Microvessels Integrate Into The Infarcted Coronary Vasculature Promoting Perfusion, Remuscularization And Function

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***Purpose/Objectives:** Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer an unprecedented opportunity to remuscularize infarcted hearts. However, the majority of hiPSC-CMs die post transplantation into the ischemic environment, limiting their regenerative potential. CM death occurs in the first few days post-transplantation due to ischemia. Attempts to promote intramyocardial vascularization (i.e. delivery of growth factors or cells) have not led to significant improvements due to poor cell retention and the long time required for new vessels to form and carry blood compared to the rapid death of transplanted CMs (2-3 days). Our goal is to develop an effective vascularization strategies to promote heart regeneration and remuscularization.
***Methodology:** Here, we used ready-made microvessels (MVs) harvested from adipose tissue - that form a vasculature and carry blood within the first days post subcutaneous implantation - to re-vascularize ischemic rat hearts and improve hiPSC-CM survival. We performed left anterior descending artery ligation in immunocompromised rats to model myocardial infarction (MI). hiPSC-CMs (10 x106) with MVs or without (control) were delivered by intra-myocardial injection 2 weeks post MI. Cardiac function was assessed by echocardiography and pressure-volume loop. Grafts were analyzed by immunohistochemistry. Delivery of MVs from GFP rats allowed assessment of MV persistence.
***Results:** Compared to hiPSC-CM transplantation alone, MVs promoted a ~600% increase in hiPSC-CM survival, with significant reduction in scar size. There was a significantly superior functional recovery in hiPSC-CM+MV-transplanted animals compared to hiPSC-CMs alone in all the parameters assessed. MVs showed unprecedented persistence and integration (>60%, 4 weeks), resulting in robust early graft perfusion at day 7 and significantly higher vessel density. This was achieved despite the very low number of cells (~2 $x10^5$) delivered in the form of MVs.
***Conclusion/Significance:** These findings provide a novel approach to cell-based therapies for MI whereby incorporation of ready-made MVs can serve as a personalized delivery system to improve functional outcomes in cell replacement therapies.

108 - Tissue-engineered Pediatric Pulmonary Valve In Growing Lamb Model

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***Purpose/Objectives:** A valve that can grow for pediatric patients into adulthood remains an unmet need. Here we present a novel valve design, with growth potential, along with initial data in a growing lamb model.
***Methodology:** The valve is constructed from three biologically-engineered tubes of collagenous matrix with demonstrated somatic growth potential, using resorbable suture, with each tube creating a "leaflet". It was evaluated in an accelerated wear tester and in a pulse duplicator system. Assessment of

the valve implanted into the PA of a 12-20 week old lamb with compromised PV using longitudinal echo to a target of 52wk implantation was completed (n=4). A second study where a fourth tube is placed around the valve as a reinforcing sleeve to modulate diameter increase is ongoing (n=4).
***Results:** The novel tri-tube valve was evaluated for 8M cycles showing intact commissures. Hydrodynamic performance under pulmonary pressure gradients was adequate. In the first lamb, central regurgitation became moderate at 36wk, when the diameter had increased from 19mm to 26mm, 2-3mm beyond the expected value based on normal PA growth. The explanted valve showed a highly recellularized root and modestly recellularized leaflets (cells invading from the base), which were still strong, thin, and absent any calcification. The second lamb exhibited a stable 26mm valve diameter with mild-to-moderate central regurgitation at 52wk. The leaflets at explantation were similar to the first lamb. The other two lambs exhibited a rapid diameter increase and moderate-to-severe central regurgitation by 12wk for reasons unclear. The first two lambs in the second study exhibited only trivial regurgitation as of 20wk.
***Conclusion/Significance:** As in our growing lamb PA replacement study (Syedain et al. 2016), the valve root exhibits growth. The leaflets recellularize more slowly than the root, too slowly for a lamb but potentially fast enough for a child. The cause of central regurgitation is due to the root growing to a diameter greater than expected, suggesting a different mechanoregulatory signal in the implanted valve as compared to simple PA replacement. The novel tri-tube design based on biologically-engineered tubes can function for at least 52wk in a growing lamb, with growth potential demonstrated.

109 - Anisotropic Nanofibrous Cardiac-specific Extracellular Matrix Scaffold For Cardiac Tissue Engineering

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***Purpose/Objectives:** Tissue-engineered cardiac patches with cardiac specific extracellular matrix (ECM) and mature cardiomyocytes (CMs) have great potential to promote cardiac remodeling after myocardial infarction (MI). In native myocardium, cardiac fibroblasts (CFs) regulate ECM deposition and cell communications. In addition, they display a unique molecular profile with CM ontogenesis associated cardiogenic transcription factors compared to non-CF fibroblasts. However, the application of primary CFs (Pri- CF) has been limited by their resources and slow proliferation rate. Human pluripotent stem cell (hPSC) derived CFs (hPSC-CFs) can potentially overcome these limitations. Another unmet challenge of cardiac tissue engineering is the differentiation and maturation of human induced PSC-derived CMs (hiPSC-CMs), due to their inferior action potential and contractile force generation compared to native CMs. We hypothesized that CF-derived ECM can provide a cardiac tissue-specific microenvironment to enhance iPSC-CM's structural and functional maturation. The objective of this study was to develop a completely biological, anisotropic, nanofibrous ECM scaffold carrying cardiac-specific bioactive components by decellularization of highly aligned hPSC-CF sheets.
***Methodology:** Aligned hPSC-CF and adult Pri-CF sheets were cultured for 2 weeks on nano-grated polydimethylsiloxane substrates and then decellularized. Organization of major ECM structural proteins was observed by immunofluorescence staining. ECM embedded growth factors including vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), angiotensin-II and endothelin-1 were quantified and compared between ECM scaffolds derived

from both CF cell sources. iPSC-CMs were grown on these ECM scaffolds to determine their alignment, contraction, and expression of CM specific structural proteins including sarcomeric alpha actinin, cardiac troponin and Connexin43.
***Results:** Highly aligned nanofibrous ECM scaffold was successfully developed after decellularization of both hPSC-CF and Pri-CF cell sheets. hPSC-CF derived ECM had similar amount of collagen, elastin, angiotensin II and endothelin 1 compared with Pri-CF derived ECM. However, hPSC-CF showed significantly higher amount of fibronectin, VEGF and IGF compared to Pri-CF derived ECM ($p < 0.01$). hiPSC-CMs cultured on both of these decellularized ECM sheets showed successful alignment following ECM anisotropy and matured organization of structural proteins. CMs started beating after 5 days of culture on the both types of ECM scaffolds.
***Conclusion/Significance:** A cardiac-specific, anisotropic hPSC-CF derived ECM scaffold with structural proteins and matrix bound growth factors that resembled a cardiac-specific microenvironment supported iPSC-CMs' alignment, contraction, and matured organization of structural proteins. The influence of the cardiac-specific ECM on hiPSC-CM's physiology regulation as well as the engineering of a highly biomimetic cardiac patch using this cardiac specific ECM and hiPSC-CMs are currently ongoing.
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110 - Extracellular Matrix Hydrogel Therapy For Intracoronary Infusion

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***Purpose/Objectives:** cellular matrix (ECM) hydrogels are capable of gelation upon injection, making them amenable to minimally invasive delivery. A cardiac ECM hydrogel, termed myocardial matrix, has shown improvements in cardiac function following intramyocardial injections in preclinical myocardial infarction (MI) models. This material has advanced to clinical trials using a transendocardial delivery approach (ClinicalTrials.gov Identifier: NCT02305602). Cardiac injections carry risks of arrythmias and left ventricular rupture in acute MI patients; therefore, a new ECM hydrogel was developed for intracoronary infusion. Our objective was to develop and apply a new ECM hydrogel for intracoronary infusion in small and large animal models of acute MI.
***Methodology:** Isolated porcine left ventricle tissue was chopped, decellularized, lyophilized, milled, partially pepsin digested, and neutralized to yield an ECM pre-gel liquid capable of gelation upon injection. This pre-gel liquid was fractionated through high speed centrifugation to separate soluble and insoluble fractions. The soluble fraction was isolated and will be referred to as soluble myocardial matrix (SolMM). SolMM was subcutaneously injected in rats to test for gelation. Safety was assessed through hemocompatibility with human blood (n=4 donors). Intracoronary infusion was tested in a rat aortic cross-clamp model following ischemia-reperfusion (n=17) and was subsequently tested in a porcine ischemia-reperfusion model using a clinically relevant balloon infusion catheter (n=2). In the large animal model, satellite organs (lungs, liver, brain, kidney, and spleen) were isolated 1-hour post-infusion and assessed for signs of material, ischemia, or inflammation. Cardiac function was assessed at 24 hours and 5 weeks in the rat ischemia-reperfusion model following infusion of either SolMM (n=10) or saline (n=11). Vascularization, assessed by smooth muscle actin and isolectin, was analyzed 5 weeks post-

infusion. Data (mean±SEM) were compared between groups using a Student's t-test, with significance set as $p<0.05$.
***Results:** SolMM was successfully isolated and formed a gel upon subcutaneous injection. SolMM did not affect blood coagulation times suggesting the safety of SolMM for intracoronary infusion. SolMM was successfully delivered in both small and large animal models as evident by the presence of tagged material in the infarct. There were no signs of material, ischemia, or inflammation in porcine satellite organs. In the rat MI model, at 24 hours post-infusion, end diastolic volumes (EDV) and systolic volumes (ESV) were significantly lower (EDV: SolMM 289.3±12.6 µl vs saline 347.8±13.1 µl, $p<0.01$, ESV: SolMM 123.7±11.2 µl vs saline 164.5±9.0 µl, $p<0.01$) alongside a trending increase in ejection fraction (SolMM 57.7±2.3% vs saline 52.7±1.7%, $p<0.10$). At 5 weeks post-infusion, end systolic and diastolic volumes were significantly lower (EDV: SolMM 383.7±13.0 µl vs saline 474.9±16.5 µl, $p<0.001$, ESV: SolMM 166.2±14.0 µl vs saline 229.4±17.3 µl, $p<0.05$), and arteriole density in the infarct was significantly increased (106.7±6.7 per mm^2) compared to saline (80.3±5.8 per mm^2) infused controls ($p<0.01$).
***Conclusion/Significance:** An ECM hydrogel therapy for intracoronary infusion was developed and delivered in small and large animal acute MI models. Proof-of-concept for the feasibility and efficacy of ECM hydrogels for intracoronary infusion was shown, suggesting its potential as a new delivery strategy for tissue engineering and regenerative medicine applications.

111 - The Role Of Monocytes In Endothelium Regeneration

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***Purpose/Objectives:** Recently our group demonstrated that immobilized VEGF can capture circulating endothelial cells from the blood *in-vitro*. Furthermore, we have demonstrated proof of concept by implanting a-cellular tissue engineered vessels (A-TEVs) comprised of SIS immobilized with heparin and vascular endothelial growth factor (VEGF) into the arterial system of sheep which remained patent (92%, n=12) for 3mo. Upon analysis, the lumen of these grafts was comprised of a fully functional endothelium as early as 1mo post implantation. This study sought to identify the type of cells that are captured by VEGF on the lumen of A-TEVs *in-vivo* and understand how these cells turn into an endothelial (EC) monolayer that is capable of maintaining patency *in-vivo*.
***Methodology:** A-TEV implantations were performed as previously published. *In-vivo Analysis:* Fixed explants of 1wk, 1mo, 3mo, and 6mo VEGF functionalized A-TEVs are assessed via IHC for MC and EC markers. Blood borne mononuclear cells that are captured on surface immobilized VEGF are coaxed to differentiate into EC with a combination of soluble and biophysical signals, including shear.
***Results:** A-TEVs were implanted as interpositional grafts into the arterial circulation of an ovine animal model. As early as 1mo post-implantation, the graft lumen was fully endothelialized as shown by IHC for EC markers, CD144 and eNOS. At the same time, luminal cells co-expressed leukocyte markers CD14 and CD163. To understand these results, we performed cell capture experiments under flow using microfluidic devices. Interestingly, blood mononuclear cells expressing high levels of VEGF receptors were captured on CHV surfaces with high specificity under a range of shear stresses. Initially, these cells expressed high levels of CD14 and CD16. Under the right conditions they were coaxed to differentiate into an EC phenotype as shown by expression of CD144, VEGFR2, and eNOS. Additional IC analysis, qRT-PCR, and flow cytometry also confirmed this observation. We will also discuss the role of soluble signals

and biophysical forces in transdifferentiation of blood cells into EC that maintain graft patency.
***Conclusion/Significance:** In this study we report, for the first time, direct incorporation of monocyte derived endothelial macrophages, M2e, as a functional endothelium in a large animal model. In addition, we establish a protocol to generate M2e cells in-vitro through activation of the WNT pathway. While rare EPCs are known to be involved in endothelium repair, our study indicates that the highly prevalent circulating monocytes may provide a more direct method of endothelialization of acellular grafts.

Session Number: 26
Stem Cell-Based Articular Cartilage Engineering
Thursday, December 5, 2019, 10:00 AM - 11:30 AM

112 - Engineering Cartilage From Aged Mscs: In Vitro Modeling Of Osteoarthritis

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***Purpose/Objectives:** Osteoarthritis(OA) is a degenerative joint disease of high incidence, with articular cartilage(AC) degradation as its main characteristic. There are no disease-modifying OA drugs(DMOADs) avalaible, likely due to the insufficiency of drug screening systems in modeling OA. Since the use of native chondrocytes is limited by availability and dedifferentiation during *in vitro* expansion, we aim to generate a robust *in vitro* OA model using human mesenchymal stem cells(hMSCs). Because aging represents the highest risk factor of OA and chondrocytes isolated from OA samples display a senescence-associated phenotype, we hypothesized that cartilage constructed from senescent hMSCs would display a similar phenotype to OA cartilage, serving as a robust system to study the pathology of aging-associated OA and test potential DMOADs.
***Methodology:** Chondrocytes were harvested from healthy young(<35 years) and old(>70 years) donors, or from old donors(>70 years) with the presence of OA. hMSCs were isolated from young(<35 years) and old(>70 years) donors. All harvests were conducted with Institutional Review Board approval(University of Pittsburgh and University of Washington). We verified hMSCs' stemness via flow cytometry, trilineage differentiation, and colony-forming unit assay. Next, hMSCs were induced to a senescent state through extensive in vitro expansion(up to passage 10 (P10)). Then, both native chondrocytes(young or old, healthy or OA) and hMSCs(young or old, P4 or P10) were subjected to pellet culture in chondrogenic medium for 21 days. The phenotypes of cartilage constructs derived from these 7 groups were assessed with real-time PCR, RNA-sequencing, western blot, histology, β-galactosidase staining, and telomerase length evaluation.
***Results:** Both natural aging and *in vitro* replicative senescence impaired the stemness of hMSCs. However, all hMSCs subgroups possessed the potential for chondrogenic differentiation. β-galactosidase(β-Gal) staining and telomere length evaluation showed that P10 hMSCs from old donors had the most β-Gal positive cells and the shortest telomere length. Interestingly, cartilage generated from OA chondrocytes also displayed a similar phenotype, which was further confirmed by real-time PCR and histology. For example, OA and Senescence-Associated Secretory Phenotype (SASP)-related genes(Interleukin-1 β , Matrix metalloproteinase 13, Interleukin-8 et al.) were highly expressed in cartilage from P10 old hMSCs and OA chondrocytes. Safranin O/Fast green staining also showed significantly less GAG in these 2 groups than in the other 5 groups. In addition, RNA-sequencing data revealed that cartilage generated from P10 old hMSCs and OA chondrocyte shared similar RNA expression heatmap profiles. These results suggested the successful generation of an aging-associated OA model from P10 old MSCs, which will be further validated in the future study.
***Conclusion/Significance:** The novel aging-associated OA model developed in this study will help us to have a more comprehensive understanding of OA progression and screen potential DMOADs.

113 - Automated And Quantitative Assessment Of Clonal Cell Populations Derived From Human Articular Cartilage For Selection And Expansion In Vitro To Improve Cartilage Cell Therapy Products

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***Purpose/Objectives:** Enormous heterogeneity in autogenous tissue sources of mesenchymal stromal cells (MSCs) with respect to the quantity and quality of the stem and progenitor cells that serve as the starting materials for cellular therapeutics limits delivery of safe, effective and reliable cellular therapies. The goal of this study is to identify the subset of the progenitors with systematically characterized critical quality attributes (CQAs) that can be selectively expanded to obtain clonal cell populations with better quality and reproducibility that can help improve stem cell therapies.
***Methodology:** Articular cartilage (Outerbridge grade 1-2) obtained from six knee arthroplasty patient's were enzymatically digested to isolate cells for 2-D cell culture assay. Large field of view images were acquired daily to capture images of the progenitors and their progeny for standardized ASTM-based automated image analysis to define and quantify CQAs of the stem/progenitor cells. Based on the CQAs identified, 24 clonal populations from each patient were picked using Cell X^{TM} robotic device in rapid, precise, repeatable and rigorously documented manner. Of the 24 clonal colonies, twelve fastest growing clones were expanded to 20 doublings for trilineage differentiation assay and RNA sequencing analysis.
***Results:** Preliminary assessment indicated wide variation in morphological and biological characteristics of progenitor and their progeny: circularity (median: 0.665; range:0.15-0.93), area (median: 116.4µm^2; range:51.9-204.2µm^2), doubling time (median:32.9h; rangse:26.9-41.2h) and colony density (median:6.5%;range:2.3-20.7%). Differences seen between two representative clonal populations with respect to the trilineage differentiation potential and RNAseq data obtained at 20 doublings . A complete detailed assessment of all the clonal populations and their culture-expanded population (n=72) will help us identify the CQAs that will aid in selection of quality clones.
***Conclusion/Significance:** An improved understanding of the heterogeneity of progenitor cells resident in adult cartilage will also improve the rigor of cell sourcing and targeting decisions for pharmacological and cellular therapies. The automated methods for rapid and high precision analysis and management of cell populations that we have built into Cell X™ represent an important advancement in the tools that are available in the field for stem cell research and manufacturing.

Session Number: 27
Tools for Imaging and Assessment of Engineered Tissues and Biomaterials
Thursday, December 5, 2019, 10:00 AM - 11:30 AM

114 - In Vivo Mapping Of Immune Cell Infiltration Into Extracellular Matrix Hydrogel In A Rat Model Of Stroke Using ^{19}F Magnetic Resonance Imaging.

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***Purpose/Objectives:** Magnetic resonance imaging (MRI)-based guidance of extracellular matrix (ECM) hydrogel affords its implantation into a tissue cavity caused by a stroke. A major immune response occurs that leads to the biodegradation of the implanted hydrogel and a reconstitution of brain tissue ensues. To improve our understanding of the spatio-temporal dynamics of the macrophage infiltration into ECM hydrogel, we used ^{19}F MR imaging to trace macrophages that were systemically labeled using perfluorcarbon (PFC) nanoemulsions.
***Methodology:** Rats underwent middle cerebral artery occlusion to induce a stroke that lead to tissue cavitation. To tag circulating macrophages, PFC nanoemulsions (CelSense) were injected through the tail vein 12 days following stroke. On day 13 post-MCAo, a pre-implantation ^{19}F and T_2-weighted MRI scans (9.4T) were acquired to visualize a baseline distribution of macrophages, as well as to verify the injection of PFC (i.e. blood vessels containing ^{19}F signal). On day 14, urinary bladder ECM (4mg/mL) was implanted into the stroke cavity. On day 15, 24 hours post-implantation ^{19}F and T^2-weighted images were acquired to investigate the invasion and distribution of macrophages in vivo. Straight after MRI, animals were perfusion-fixed for histological analyses.
***Results:** Baseline ^{19}F images revealed no infiltration of labeled macrophages into the stroke-damaged brain, but verified that PFC was circulating with a strong ^{19}F signal present within major blood vessels. 24 hours post-implantation, a strong ^{19}F signal was evident within the lesion cavity implanted with ECM hydrogel, as well as the peri-infarct parenchyma. Moreover, PFC-labeled macrophages also produced a strong signal in the surgical wound on top of the head, providing a positive control for a macrophage response to tissue damage. These results further indicate that the main immune response to ECM hydrogel implantation in the acute phase is mediated by invasion of peripheral macrophage into the brain, rather than through a recruitment of local microglia.
***Conclusion/Significance:** The spatio-temporal dynamics of macrophage invasion into ECM hydrogel after implantation into stroke are difficult to capture using histological methods. However, we here demonstrated that ^{19}F MR imaging can potential provide this type of information to improve our understanding of the contribution of macrophages to hydrogel biodegradation and tissue restoration.

115 - Multimodal Assessment Of In-situ 3d Net Mold System-derived Myocardial Patch

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***Purpose/Objectives:** In-situ approaches that embed tissue-engineered cardiac patches offer potentially promising methods to restore both constructive and functional properties of the myocardium [1]. In this work, we propose a multimodal imaging-based assessment with Cardiac Magnetic Resonance (CMR),

Electromechanical mapping (EM), and histology for assessing a new in situ cardiac patch derived from a novel 3D net mold system with scaffold-free 3D structure and the use of a swine animal model.
***Methodology:** The proposed scaffold-free patch design incorporated H9C2 rat cardiomyoblast cell line, which was inserted and grown in a custom mesh-based, 3D net mold system (NM25-1). This synthesis employed a rotation culture system with a 14-day incubation period, with fibroblast added to stabilize the 3D tissue cell patch synthesis, followed by 3D scaffold removal, and was incubated to full maturity. The synthesized *biopatch,* which was further reinforced by extracellular matrix (ECM) sheet on both endo- and epicardial surfaces upon implantation, was inserted into swine model of right ventricular free wall (Landrace breed, female 20-30 kg; n=3; 2 additional controls for normal value assessment) under anesthesia via right anterolateral thoracotomy. After 60 days, the animals were subjected to Cardiac Magnetic Resonance (CMR) via cine-, strain-, myocardial T1 mapping, and Late gadolinium enhancement (LGE); and EM. Further tissue examinations were performed ex-vivo using histology (immuno-chemical staining using antibodies of actinin, tropomyosin, and von Willebrand factor; qRT-PCR), and rheometry for ECM viscoelasticity assessment, for which tissue at the patch, right ventricular (RV) free wall, and normal left ventricular myocardium (LV) were prepared with a 3 step decellularization protocol [2], followed by oscillating disc rheometry for shear storage and loss modulus assessment by a frequency sweep.
***Results:** All 5 swine experiments completed successfully. Assessment by Cardiac MR yielded no visible reduction in RV wall motion for either groups using cine-imaging, while changes were observed in the patch region against surrounding normal RV in reduced longitudinal myocardial strain quantified by SENC at end-systolic phase (by ~20-50% within manually selected ROI), Myocardial T1 (reduced overall T1 and increased ECM volume fraction via pre- and post ECM mapping) and LGE (presence of scar tissue). EM assessment yielded positive electrical conductivity in the patch area (as blinded to the EM reader), with both contiguous and heterogeneous voltage with the surrounding area at 1.11±0.8 mV against 4.7±2.8 mV. Hematoxylin-eosin staining findings and qRT-PCR were indicative of myocyte repopulation. Rheometer measurements (on n=3 swine) at initial 0.1 rad/sec angular frequency were: for RV patch, ECM shear storage modulus 130±70 Pa, and shear loss modulus 65±36 Pa respectively. For LV 420±220 Pa and 130±66 Pa (4 samples/angular frequency).
***Conclusion/Significance:** This initial multi-modal imaging approach to assess bioengineered cardiac tissue synthesis using 3D net molding enhanced our ability to characterize and validate new tissue properties using an in situ animal model insertion with both in- and ex-vivo experimental components. While additional sample size (n=3 and n=2 control) is warranted for statistically definitive assessments, this initial feasibility work shows a promising approach to test and evaluate novel biopatches.

116 - Enabling Non-invasive Cell Tracking For Patient-specific Vascular Endothelial Cells

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***Purpose/Objectives:** The clinical translation of stem cell-based therapies for vascular regeneration and other regenerative engineering applications is currently hindered by the lack of suitable imaging approaches that allow long-term monitoring of transplanted cells. Nuclear imaging techniques together with reporter gene transgenic expression provide a highly sensitive, non-invasive tool to monitor the fate of viable transplanted cells *in vivo*. We have previously demonstrated that ectopic expression of human sodium iodide symporter (hNIS) in vascular endothelial cells (ECs) enables live cell tracking via

Single Photon Emission Computed Tomography/Computed Tomography (SPECT/CT). We have also successfully developed multiple patient-specific EC lines using induced pluripotent stem cell (iPSC) technology, from patients with peripheral artery disease (PAD). Those cells may serve as a novel cell source for autologous vascular regeneration for PAD patients. The objective of this study is to engineer PAD patient-specific iPSC-ECs that enable long-term, non-invasive cell tracking with a clinical-translatable imaging method.
***Methodology:** Patient-specific iPSCs were reprogrammed from peripheral blood mononuclear cells of 4 PAD patients (age 56-80, 2 male, 2 female). Lentivirus encoding hNIS with reporter gene eGFP was added to the iPSC culture at various multiplicity of infection (MOI) for 48 hours. $hNIS^+$ iPSCs were differentiated into ECs and purified with magnetic-activated cell sorting for $CD144^+$ cells. $hNIS^+$ ECs (1 million cells/injection) were injected to the gastrocnemius muscle of Nu/Nu mice and imaged with SPECT/CT on days 0, 2 and 7 after the injection. ^{99m}Tc (0.5 mCi) was administrated via tail vein injection and allowed for cell uptake for 1 hour before imaging.
***Results:** Through lentiviral transduction, we engineered patient-specific iPSCs to ectopically express eGFP and hNIS. The co-expression of hNIS with eGFP allows for direct assessment of transduction efficiency in living iPSCs via fluorescence microscopy and flow cytometry. The expression of eGFP-hNIS retains after iPSC differentiation into ECs, due to the integration of transgene via lentiviral vectors. Radioactive signals were detected at the limbs 1 hour after the cell injection, indicating the feasibility of using SPECT/CT to track live ECs *in vivo*. Moreover, co-delivery of $hNIS^+$ECs with PPCN-A5G81 prolonged the presence of signals detected by SPECT/CT up to 7 days, when compared with $hNIS^+$ECs delivered in PBS, indicating PPCN-A5G81 supported EC survival, which could be tracked non-invasively via SPECT/CT.
***Conclusion/Significance:** We established a clinically applicable imaging method that can track the survival, engraftment, and distribution of patient-specific ECs, advancing their potential for future clinical translation.

117 - Resonant Acoustic Viscoelastography For The Mechanical Characterization Of Soft Biomaterials

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***Purpose/Objectives:** The mechanical properties of the cellular microenvironment have been identified as an important regulator of cellular phenotype. However, these matrix properties are not static, undergoing continuous change due to cell-mediated mechanical forces and remodeling. Traditional approaches for the characterization of soft, viscoelastic materials, such as shear rheometry or indentation, are destructive, limiting the ability to track temporal changes in an engineered tissue. Ultrasound elastography techniques, such as acoustic radiation force impulse (ARFI) imaging and shear wave elasticity imaging (SWEI), are widely used to measure tissue mechanics in a clinical setting. While these techniques are non-destructive, their application to tissue engineered constructs is limited, respectively, by semi-quantitative measurements and large sample area requirements. This study demonstrates a new approach for the viscoelastic characterization of engineered tissues, utilizing the oscillatory behavior of soft hydrogels following an acoustic radiation force impulse.
***Methodology:** Dynamic mechanical characterization of in-vitro hydrogels was performed using two coaxially aligned focused ultrasound transducers. A 2 MHz annular focused ultrasonic transducer generated a single high-intensity pulse to induce a displacement in the hydrogel sample via acoustic radiation force (ARF). The length and intensity of the pulse were varied to find an optimal displacement

for a variety of soft materials relevant to tissue engineering. A 10 MHz transducer was used to transmit a series of lower intensity pulses at a pulse repetition frequency (PRF) of 2 kHz after the ARF pulse, tracking the response of the sample over a 0.25 second measurement time. Displacement magnitudes were calculated using a two-step cross-correlation.
***Results:** Non-contact imaging and mechanical characterization of soft biomaterials was easily performed over a range of concentrations. Multiple tests could be performed on a single sample to collect both regional and aggregate data. Displacement over time curves for agarose and fibrin hydrogels following the initial ARF pulse showed an oscillatory behavior with a rapid decrease in amplitude, indicating under-damped oscillatory systems. This behavior was fit to a damped harmonic oscillator model to extract values for the amplitude (A), damping coefficient (γ), and frequency (f_0). While the amplitude values increased with increasing ARF intensity, the damping coefficients and frequencies were constant for ARF spatial peak pulse average (I_{SPPA}) intensities from 300-500 W/cm^2. The frequency values increased with increasing hydrogel concentration and with stiffer materials, while the damping coefficient did not follow this trend. Predicted shear modulus values were calculated from extracted resonant frequencies, ranging from 0.32 ± 0.09 kPa for 2.5mg/mL fibrin gels up to 1.2 ± 0.095 kPa for 10 mg/mL fibrin gels.
***Conclusion/Significance:** These results demonstrate that advanced ultrasound techniques can successfully be applied to characterize the mechanical properties of a hydrogel material in a force-independent manner. Importantly, this approach is non-invasive and non-destructive, allowing longitudinal monitoring of biomaterial properties. Future studies will explore the capability of this technique to monitor the dynamic mechanical properties of cell-seeded engineered tissues.

118 - Effects Of Freeze-thaw Cycling On Articular Cartilage Anisotropy Measured Using Ultrasound

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***Purpose/Objectives:** Depth-dependent structural variations render articular cartilage (AC) structurally anisotropic and inhomogeneous. Ultrasound can be used to non-destructively evaluate acoustic anisotropy of the tissue. In tissue engineering applications, anisotropy is useful as a metric for the similarity of engineered cartilage to native tissue. In this study, we adapted our previously developed technique to measure anisotropy in AC samples and investigate possible adverse effects of freeze-thaw on acoustic properties.
***Methodology:** A device for measuring speed of sound (SOS) in two orthogonal directions was developed. Tissue samples were held in an L-shaped magnetic stainless steel bracket. The holder was secured magnetically to the apparatus in either of two positions 90 degrees from each other to allow two perpendicular measurements to be taken. Ultrasound evaluation of samples was performed using a focused transducer. A 1-2 mm wide cartilage slice, cut perpendicular to the articular surface of a normal bovine calf femoral condyle, was placed in the inside corner of the sample holder, then the ends were glued to the holder using cyanoacrylate glue. Time of flight (TOF) between US transducer and the metal surface was recorded using an oscilloscope, then two echoes, one from the surface of the cartilage, the other from surface of the metal holder after passing through the cartilage strip were recorded. After these measurements, the stainless steel holder was flipped 90 degrees and the procedure was repeated

to measure the TOFs in this direction. For 6 samples, we placed the sample/holder assembly in a conical tube and submerged it in liquid nitrogen to freeze the sample. After 30 seconds, the assembly was thawed in a room-temperature PBS bath. The freezing-thawing process was repeated 10 times. Afterwards, samples were returned to the US apparatus to again measure TOFs in the two orthogonal directions.
***Results:** SOS in PBS was 1,509±12 m/s (N=14), and was used in the calculation of SOS in AC. In all cases, SOS was greater perpendicular to the articular surface compared to parallel to the articular surface (P = 0.0001, Wilcoxon signed rank test). Average SOS was 1,771 ± 114 m/s perpendicular to the articular surface, and 1,618 ± 58 m/s parallel to it. For the freeze/thaw samples, we observed fluctuations in SOS and thickness before and after the freeze/thaw process, but they were not statistically significant (P=0.99 perpendicular to the articular surface and P=0.28 parallel direction, paired t-test). Small increases in normalized mean thickness were observed (4.6% perpendicular to the articular surface and 3.3% parallel to it after the cycling).
***Conclusion/Significance:** In conclusion, measured SOS was within the range of previous studies, and SOS was always higher perpendicular to the articular surface. This work provides benchmark targets for engineered cartilage. There was no significant effect on SOS before and after multiple freeze-thaw cycles. The lack of effect on SOS suggests a lack of change in mechanical properties, and has implications for cryopreservation of natural and engineered tissues for implantation.

119 - Quantifying Bladder Tumor Induced Changes In Stromal Collagen Architecture

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***Purpose/Objectives:** Bladder cancer is typified by "field cancerization" whereby tumors, after therapy, have an abnormally high chance of recurrence. Most studies implicate factors within the cancer cell, such as genetic or epigenetic alterations that produce a growth benefit in the bladder. However, recent studies of the tumor microenvironment (TME) have implicated it as a strong proponent of oncogenesis. Mesenchymal cells, growth factors, and extracellular matrix (ECM) are components of the TME that individually interact with cancer cells, but the sum of these interactions determines a cancer cells' final fate - whether pro- or anti-oncogenic. We *hypothesize* the TME may contribute to field cancerization through alterations to stromal architecture. Initial tumor populations may cause irreversible changes to the TME which in-turn produce a pro-oncogenic environment leading to increased rates of local or regional recurrence.
***Methodology:** In this study, we produce bladder cancer tissue equivalents (organoids) composed of individual tumor and stroma compartments within a collagen-based matrix. Bladder tumor cells interact, through paracrine signaling, with the stromal cells (bladder smooth muscle cells) causing changes in stroma-mediated organization of the collagen matrix. After imaging collagen matrix using polarized signal of picric acid staining on an upright Leica, widefield microscope (40x), we developed novel image analysis techniques to accurately and consistently quantify these images and extract relevant information to better understand changes in stromal collagen architecture, such as lengthening, thickening, and bundling (binding of adjacent fibrils) during the bladder cancer development process.
***Results:** First, we segmented polarized picric acid images by color to generate pixel-by-pixel definitions of fiber bundling/thickening. We developed image analysis methods to quantify the distributions,

recurrence, and regularity of the colors in images which yields information about heterogeneity of collagen remodeling by stromal cells. Then, we further characterized the structural changes in the collagen using Histogram Oriented of Gradient (HOG), an object detection methodology which divides the image into small (9 x 9 pixel) regions of interest called image composing units (ICUs), then calculates the gradient (contrast) of each cell to provide a direction and magnitude of the ICU. The individual ICU information can then be collated to provide directionality and length data of individual collagen fibers. ***Conclusion/Significance:** In all, these results indicate cancer cells can cause local alterations to stromal architecture, and possibly create an environment more conducive to further cancer recurrence and growth. This finding can be applied to biopsied samples to use ECM organization as a diagnostic and prognostic marker to predict whether a bladder cancer will recur and/or progress.

Session Number: 29
Biobased Materials for Regenerative Medicine
Thursday, December 5, 2019, 1:00 PM - 2:30 PM

120 - Magnetically-actuated Alginate Scaffold: Effects On Macrophage Function And Angiogenesis

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***Purpose/Objectives:** Artificial tissue can restore function in failing organs. Engraftment of artificial tissue requires a coordinated interaction between the implanted material and the host innate immune response. Macrophages regulate the host-material interaction immediately following implantation, and these cells influence downstream scaffold vascularization by displaying a multi-phase response that first primes the environment and then remodels ongoing regenerative processes. We investigated a novel approach for user-controlled immunomodulation after implantation that relies on the material-delivered cyclic strain. We hypothesized that cyclic strain applied during early priming phases will impact the mechanosensitive host-macrophage population by enhancing the classically activated (M1) phenotype and promoting downstream angiogenesis.
***Methodology:** We used magnetically responsive scaffolds in combination with an alternating uniform magnetic field to deliver cyclic strain to scaffold associated cells. Cell-free scaffolds were subcutaneously implanted into male, BALB/c mice, N=5. Mice were exposed to a magnetic field (30 Gauss, 0.5Hz) during the early (day 1-3 post-implantation (p.i.)), middle (day 5-8 p.i.), or late (day 12-15 p.i.) phase of the host macrophage response or kept as unexposed controls. The identity and phenotype of infiltrated host-cells was assessed using flow cytometry. To assess scaffold vascularization at day 21 p.i., we quantified the number of perfused blood vessels on histological samples.
***Results:** Magnetically actuated scaffolds at day 5-8 p.i. showed an increase in the percentage of cells expressing the macrophage marker F4/80 (67.4% ± 2.29 stimulated vs. 58.9% ± 1.05 control ($p<0.05$), n=5). Furthermore, we observed an increase in the percentage of macrophages expressing the M1 marker CD86 (37.5% ± 2.93 stimulated vs. 23.4% ± 2.78 control ($p<0.05$), n=5) by t-test. Magnetic stimulation during the early (1.25 vessels/mm^2 ± 0.38 ($p=0.052$), n=10) or middle (1.22 vessels/mm^2 ± 0.32 ($p=0.059$), n=10) phase promoted angiogenesis compared to control (0.25 vessels/mm^2 ± 0.16, n=7) using the Kruskal-Wallis test.
***Conclusion/Significance:** We demonstrate that user-controlled, mechanically active materials can modulate the host-macrophage response after implantation and promote angiogenesis.
Acknowledgments: This study was funded by the BSF Foundation, the Commonwealth of Pennsylvania CURE grants, and Drexel University Dean's Fellowship for Excellence in Collaborative or Themed Research Training.

217 - Decellularized Vocal Fold Lamina Propria-ECM And Associated Matrix-bound Vesicles: Proteomic And In Vitro Characterization Of The TGF-β1 Mediated Fibrotic Response In Human Fibroblasts

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***Purpose/Objectives:** Vocal fold lamina propria (VFLP) is the outermost layer of the vocal fold (VF) and is highly susceptible to injury. Voice disorders affect about 20 million people in the U.S. alone and there is currently no clinically available biomaterial with significant satisfactory wound healing outcomes. During injury, local cells of the VFLP (e.g., fibroblasts) respond by overproducing extracellular matrix (ECM) proteins increasing the tissue stiffness and leading to diminished function. Decellularization of porcine tissues can yield scaffolds that contain immunomodulatory properties with low risk of immunogenicity, while allowing local cells to recognize the protein and non-protein composition with potentially superior recovery[1,2]. However, the factor(s) in the acellular-ECM scaffold responsible for the phenotypic response remains unknown. A recent study identified Matrix-Bound Vesicles (MBVs) isolated from decellularized-ECMs that are able to recapitulate the macrophage polarization effects[3]. We hypothesize that decellularized porcine VFLP-ECM scaffold will have a unique proteomic composition and modulate the TGF-β1 mediated fibrotic response in vocal fold fibroblasts (HVOX). In addition we also hypothesize that MBVs and macromolecules isolated from the VFLP-ECM scaffold will also mediate the fibrotic activation of fibroblasts.

***Methodology:** VFLP and urinary bladder matrix (UBM) were obtained via established methods and processed according to a discovery proteomics workflow. VFLP-ECM hydrogel (VFLP-ECMh) was used for the *in vitro* testing of HVOX upon TGF-β1 stimulation. MBVs and macromolecules larger than 100 KDa isolated via ultra-filtration of the digested acellular VFLP-ECM.

***Results:** The VFLP-ECMh showed characteristics analogous to other gels in clinically use. However, the proteomic discovery analysis revealed a unique composition in the VFLP-ECM scaffold when compared to UBM-ECM. Significant differences were found in collagens, elastin, aggrecans, proteoglycans, and other ECM proteins. Gene correlation analysis identified proteins (e.g., *LTBP4* and *Col18A1*) that might play a role in the observed HVOX response. The downregulation of the expression of *ACTA2* and *Col1A1* suggests a modulation of the HVOX phenotype seeded on VFLP-ECMh. In addition, α-SMA staining showed a reduced expression in HVOX seeded on VFLP-ECM in support of the gene expression results. By supplementing MBVs and macromolecules to culture media, we were able to recapitulate the downregulation of *ACTA2*, which is a critical marker for initiation of scar formation.

***Conclusion/Significance:** VFLP-ECMh has a specific composition when compared to UBM-ECM. In addition, VFLP-ECMh is able to downregulate α-SMA as well as affect ECM-related genes when stimulated by TGF-β1.

Acknowledgments: We thank our funding sources: NIH R01DC017139, The UNC-Chapel Hill/NCSU Joint Department of Biomedical Engineering, and Comparative Medicine Institute, NCSU.

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122 - 3d Bioprinting Of Biomimetic Pancreas With Biocompatible Hydrogel, Adipose-derived Stem Cells And Islets

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***Purpose/Objectives:** Diabetes is a global disease affecting more than 400 million people. The burden of the disease by life- threatening secondary complications has led to the disease becoming the seventh leading cause of death worldwide. Autoimmune destruction of endocrine function results in Type 1 diabetes (T1D). Clinical islet transplantation is superior to exogenous insulin supplementation in the treating of T1D patients with difficulties to control their blood glucose levels. However, the efficiency and long-term outcome is limited by loss islets at the current intraportal transplant site. We aim to develop implantable device for delivery of pancreatic islets and adipose-derived stem cells by 3D bioprinting using nanocellulose isolated from tunicates as biocompatible biomaterial to overcome this limitation.
***Methodology:** Cellulose nanofibrils (TUNICELL) were isolated from tunicates at Ocean Tunicell AS, Norway. Samples were prepared using manufacturing practice (GMP) processing steps. The final concentration of nanocellulose hydrogel was adjusted to 2.5% by weight, and samples were sterilized. Clinical grade alginate SLG100 (DuPont NovaMatrix, Norway) was used to provide crosslinking using 100mM $CaCl_2$. Lipoaspirate was obtained in the operating theater under sterile conditions, and the stromal vascular fraction (SVF) was isolated using the automated Celution® system (Celution 800/CRS, Cytori Therapeutics Inc., USA) according to the manufactures instructions. Clinical grade adipose derived stem cells (ASC) were isolated and expanded using the automated Quantum ® Cell Expansion Systems (Terumo BCT, Japan). Hydrogel bioinks based on TUNICELL and alginate mixed with ASC were printed in various configurations using 3D Bioprinter INKREDIBLE from CELLINK, Sweden.
***Results:** Selected constructs were designed using CAD software and 3D bioprinted with or without ASCs in the TUNICELL nanocellulose based bioinks at different compositions. TUNICELL nanocellulose hydrogel consists of highly crystalline nanofibrils with robust nanofibril morphology which results in much higher viscosity compared with other nanocellulose hydrogels such as plant and bacteria produced. This could result in much higher pressure in the nozzle. In our experiments we found preserved viability of 3D bioprinted ASCs stained by FDA/PI (live/dead). The best architecture of construct for in vitro validation was found to be a “sandwich” structure where ASC were placed in dense bottom layer and could function as a support layer for the islets (grid layer) to avoid stress and increase the oxygen and nutrient transport. Our focus will further be on design of vascular channels in the 3D bioprinted cell laden constructs which could be in situ vascularized after implantation to further increase the revascularization process. 3D bioprinted vascularized islet scaffolds with ASCs as supporting cells could represent an extraheaptic and safe site for islet transplantation to cure T1D.
***Conclusion/Significance:** We have shown that medical grade hydrogels based on nanocellulose isolated from tunicates (TUNICELL) are suitable for 3D Bioprinting of a device consisted of ASCs. The next step will be in vitro and in vivo evaluation with islets and design of a larger size device with vascular tree.

123 - Aging Affects The Immune Response To Synthetic And Biological Implants Through Divergent Mechanisms

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***Purpose/Objectives:** Introduced over 50 years ago, polypropylene meshes are the most commonly used ones for abdominal wall repair. Nonetheless, these synthetic materials present with several complications related to the foreign body reaction which is a predictor of implant success or failure. In past decade, biological materials have been introduced as alternatives. One of the major advantages of these ECM based materials is their potential for tissue integration and induction of regeneration. This divergence in immune response wherein synthetic materials are characterized by chronic inflammatory response while non-crosslinked biomaterials present with more pro-regenerative response has been studied. However, one very important consideration that warrants more attention is factoring in aging as a possible determinant influencing the differential immune response. Aging as a determinant factor is of particular importance in the field of biomaterial implantation as a vast majority of patients in need of these implantation are aged and with increasing lifespan, the number as well as proportion of aging population are on the rise.
***Methodology:** This study focused on characterizing host response to synthetic and ECM-based biological material, in this case polypropylene mesh (PPM), and decellularized urinary bladder matrix (UBM), respectively, as a function of aging. We characterized the immune response at the implantation site at 3, 7, 14 and 90 days post-implantation in both young (4 months) and aged (18 months) mice in a muscle injury model. We also evaluated how aging impacts the phenotypic modulation of the macrophage populations and differential ability of PPM vs UBM to promote ECM deposition and tissue remodeling.
***Results:** While, in the young mice, the biological material tends to reduce the cellular infiltration after day 7, aged mice fail to achieve this reduction until 90 days. However, ECM deposition induced by the biological material is higher compared to that in synthetic material despite the age of mice indicating that the constructive tissue remodeling by biological material is not significantly affected by aging. This can be explained by our observation that there was significantly higher infiltration of arginase producing macrophages in the UBM group vs the PPM group both in young and aged mice. Since arginase production has been used widely as a marker of pro-regenerative M2 macrophages in mice, we wanted to further characterize this cellular response at molecular level. Towards this end, we performed next generation sequencing for evaluating differential gene regulation. At day 7, many inflammation-related and functional pathways associated with cell movement and activation were upregulated in both UBM and PPM. Nevertheless, response to UBM showed upregulation of many anti-inflammatory molecules in young mice whereas downregulation of inflammatory molecules in aged mice.
***Conclusion/Significance:** In conclusion, the phenotypic and transcriptome analyses showed mechanistic divergence in immune response to biological vs synthetic implant material as a function of aging.

124 - In Vitro Assessment Of Decellularized Human Skin As A Stand-alone Scaffold For Cardiac Regeneration

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***Purpose/Objectives:** The induction of cardiac regeneration by the means of three factors, namely cells, scaffold and signals, is currently the target of cardiac tissue engineering (CTE). The cardiac extracellular matrix (ECM) is part of the non-cellular compartment of the myocardium that provides structural support and delivers biochemical signals. Therefore, cardiac ECM alone provides two of the three pillars of CTE. However, since the organ donor shortage dramatically affects the availability of human native cardiac ECM, finding alternative scaffolds is a top priority in CTE. We have recently demonstrated that the decellularized human skin (d-HuSk) is an easily accessible biological scaffold with the capability of supporting human cardiac progenitor cell (hCPC) survival and differentiation towards cardiac myocytes [1]. With the present study we aim at assessing whether d-HuSk might be also exploited as a stand-alone scaffold for CTE by evaluating its capability of recruiting resident hCPCs and promoting cardiac regeneration *in vitro*.
***Methodology:** To test our hypothesis, we prepared ECM scaffolds by decellularization of specimens of myocardium from hearts of patients receiving heart transplant and from skin specimens of patients undergoing tummy tuck surgery, following previously described protocols [1, 2]. Additionally, resident hCPCs were isolated from the samples of myocardium. To evaluate the ability of d-HuSk to attract resident hCPCs and support their engraftment and differentiation *in vitro*, we prepared three-dimensional cultures by seeding hCPCs on decellularized human myocardium (d-HuM) and cultured them for two weeks. Afterwards, acellular scaffolds of d-HuSk were placed in culture in close proximity of hCPC-d-HuM scaffolds and cultured for two additional weeks. The effects of d-HuSk on hCPC migration, engraftment and differentiation were then evaluated by time-lapse microscopy, SEM, immunocytochemistry and gene expression analyses.
***Results:** Interestingly, d-HuSk attracted hCPCs from the cardiac native matrix and provided a suitable environment for their engraftment and differentiation towards cardiac cell lineages. Indeed, time-lapse microscopy showed that hCPCs *in vitro* migrated from d-HuM to reach d-Husk scaffolds within a time ranging between 90 and 116 hours, while SEM analysis showed the engraftment of hCPCs onto d-HuSk. Additionally, as highlighted by immunocytochemistry and real-time PCR, hCPCs engrafted onto d-HuSk expressed markers typical of cardiac cell lineages, both at protein and gene level. Notably, the transcription of genes typical of cardiac myocytes, smooth muscle and endothelial cells, like ACTC1, ACTA2, GATA6 and FVIII resulted significantly upregulated in hCPCs migrated to and engrafted onto d-HuSk.
***Conclusion/Significance:** Our study provides evidence that d-HuSk is an easily obtained autologous scaffold that might be exploited as stand-alone scaffold to boost cardiac regeneration by recruiting resident cardiac progenitor cells. [1] Castaldo C, et al. Tissue Eng Part A. 23 (2017) S67 [2] Di Meglio F, et al. Tissue Eng Part C Methods. 23 (2017) 525-539

125 - Non-neoplastic Extracellular Matrix Components Mitigate Primary Human Glioma Cell Growth

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***Purpose/Objectives:** The cell-centric somatic mutation theory of carcinogenesis has been increasingly challenged as data that does not fit within the paradigm accumulates. One alternative theory, the tissue organization field theory (TOFT), explains much of this data. TOFT is based upon the concept that cancer is a tissue-based disease and tumor growth is driven primarily by the microenvironment, especially the extracellular matrix (ECM). This theory predicts that manipulating the tumor microenvironment would modulate growth and malignancy. Indeed, recent reports describe suppressive and/or lethal effects of mammalian ECM hydrogels derived from non-neoplastic sources upon various cancer types. We applied these principles to in vitro and in vivo models of glioblastoma multiforme.
***Methodology:** Viability of non-neoplastic and glioma cells were tested in the presence of various ECM preparations in vitro. In vivo studies probed recurrence of resected flank tumors and longevity in athymic nude mice, and longevity and intracranial tumor volume in Wistar rats.
***Results:** ECM hydrogels derived from normal, healthy porcine dermis, small intestine, or urinary bladder all decreased primary glioma cell viability in vitro, with urinary bladder extracellular matrix (UBM) having the most dramatic effect. A saline-soluble fraction of UBM (UBM-SF), devoid of the fibrillar, macromolecular components, recapitulated this effect. In a cell viability assay normalized to the media treatment, non-neoplastic CHME5 and N1E-115 cells averaged 103% and 114% 48 hours after treatment with UBM-SF. Two primary high-grade glioma cell types averaged 17% and 31% after the same treatment (N=2). Time-lapse video showed CHME5 cells thriving in UBM-SF-spiked media while most glioma cells shriveled and died within 18 hours. Videos with Nucview dye, fluorescent upon cleavage by active caspase-3, confirmed glioma cells underwent apoptosis. In animal experiments, large glioma flank tumors in athymic nude mice were resected and replaced with either UBM-SF or Matrigel (an ECM product of neoplastic cells). After 7 days the resection sites with UBM-SF had minimal tumor regrowth compared to the dramatic recurrence seen at the Matrigel injection sites (N=2). Additionally, survival times of mice receiving UBM-SF as intratumoral injections were significantly greater (26 days) than saline controls (10 days, N=3). Finally, intratumoral injections of UBM-SF in Wistar rats with intracranial C6 glioma tumors dramatically increased lifespan. Control animals survived 20 days on average (N=7), treatment animals survived 39 days on average (N=9). Importantly, weekly MRIs of three of the treatment animals showed no sign of residual tumor in the brain and, although healthy, they were electively taken off protocol at day 70. This longevity correlated with tumor volume. At time of death or sacrifice, average tumor volume for control animals was 299mm^3, while treatment animals averaged 58mm^3.
***Conclusion/Significance:** These findings indicate that UBM-SF prepared from healthy tissue contains potent signals capable of mitigating glioma cell viability in vitro and in vivo. Delivering soluble fractions of ECM derived from normal porcine urinary bladder to a glioma tumor site may represent a novel therapeutic approach for glioblastoma, sidestepping traditional cytotoxic therapies.

Session Number: 31
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Thursday, December 5, 2019, 1:00 PM - 2:30 PM

194 - Hypoxia-induced Choroidal Neovascularization In 3D Bioprinted Model Of Retinal Pigment Epithelium-Choroid Interface

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Disruption of the interactions between retinal pigment epithelium (RPE) and underlying choroidal vasculature (CV) is a driving force of vision loss in macular degeneration. Hypoxia has been suggested as a dominant factor in stimulating increased proangiogenic cytokine secretion from the basolateral region of RPE. This induces choroidal neovascularization (CNV), the excessive angiogenic expansion of CV into the subretinal space. Currently, mechanisms of RPE/choroid interactions during CNV development require clarification.
We have developed an engineered tissue model of the choroid and RPE that consists of three layers: 1) a bio-printed “choroid” consisting of endothelial cells, pericytes, and fibroblasts that self-assemble into microvasculature; 2) an electrospun sheet of poly(lactide-co-glycolide) nanofibers that provides mechanical support to the tissue; 3) a RPE monolayer that forms on top of the nanofiber sheet. Hypoxia was induced in the engineered tissue model by treating the RPE with an iron chelator (ML228) and Dimethyloxalylglycine, which together increased activation and nuclear localization of Hypoxia-inhibitory Factor 1α (HIF-1α) and subsequent Vascular Endothelial Growth Factor (VEGF) secretion from the basolateral region of RPE to the “choroid". This drove angiogenic expansion of CV toward the RPE. Decreased transepithelial resistance (TER) in the tissue indicated RPE barrier disruption with HIF-1α induction. Avastin, an anti-VEGF antibody, reduced CNV within the tissue in presence of the HIF-1α inducers and partially rescued TER. These results demonstrated that our engineered CNV model provides critical insights into RPE-choroid interactions that are expected to uncover target molecules for treating CNV,

127 - High-purity Photoreceptor Precursors From Human Induced Pluripotent Stem Cells For Photoreceptor Replacement Therapy

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***Purpose/Objectives:** Inherited retinal degenerative diseases such as retinitis pigmentosa, cone-rod dystrophies, and Leber congenital amaurosis may be addressable by photoreceptor precursor (PRP) transplantation. Photoreceptors are responsible for light sensing; their dysfunction leads to vision loss and ultimately blindness. As human photoreceptors do not naturally regenerate, we have developed a cell manufacturing platform to differentiate human induced pluripotent stem cells (hiPSCs) into high-purity PRPs as an allogeneic photoreceptor replacement therapy.

***Methodology:** This platform includes hiPSC reprogramming and culture, retinal differentiation, PRP enrichment by magnetic-activated cell sorting (MACS), process scale-up, and quality control assays. To test the therapeutic potential of highly purified PRPs, we performed subretinal transplantation studies in an immunocompromised rat model of photoreceptor degeneration (Foxn1/S334ter-3).
***Results:** We successfully differentiated hiPSCs into an enriched population of PRPs at a scale of several billion cells per batch. Before enrichment, 60-90% of the cells expressed the early photoreceptor marker recoverin, but after enrichment, and following cryopreservation, the recoverin-positive fraction increased to over 90%. When these cells were transplanted into the subretinal space of S334ter rats, engrafted PRPs reformed an outer nuclear layer, integrated with the host retina, and produced the appropriate synaptic machinery. After 3 months *in vivo*, the transplanted cells expressed mature rod and cone photoreceptor markers. Protocol and process improvements yielded long-term grafts with virtually no proliferative and minimal off-target cells. In parallel *in vitro* studies, cultured PRP could be further differentiated and showed a similar profile of mature rod and cone photoreceptor markers.
***Conclusion/Significance:** This work demonstrates the development of a robust manufacturing platform for high purity hiPSC-derived PRP. Our results show that PRP transplanted into the subretinal space of rodent degenerative models can engraft and mature into both rod and cone photoreceptors. This development is a key step towards an ocular therapy to replace photoreceptors lost to disease at a clinically-relevant scale.

128 - Hydrogels For Enhanced Transplanted Retinal Ganglion Cells Survival

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***Purpose/Objectives:** Neurofibromatosis type 1 (NF1) is caused by a mutation in the NF-1 gene that functions as an inhibitor of RAS, a key controller of cell growth and survival. Patients with NF-1 are prone to low-grade tumor development in the optic nerve, tract, chiasm and radiations known as optic pathway gliomas (OPGs), which can cause loss of vision through the death of retinal ganglion cells. One area of current research aims to preserve or replaced lost cells in the retina by transplanting stem cell derived retinal ganglion cells (RGCs). Here we attempt to improve transplanted RGCs survival through the use of hydrogels to aid cell delivery.
***Methodology:** We screened different polymeric matrices that could support and enhance RGC survival in-vitro and in-vivo. Polymers chosen for this work were 2% Gelatin-HPA, 4% Collagen, 2% hyaluronic acid-tyramine and high molecular weight shear thinning un-crosslinked hyaluronic acid. These polymers were chosen due to their biocompatible, biodegradation characteristics along with the ability to support cell attachment and survival. We evaluated the response of RGCs derived from human embryonic stem cells to the hydrogel by assessing viability (live-dead assay), apoptosis (Cas3/9 staining) and cellular phenotype (flow cytometer). Based on the in-vitro assay 2% Gelatin-HPA and 2% hyaluronic acid-tyramine was chosen for the in-vivo testing. Gels mixed with RGCs were injected into the vitreous of the non-immune suppressed Long Evans rats. Animals were observed for 2 weeks post surgery and eyes were further processed to determine the extent of cell engraftment in-vivo.
***Results:** Animals that received RGCs in PBS showed the least integration and viability. Both polymers

performed better than the control group suggesting hydrogels support RGCs survival in-vivo, thus allowing these cells to migrate and integrate into the RGC layers of the retina.
***Conclusion/Significance:** Using hydrogels as a delivery vehicle protects cells from the shear stress exerted during the surgery and allows cells to survive longer. This offers the opportunity to use grafted RGCs to preserve and replace host RGCs in the diseased retina.

129 - Matrix Bound Nanovesicles (mbv) Prevent Retinal And Optic Nerve Damage Following Acute Ocular Injury

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***Purpose/Objectives:** Traumatic injuries to retinal ganglion cells (RGC) often result in permanent vision loss due to progressive retinal and optic nerve degeneration. RGC, which constitute a thin cellular layer within the retina with axonal extensions to the brain via the optic nerve, transform light stimuli into electrical signals for image generation in the visual cortex. The acute innate immune response to RGC injury is one of the main causes of cell degeneration and death. The pro-inflammatory (M1-like) events exacerbate the original injury, often leading to irreversible vision loss. The use of extracellular matrix (ECM) bioscaffolds for constructive tissue remodeling and functional repair has been shown to be associated with downregulation of the pro-inflammatory signals and promotion of the pro-remodeling (M2-like) activation pathways. Recently, matrix-bound nanovesicles (MBV) have been identified as a key component of ECM bioscaffolds, capable of recapitulating many of the functional, phenotypic, and immunomodulatory effects of the parent ECM. The present study evaluated the neuroprotective effect of MBV upon RGC following intraocular pressure (IOP) induced ischemic injury in the eye.
***Methodology:** A rat model of IOP-induced ischemia was used to evaluate the effects of MBV injection following injury. RGC viability and axon integrity were evaluated by histology and manganese enhanced MRI; GFAP and GAP-43 expression in the optic nerve were determined; and retinal function was measured by electroretinography (ERG) after 14 days. *In vitro* studies were conducted to determine the cellular mechanisms by which MBV provide protection to retinal ganglion cells. The pro-inflammatory activation state of microglia and astrocytes was evaluated in response to MBV.
***Results:** After acute IOP elevation, MBV significantly preserved RGC survival and axon integrity along the optic nerve. Preserved axon integrity correlated with decreased GFAP and increased GAP-43 expression in the optic nerve. Functionally, MBV prevented IOP-induced decreased retinal photopic negative response (PhNR) and increased in PhNR latency. In vitro, MBV were shown to significantly decrease pro-inflammatory cytokine secretion from isolated microglia and astrocytes, increase RGC neurite growth without altering survival, and neuroprotect RGCs in media conditioned by pro-inflammatory astrocytes. Related molecular studies identified IL-33 encapsulated within MBV as a key mediator in the activation of a reparative and pro-remodeling macrophage phenotype via a novel, ST2 receptor-independent pathway.
***Conclusion/Significance:** Results of the present study suggest that ECM-derived nanovesicle can provide a therapeutic option for preservation of RGC viability and preservation of vision following traumatic injury.

130 - Antimicrobial Drug Eluting Hydrogels For The Treatment Of Bacterial Keratitis

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***Purpose/Objectives:** Blindness due to corneal ulcers represent 5% of all cases worldwide. These ulcers are caused by a range of conditions from autoimmune diseases to infections such as fungal and bacterial keratitis. Bacterial keratitis is often contracted through the improper use of contact lenses and treatment regimens often include broad spectrum antibiotic drops, and sometimes the application of a bandage lens to protect the wound. This method of delivery of the drug however is not efficacious as less than 7% of the active agent reaches the site of injury due to the method of administration. Moreover, in recent years, there has been a drive to reduce the use of antibiotics owing to the growing epidemic of antimicrobial resistance. Nitric oxide (NO) is an endogenously produced molecule which is one of the body's natural responses to fighting infection. This work describes tethering NO donor contact lenses capable of releasing a controlled and sustained dose of NO to target biofilms on infected wounds.
***Methodology:** In this work poly-ε-lysine (pεK) is cross-linked with bis-carboxy fatty acids and functionalised with diazeniumdiolate to produce nitric oxide releasing contact lens gels with a high water content and excellent transparency. The chemical properties of the gels were determined using X-ray photoelectron spectroscopy, Fourier transform infrared spectroscopy and UV vis spectroscopy. The NO released was determined using a chemiluminescent NO detector. The antimicrobial efficacy of the gels against S. aureus and P. aeruginosa was determined after 4 and 24 hr incubation and an indirect cytotoxicity assay was carried out to determine if released NO negatively affected a human corneal epithelial cell line (HCE-T cells).
***Results:** NO release from the functionalised contact lens bandages was evaluated at varying pHs in three different solutions; buffer, cell culture media and nutrient broth. The gels demonstrated a burst release at pH 4, and a lower and more sustained release profile at physiological pH 's (pH 7). The antimicrobial efficacy of the contact lenses was observed as reduction colony forming units of S. aureus and P. aeruginosa using a bactericidal assay. A 3-4 log reduction in S. aureus and up to 1 log reduction with P. aeruginosa was observed after incubation with the NO releasing gels. The indirect cytotoxicity assay demonstrated that released NO did not negatively affect a human corneal epithelial cell line (HCE-T cells).
***Conclusion/Significance:** The contact lens gels exhibit excellent optical and mechanical properties and can release NO under physiological conditions. The gels displayed excellent antimicrobial activity against two of the most common pathogens associated with bacterial keratitis- S. aureus and P. aeruginosa and did not exhibit significant cytotoxicity against a human corneal epithelial cell line. These contact lens gels could be a promising alternative to current antibiotic eyedrop treatments, that are often inefficient and laborious. The use of NO would not contribute to the growing epidemic of antimicrobial resistance and as the delivery of the treatment is direct to the site of infection, less treatments would be required which would ultimately improve patient compliance.

131 - Characterization Of The Immune Pathways In Corneal Scarring And Immunomodulation To Promote A Healthy Repair

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***Purpose/Objectives:** Corneal injuries are common causes of visual impairment. Typical events which can cause cornea damage include surgical trauma, accidents, chemical burns, and military-related injuries. With advances in understanding of biomaterials and immune response, promising solutions for corneal reconstruction are possible. Moreover, regenerative immunotherapies may increase the efficacy and reduce the failure rates of current keratoplasty procedures for complex ocular wound management. In this study, we redirected the immune response with biomaterials to enhance corneal repair. Specifically, Type-2 immunity stimulants are used to promote a pro-regenerative immune environment which will favor the regenerative outcome for cornea reconstruction.
***Methodology:** Mouse corneal debridement wounds of 1.5 mm in diameter were made with a 1.5 mm flat blade. Epithelial debridement wounds were produced by scraping off the epithelium over the central 1.5 mm of the mouse cornea. Once the epithelium is removed, a second scrape will be made, this time applying more pressure to remove the basement membrane and 10 to 15 µm of anterior stromal tissue. After wounding the corneas, 50µl of PBS, decellularized extracellular matrix (ECM) and schistosome egg antigen (SEA) solution were injected into the subconjunctival space. The mice were harvested at day 2, day 7 and day 14 post surgery for early, mid, and late time point examination. Flow cytometry, morphological analysis, corneal haze, and neovascularization were evaluated.
***Results:** In PBS treated (control) cornea wounds, the corneal scar area occupied 5~15% of the entire cornea by day 14. Both ECM and SEA treatments significantly reduced the corneal haze, and the area ratio of the cornea haze was lower than 3%. The α-SMA staining further demonstrated clear neovascularization invaded into the central area of the cornea in PBS group, and no blood vessel invasion was seen in ECM and SEA treated groups. Flow cytometry revealed the immune cell recruitment to the wound site and draining lymph node during wound healing. By day 7 post surgery, ECM and SEA treated corneas recruited a greater density of CD4+ T cells, and SiglecF+ eosinophils. In draining lymph nodes, we found significantly higher IL-4 production (Type-2) in 4Get mice and BALB/c mice treated with ECM and SEM at day 7 and day 14 post surgery. To further highlight the critical role of IL-4 to corneal wound healing, we used GATA1 knockout mice, which had eosinophils (produce the majority of IL-4 cytokine) depleted. The gross observation showed a severe scar formation of 10~25% scar ratio compared to 5~15% in wild type mice, and SEA treatment did not reduce the corneal haze in the GATA1 KO mice.
***Conclusion/Significance:** We took a novel approach by applying Type-2 immunity stimulants to a corneal wound to reduce haze formation. We found an increase in IL-4 production in wounded corneas and draining lymph nodes in the treated mice, resulting in an enhanced wound healing with minimum corneal haze formation. Depletion of eosinophils, the main producer of IL-4, significantly impaired wound healing.

Session Number: 32
Applications and Challenges for Dental Pulp Stem Cells use in Tissue Engineering
Thursday, December 5, 2019, 1:00 PM - 2:30 PM

132 - Bioengineered Alveolar Bone And Tooth Constructs

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***Purpose/Objectives:** Craniofacial bone defects are a common birth defect (1:700), and can significantly influence the physical and psychological health of affected individuals. Our long-term goal is to develop therapies to bioengineer functional alveolar jawbone and teeth, to repair craniomaxillofacial defects in humans. The coordinated repair of alveolar jawbone and teeth is anticipated to reduce surgical costs, improve patient recovery times, and result in improved tissue formation and function. Since craniofacial injuries account for 80% of all battlefield injuries, these studies address an important medical need for both military personnel and civilians afflicted with birth defects, trauma and surgical resection. The purpose of this study was to test the utility of neural crest derived human dental pulp cells (hDPSCs) seeded onto well-characterized tyrosine-derived polycarbonate scaffolds, referred to as E1001(1K)-bTCP) to regenerate alveolar jaw bone in an in vivo rabbit mandible defect repair model.
***Methodology:** Five implants were placed at each of two time points, three hDPSC seeded and two acellular. After 1 and 3 months, rabbit mandibles were harvested, analyzed by MicroCT, demineralized, processed for paraffin embedding and sectioning, and analyzed by histological and immunohistological methods.
***Results:** Our results demonstrated the formation of osteodentin-like mineralized jawbone tissue resembling that of natural rabbit jaw bone. The newly formed bone in cell-seeded samples showed much more homogeneous bone formation throughout the implants as compared to acellular constructs, suggesting that hDPSCs contributed to new bone formation. Active remodeling was observed, with Osteoblasts and Osteoclasts present on newly formed bone surfaces.
***Conclusion/Significance:** In conclusion, these studies demonstrated the potential use of hDPSC seeded E1001(1K)-bTCP scaffolds for alveolar bone regeneration, and in the future, for coordinated alveolar bone and tooth regeneration.

133 - Enhancing Peripheral Nerve Regeneration Using Scaffold-free Dental Pulp Stem Cell Sheets

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***Purpose/Objectives:** Peripheral nerve defects can result from trauma, disease or surgical injury. Current methods of nerve repair include end-to-end suturing for smaller defects and larger defects are bridged using autologous nerve grafts; however, these treatments involve prolonged repair times and full functional recovery is not achieved. Neurotrophic factors (NTF) are molecules known to enhance axon regeneration and growth. The dental pulp, the soft tissue found at the center of the tooth, contains a population of stem/progenitor cells known to endogenously express high levels of NTFs, a

characteristic likely due to their neural crest origin. Scaffold-free cell sheets are layers of tissue consisting of only cells and their endogenous matrix. The goal of this study was to develop scaffold-free DPC sheets as NTF delivery systems that could be wrapped around facial nerves following standard surgical treatment methods to accelerate healing and enhance functional recovery.
***Methodology:** Human DPCs were obtained from extracted third molars. The DPCs were cultured in wells of a 6 well plate in growth medium to form a cell sheet. The expression of NTF genes and proteins including brain-derived neurotrophic factor (BDNF), glial cell-derived neurotrophic factor (GDNF), and neurotrophin-3 (NT-3) were assessed using quantitative RT-PCR and ELISA, respectively. The functional effect of DPC sheets on neurons was assessed in vitro by culturing SH-SY5Y neurons with DPC sheet conditioned medium; the formation and extension of neurites by the neurons in response to the DPC conditioned medium was quantified. The regenerative effects of DPC sheets were evaluated in vivo by wrapping DPC sheets around crush injuries created in the buccal branch of the rat facial nerve. The nerves were histologically assessed at a 3 week time point. Sections were stained with hematoxylin and eosin to assess tissue structure and immunostaining against beta-tubulin was performed to evaluate axon extension through the defect site.
***Results:** DPCs formed a robust cell sheet that could be easily detached from the culture dish and handled. The DPCs in the cell sheets express high levels of NTF genes and proteins. SH-SY5Y neurons cultured with DPC sheet conditioned medium had quantitatively more neurite extensions and longer neurite extensions compared to those cultured with control medium. These effects on the neurons were reversed by inhibiting NTFs verifying that DPC sheets have a significant positive functional effect on neurons in vitro through the production of NTFs. In vivo, crush defects treated with DPC sheets exhibit continuous axon extension through the defect site, whereas defects lacking DPC sheets show a distinct discontinuation.
***Conclusion/Significance:** We have developed a method to direct DPCs to form manipulatable NTF secreting cell sheets that are capable of inducing axon extension in vitro and enhance regeneration in a nerve defect model in vivo. These cell sheets present a promising NTF delivery system that could improve current methods of nerve therapy by accelerating regeneration and enhancing functional outcome.

134 - Transplantation Of Islets Derived From Dental Pulp Stem Cells Into Diabetic Rats

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***Purpose/Objectives:** In this study, we aimed to establish the differentiation protocol of dental pulp stem cells (DPSCs) into pancreatic islets using a three-dimensional (3D) structure.
***Methodology:** DPSCs were differentiated in a 3D culture system using a stepwise protocol. Expression of β-cell markers, glucose-stimulated insulin secretion, and PI3K/AKT and WNT pathways were compared between monolayer-cultured pancreatic cells and islets. Diabetes was caused by streptozotocin in rats, and was transplanted.
***Results:** Islet formation increased insulin and C-peptide production and enhanced the expression of pancreatic markers. Glucose-dependent secretion of insulin was increased by islets. Pancreatic endocrine markers, transcriptional factors, and the PI3K/AKT and WNT pathways were also upregulated. Diabetes caused by streptozotocin in rats was recovered by transplantation. Pancreatic islets were generated from DPSCs in a 3D culture system.

***Conclusion/Significance:** This system could provide novel strategies for controlling diabetes through regenerative medicine.

135 - Decellularized Dental Pulp Extracellular Matrix For Pulp Regeneration

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***Purpose/Objectives:** Aiming dental pulp regeneration, the goal is to provide a suitable environment for cellular infiltration, proliferation, and differentiation. The extracellular matrix (ECM) represents a natural scaffold material resembling the native tissue chemical and mechanical properties. ECM-based scaffolds have shown ability for progenitor cells recruitment, promotion of constructive remodeling, and modulation of host response.
***Methodology:** In this work, we have decellularized and characterized ECM derived from porcine dental pulp. In addition, we have conducted a pilot in vivo study where the matrix was implanted for 8 weeks in beagles' root canal model.
***Results:** Our in vitro and preliminary in vivo data show that the decellularized ECM supports cellular infiltration together with the expression of pulp-dentin and vascular markers (DSP and CD31) compared to the controls.
***Conclusion/Significance:** We conclude showing the feasibility of the production of a decellularized dental pulp ECM scaffold and the validity of its application for dental pulp regeneration.

136 - Utilization Of Antioxidant Agents For Thawing Of Stem Cells For Use In Regenerative Medicine

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***Purpose/Objectives:** Cryopreservation of stem cells (SC) allows for the maintenance of cells at low temperatures to maintain their viability indefinitely for utilization in tissue engineering. During freezing, many cells undergo apoptosis which could be attributed to activation of caspases, dehydration and the production of oxygen radicals.
***Methodology:** As L-Ascorbic Acid 2-phosphate (ASAP) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) are antioxidant agents, the aim of this work has been to evaluate the effect of different concentrations of ASAP or Trolox biochemically and on cell viability after thawing. SC isolated from teeth, characterized and cryopreserved with 10% DMSO in fetal bovine serum were thawed in culture media, composed of DMEM low glucose supplemented with 20% FBS at room temperature. The SC were cultivated with culture media supplemented with 375, 750 or 3,000 µM of ASAP or Trolox. Viability was assessed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the oxidative stress was evaluated by the concentration of reduced glutathione (GSH) and assessment of lipid peroxidation by measuring the malondialdehyde (MDA).
***Results:** The concentration of 3,000 µM of ASAP and Trolox decreased cell viability after 7 days. Thus, the concentrations and cultivation time were reduced. On day 5 after thawing, the results demonstrated

that the cultivation with ASAP and Trolox increased the cellular viability, but not statistically (p=0.91), when compared to the control. The mean absorbance and mean standard error at day 5 for the control (0) were 0.291 ± 0.010; for 375 and 750 µM of ASAP 0.299 ± 0.027; 0.329 ± 0.020 and for 375 and 750 µM of Trolox 0.306 ± 0.036 and 0.304 ± 0.027, respectively. The utilization of 750 µM of Trolox statistically increased the GSH, while the antioxidant agents did not affect the MDA. The percentages of the normalized results of GSH were 100 ± 16 (control), 165 ± 25 (750 µM of ASAP) and 533 ± 56% (750 µM of Trolox, $p < 0.01$), and the results of MDA were 100 ± 8 (control), 83 ± 7 (750 µM of ASAP) and 98 ± 7% (750 µM of Trolox).
***Conclusion/Significance:** The results show that lower doses of Trolox and ASAP did not affect cell viability compared to the control. High doses (3,000 µM) of Trolox and ASAP reduced cell viability, showing that these vitamins, at a high concentration may act as an oxidation stimulator rather than an antioxidant. MDA was not affected by cell cultivation with the analogous of the vitamins. Additionally, GSH was increased on the SC treated with 750 µM of Trolox, but this effect was not enough to enhance cell viability, probably due to the lipid peroxidation caused during freezing, which was not decreased. Because of this, the cells could not overcome the apoptosis rate and improve the recovery rate. An increase in post-thaw ability could be achieved in further experiments by treating the cell cultures with antioxidants together with protease inhibitors in freezing medium. Financial support: MCTIC, FINEP, CNPq, UFRGS and Instituto de Pesquisa com Células-tronco (IPCT).

137 - Processed Lipoaspirate Cells And Dental Pulp Stem Cells The Mirna Expression Profile

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***Purpose/Objectives:** To understand the molecular mechanisms and the signaling pathways activated during the human mesenchymal stem cell (MSC) differentiation process are very important to choose the best MSC source to be used in tissue engineering for clinical use. To try to understand if microRNA has an influence on this better osteogenic potential of DPSC we compared the microRNA expression profile between PLA and DPSC during the osteogenic differentiation.
***Methodology:** Dental pulp stem cells (DPSC) and cells isolated from human lipoaspirates (PLA) were cultured in osteogenic medium during 21 days. Total RNA was extracted during four different osteogenic stage point (0, 7, 14 and 21 days) and submitted to RNA-sequencing. Expression profiles of miRNA networks were determined.
***Results:** Our results showed the 21 miRNAs that alter expression during the bone formation process and which are independent of the cell of origin (DPSC or PLA). However, we identify 4 miRNAs common to DPSC and PLA that alter expression during cell differentiation.
***Conclusion/Significance:** These findings provide better discernment of the molecular bases behind MSC osteogenic plasticity and open up new perspectives to influence the osteogenic potential in MSCs by modulation of a specific miRNA.

138 - Design And Characterization Of A Chitosan Hydrogel Scaffold For Dental Pulp Regeneration

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***Purpose/Objectives:** The objectives of the present study were: (1) to develop natural injectable hydrogel scaffold loaded with bioactive molecule to cell homing; and (2) to characterize the scaffold biologically.
***Methodology:** The hydrogel was developed with thermosensitive chitosan/β-glycerol phosphate disodium salt chitosan and the rheological and biological properties were analyzed. Stem cells from apical papilla (SCAPs) were characterized by flow cytometry and used to analyze the adhesion and proliferation of the hydrogel. Cell adhesion was observed by scanning electron and proliferation was investigated by alamarBlue® assay. Chitosan scaffold (CS) was loaded with 100ng of VEGF (CS-VEGF). ELISA confirmed the kinetic release of VEGF in hydrogel for 7 days
***Results:** The cell population showed stem cell characterization. The chitosan scaffold constituted of dense cellular network with the interconnected pores. Hydrogel presented low viscosity (7,6cP) and promoted adhesion and proliferation of SCAPs. A peak release of VEGF was observed in 24 h (1,4 ng/ml), and a controlled release was observed until 7d
***Conclusion/Significance:** It can be concluded that the chitosan scaffold is biocompatible and favors survival and proliferation of SCAPs. The CS-VEGF scaffold may be a promising biomimetic scaffold for regenerative endodontics.

139 - Novel Strategy Of Tissue Engineering ConstructFor Cartilage RegenerationFrom Dental Pulp Stem Cells

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***Purpose/Objectives:** Chondral lesion is a very prevalent disease, reaching as much as 60% of population. It may result in underlying subchondral bone injury when left untreated leading to disturbances in biomechanics. This process may result in wear and osteoarthritis.Mesenchymal stem cells (MSCs) therapy has a relative ease handling of tissue harvesting, subsequent cell expansion, differentiation and is safe. Dental Pulp Stem Cells (DPSCs) show higher proliferative and immunomodulatory capacities than other lineages. They are collected from deciduous teeth and extracted third molars.
In addition of cell source selection, local delivery is another concern for cell therapy. MSCs monolayers cultured into ascorbic acid led to 3D structure by contraction. It's defined as scaffold-free Tissue-Engineered Construct (TEC). To our knowledge, there is no TEC derived from DPSCs.
The aim of this study was to evaluate the viability of DPSCs in GMP facilities to produce Tissue-Engineered Constructs for cartilage restoration.
***Methodology:** Translational study with miniature pig animal model. The authors performed harvesting, isolation and expansion of DPSCs. A 6 mm full thickness chondral defect was produced in the medial femoral condyle in both posterior limb. One defect was implanted with TEC (DPSCs) and the other was left empty. Histology, immunohistochemistry, RT-PCR, radiology (7-T MRI) and mechanical indentation test were evaluated after six months follow-up.
***Results:** Seven MSCs lineages were acquired from deciduous teeth and a scaffold-free TEC were manufactured. Initial outcome showed differences between both side defects treated with and without TEC derived from DPSCs on MRI, macroscopically and histological evaluations, and indentation test.

***Conclusion/Significance:** Evidence of the viability of extracellular matrix construct (TEC derived from dental pulp) in pre-clinical large animal model enable the translational application of this scaffold free tissue engineering compound in a clinical trial phase I/II in humans.