Poster Session 1
Monday, December 2, 2019, 6:00 PM - 8:00 PM

140 - 3D Hydrogels and Bioinks for Realistic In Vitro Modelling and Bioprinting

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***Purpose/Objectives:** Biogelx have developed innovative biomaterials which offer artificial tissue environments for a range of 3D cell culture and bioprinting applications. The technology platform is based on peptide hydrogels and bioinks which contain ECM-relevant ligands and are highly tunable. They are three-dimensional (3D), 99% water and have the same nanoscale matrix structure as human tissue. Biogelx biomaterials are non-animal derived and their surface chemistry and mechanical properties can be tuned to meet the needs of any given cell or tissue type.
***Methodology:** In more detail; these novel hydrogels and bioinks form a nanofibrous network mimicking the extracellular matrix, which supports cell function, signaling, and proliferation. Moreover, they have been developed and improved to ensure the rheological properties are optimal for bioprinting applications. Additionally, they provide great 3D fidelity and do not require the use of support/sacrificial materials or curing agents. These are very versatile materials which offer important benefits for researchers by providing a base modular gel in which the stiffness and surface peptides can be adapted. The uniqueness of these bioinks is that they offer viscosity control, complete reproducibility, an easy crosslinking method and excellent printability within the same material.
***Results:** This presentation will cover the underlying chemistry of Biogelx's peptide hydrogels, highlighting the range of biochemical/biophysical modifications that can be implemented within the gels, in order to address a wide range of cell-based applications. Some examples of academic and industrial collaborative work shall also be presented, including how the gel tunable properties can be used to influence the differentiation pathway of stem cells for regenerative medicine applications.
***Conclusion/Significance:** The ability to precisely control the hydrogel properties is creating new opportunities in the fields of cancer biology, stem cell research and tissue engineering by offering synthetic-yet-biologically-relevant alternatives to traditional, animal-derived 3D matrices.

141 - Hierarchical Microchanneled Scaffolds Modulate Multiple Tissue-regenerative Processes

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***Purpose/Objectives:** A series of critical events in tissue healing including immune-responses, angiogenesis, and stem cell homing and differentiation orchestrate to relay the regeneration process. Herein, we report hierarchically structured 'microchanneled' 3D printed scaffolds.
***Methodology:** The 3D printed PCL scaffolds with hierarchical microchanneled structures were prepared as schematically shown in Figure. 1A. In particular, camphene was used in the PCL solutions to generate microchannels while 3D printing in a quenching medium. The unique properties of camphene (e.g., miscibility with PCL solvent, high volatility, low sublimation temperature) allow the camphene-PCL

solution to be 3D printable with various microchanneled structures in one step process. During 3D printing in the medium, PCL-camphene struts are co-precipitated to form interconnected networks and the camphene further sublimes to leave microchannels.
***Results:** The μ-Ch reduced the extracellular trap formation of anchored neutrophils at the very beginning of implantation while increasing the number of live cells. Among the macrophages covered the surface of μ-Ch over 7 days a major population polarized toward M2 phase which contrasted with control scaffolds where M1 phase being dominant. The MSCs recruited to the μ-Ch were significantly more than those to control. Furthermore, the neo-blood vessel formation was more pronounced in the μ-Ch. Further assays on the protein sequestration to the μ-Ch revealed a set of chemokines involved in early pro-inflammatory responses were less found whereas representative adhesive proteins engaged in the cell-matrix interactions were significantly captured. The tissue healing and regenerative capacity of the μ-Ch was then confirmed in a critically sized bone tissue model, where those series of events observed are essentially involved to relay bone regeneration process.
***Conclusion/Significance:** The μ-Ch were coherently favorable for the regeneration process of tissues, supporting the engineered scaffolds for potential therapeutic 3D platforms.

142 - Co-axial Extrusion Of Multicellular Blood-Brain Barrier

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***Purpose/Objectives:** Three-dimensional (3D) bioprinting has been widely adapted to construct *in vitro* 3D living tissues or organs. However, in vitro models of central nervous systems tissues have been limited by the low resolution and viability of bioprinting cerebral vessels. Another challenge is the formation of a single monolayer of endothelial cells with physiologically relevant tightness which is characteristic of the blood-brain-barrier. Towards the development of a more physiologically relevant model, we have employed the methodology of coaxial extrusion for single step bioprinting of an in-vitro blood-brain barrier (BBB) model consisting of endothelial cells and pericytes utilizing hybrid hydrogels of Matrigel-alginate (MA) and collagen-alginate (CA).
***Methodology:** For the determination of cellular adhesion and monolayer formation, the hydrogels were pipetted into well plates forming 3D hydrogel disks. First the mixture was thermally crosslinked at 37 °C for 1 hour. Following the thermal crosslinking the mixtures were exposed to $CaCl_2$ for one minute then washed three times with phosphate buffered saline. HCMEC/D3 endothelial cells were then plated on the 2D surface of the disks and allowed to culture for 21 days. For determination of cell viability LDH and PI assays were performed and to determine cellular attachment time for monolayer formation was measured. For construction of the cerebral vessel constructs, Matrigel or Collagen type I was mixed with sodium alginate to provide binding sites then simultaneously extruded with a crosslinking compound ($DIW/CaCl_2$) instantly polymerizing alginate resulting in the formation of hollow microfibers through a coaxial needle. The printing parameters were regulated by an extrusion-based bioprinter and a computer-controlled syringe pump. The construct was printed directly into a media bath to neutralize the cytotoxic crosslinking solution. The Matrigel or collagen was then fully crosslinked by placing at 37 °C for 1 hour to allow for the stabilization of the vessel structure.
***Results:** It was shown that the time for endothelial cells to achieve a monolayer on the surface of the MA and CA hydrogel disk was 33 and 50% longer respectively than the control of a collagen coated well. The PI assay of CA hydrogel showed a viability of 97 ± 3% after 3 weeks of culture. The LDH assay

revealed no detectable cytotoxicity for the MA hydrogel. For the preliminary testing of the coaxial extrusion vessel constructs composed of MA were achieved with an inner lumen diameter of 325 ± 12 um and an outer diameter of 612 ± 53 um. We are currently working on the testing of pericyte viability and loading of cells into the constructs achieve multicellular structures.
***Conclusion/Significance:** Our co-axial extrusion method is capable of producing 3D vessel constructs and the hybrid hydrogels provide a suitable cellular environment. Through the development of a physiologically relevant cerebral vessels future development of larger organ structures as well as production of neurovascular models for modelling disease pathophysiology and drug screening.

143 - Long-term In Vivo Stability Of 3D-Bioprinted Cartilaginous Constructs

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***Purpose/Objectives:** Cartilage restoration and repair in plastic reconstructive surgery has undergone major advances through the introduction of three-dimensional (3D)-bioprinting technology. Different methods have been established to replace lost native structures with autologous cells that can be arranged in a 3D fashion and then transplanted into the target location.
***Methodology:** We have addressed the long-term safety and stability issues associated with 3D-bioprinted constructs comprising a cellulose scaffold and human cells (chondrocytes and stem cells) over a period of 10 months in nude mice.
***Results:** Our findings showed that increasing unconfined compression strength over time significantly improved the mechanical stability of the cell-containing constructs relative to cell-free scaffolds. Additionally, the cell-free constructs exhibited a mean compressive stress and stiffness (compressive modulus) of 0.04 ± 0.05 MPa and 0.14 ± 0.18 MPa, respectively, whereas these values for the cell-containing constructs were 0.11 ± 0.08 MPa (p = 0.019) and 0.53 ± 0.59 MPa (p = 0.012), respectively. Moreover, histomorphologic analysis revealed that cartilage formed from the cell-containing constructs harboured an abundance of proliferating chondrocytes in clusters, and after 10 months, resembled native cartilage. Furthermore, extension of the experiment over the complete lifecycle of the animal model revealed no signs of ossification, fibrosis, necrosis, or implant-related tumour development in the 3D-bioprinted constructs.
***Conclusion/Significance:** These findings accentuate the potential of chondrogenesis using 3D-bioprinting technology and confirm the efficacy of this method for creating cartilage *in vivo* that exhibits long-term stability.

144 - Scaffold-free Vs. Direct Write Vs. Bioprinting Of 3d Cartilage Constructs

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***Purpose/Objectives:** Significant advances in both 3D bioprinting and self-assembly have been made but it is unclear which is the optimal strategy to produce a biological repair. Competing technologies were assessed for cartilage tissue engineering to achieve 3D constructs.
***Methodology:** Rabbit articular chondrocytes were thawed from uncultured frozen stocks (3 donors) and seeded onto devitalized synoviocyte matrix in complete media (DMEM/F12 supplemented with 10% FBS and penicillin/streptomycin). At approximate confluence, cells were trypsinized and divided between: 1) scaffold-free sheets, 2) direct write polycaprolactone mesh seeded sheets, 3) collagen hydrogel bioprinted sheets, 4) direct write polycaprolactone mesh supported bioprinted sheet, 5) scaffold-free dome, 6) direct write polycaprolactone dome, 7) bioprinted dome, 8) direct write polycaprolactone mesh supported bioprinted dome. Sheets and domes were seeded on a 3D printed acrylonitrile butadiene styrene biochamber in a 30ml Nalgene container with a 0.22 µm filter cap for gas exchange. Bioprinted groups were printed at 10 million cells/ml (Se3d r3bel mini, 22G needle syringe based extrusion) in high concentration collagen hydrogel (Lifeink Advanced Biomatrix) using the FRESH method (a gelatin support bath) that was melted (37°C, 20 min) and cultured for 24h in complete media, as this was previously found to greatly improve chondrocyte viability. All other groups were seeded in defined chondrogenic media (DMEM-HG + ITS Premix, 100nM dexamethasone, 1% Glutamax, 1% Pyruvate, 1% MEM NEAA, 130µM ascorbate-2-phosphate, penicillin/streptomycin/fungizone, 1ng/ml TGFβ1) with media exchanges every 2-3 days. Constructs were grown under physiological oxygen tension (5% O_2, 5% CO_2, 37°C) placed on a shaker at 10 rpm on day 2 and increased to 60 rpm on day 5. Constructs were grown for 28 days before harvest. Nanoindentation was performed (Piuma, Optics11) along with biochemical (glycosaminoglycan; dimethylmethylene blue and DNA; Hoechst) and histological assessments (safranin-O and type II collagen).
***Results:** Scaffold-free sheets and domes had the highest shape fidelity and were stiffer than bioprinted sheets (408 ± 937 vs 273 ±411 kPa Young's moduli respectively), in some areas approaching native stiffness. In bioprinted constructs, cells were more dense, with greater extracellular matrix deposition at the edges of both sheets and domes. Glycosaminoglycan deposition in constructs was similar in per cell terms, regardless of condition (34-49 µg/µg DNA).
***Conclusion/Significance:** Self-assembled cartilage constructs produced from chondrocytes expanded on devitalized synoviocyte matrix form cartilage with good shape fidelity, biochemical and histological properties. This has been extended to a dome shape to approximate the shape of the hip. Bioprinted constructs contracted during culture, indicating that further optimization of this process is needed. Polycaprolactone scaffolds somewhat reduced this contraction but defects experienced during printing are not 'healed' during in vitro culture.

145 - Multi-material 3d Printing With Architectural And Compositional Gradients For Directed Osteogenic Differentiation And Spatial Segregation

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***Purpose/Objectives:** Tissue engineering of complex tissue defects such as those found in critically-sized bone fractures remains a challenge. Crucial to the development of better bone tissue replacement

scaffolds is the ability to fabricate scaffolds with complex morphologies as well as an understanding of the factors affecting osteogenic signaling and differentiation. Three-dimensional printing (3DP) has emerged as an attractive technique for the development of heterogeneous scaffolds. In this study, the biochemical effects of architectural and compositional gradients within 3DP hydroxyapatite (HA)/poly(ε-caprolactone) (PCL)-based scaffolds were investigated by incorporating varying porosities and concentrations of β-tricalcium phosphate (β-TCP) and examining the osteogenic maturity of seeded MSCs.
***Methodology:** Specifically, three different porosities (32.9 ± 3.2 %, 50.1 ± 3.8 %, and 65.4 ± 2.4 %) were incorporated within scaffolds by varying the spacing of deposited fibers, and three different concentrations of β-TCP (0, 10, 20 wt%) were incorporated to determine their individual and combined contributions on the biochemical response of MSCs within 3D culture. By delaminating and sectioning the scaffolds between the gradient transition points, we demonstrated differences in the cellular phenotype within each section.
***Results:** Our results showed that increased concentrations of β-TCP were associated with upregulation of early osteogenic markers, including alkaline phosphatase (ALP) activity and osteocalcin, as well as increases in late-stage mineralization. Additionally, MSCs located within the scaffold in areas of higher porosity displayed a more mature osteogenic phenotype compared to those in areas of lower porosity.
***Conclusion/Significance:** The results in this study demonstrate the ability to leverage 3D printing to develop multiphasic scaffolds that directly influence the behavior and maturation of seeded MSCs.

146 - Development Of An Artifact For Assessing Bioink Printability

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***Purpose/Objectives:** The dearth of suitable bioinks is frequently cited as a major limiting factor for the advancement of bioprinting technology. “Printability” in particular has been overlooked in the bioink development process, with many researchers relying heavily on qualitative measures, flawed methodologies, and assessments of limited scope. Other 3D printing modalities have successfully addressed this problem by establishing artifacts, but these artifacts are ill-suited for bioprinting due to the additional limitations of hydrogel-based bioinks. The underlying factors which impact printability are also poorly understood. Several different rheological properties have been implicated, but to date, no study has identified which properties impact the various aspects of printability the most. The objective of this study was to develop an artifact specifically for bioprinting applications and to utilize it both to study the effects of various rheological properties on printability and ultimately to develop improved bioinks.
***Methodology:** The primary objectives of this artifact were to be comprehensive, require less than 10 min print time, reproducible, and applicable to all extrusion-based bioprinting. For rheological characterization, strain sweeps (from 0.2% to 1%), stress sweeps (from 10 Pa to failure), and frequency sweeps (from 0.01 to 100 Hz) were conducted on various bioinks using a 40 mm cone-plate geometry on a DHR-2 Rheometer.
***Results:** The viscoelastic, shear thinning, and yielding behaviors have been found for various single hydrogel materials with a wide range of outcomes seen. All hydrogels showed an increased storage modulus, loss modulus, yield stress, and degree of shear thinning with increasing concentrations.

Additional data has been collected for these hydrogels using a filament drop test as a pre-screening tool and all three filament types have been observed with some hydrogels transitioning from droplet formation to smooth filaments to irregular filaments as their concentrations increased. The artifact has been designed to include shape fidelity (structural integrity, overhang collapse, filament uniformity, Pr) and print accuracy (turn accuracy, material deposition, and filament continuity) measurements. Total print time for the artifact was approximately 3 min. The artifact has been successfully developed to evaluate bioink printability in an efficient, comprehensive, and standardized fashion.
***Conclusion/Significance:** In future work, the developed artifact will be applied at three different institutes with various composite bioink materials. The artifact will be evaluated based on its reproducibility between institutes, the ability to differentiate between bioinks, and for any redundancy between measures. The various outcomes from the artifact will also be related to each bioink's rheological properties to better understand the underlying mechanisms which impact printability. The further development and application of this artifact will result in more efficient and more standardized development of new bioinks, contributing to the long-term advancement of bioprinting technology.

147 - Optimizing Hypoxia Resistant 3D Aligned Cardiac Patch And Structural Maturation For Cardiac Regeneration

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***Purpose/Objectives:** Recently, three-dimensional (3D) bio-printing technology is emerging as a promising therapeutic method for the treatment of ischemic heart disease. Due to the thickness of the 3D structure resulting in a significant decrease in oxygen and nutritional supply, it is now one of the scientific priorities to precisely adjust the 3D space through 3D bio-printing technology.
***Methodology:** This study presented an innovative approach to produce cell patch human cardiomyocytes (AC16) with proliferative capacity and normal human cardiac fibroblasts (NHCFs) that secrete various ECM, and using gelma and collagen as bio-ink. The cells were encapsulated within the hydrogel fiber containing gelma and collagen, and the 3D space was adjusted with 100 μm nozzle to fabricate the heart.
***Results:** We examined the ratio of gelma and collagen at 3:1 (G3C1), 5:1 (G5C1), and 10:1 (G10C1) to obtain the optimum ratio of bio-ink. As a result, cell sprouting on gelma and collagen was significantly increased in the G3C1 group than the G5C1 and G10C1 groups. Moreover, we fabricated an aligned cardiac patch (cPatch) to prove the leptological advantage, and an agglomerated patch (aPatch) as a control. Cell viability was compared between day 0 and day 2 after cell patch production. aPatch showed decreased cell viability from 92% on day 0 to 85% on day 2, and cPatch decreased from 98% on day 0 and 94% on day 2. In addition, we examined the response of cPatch and aPatch to the hypoxic condition (2% CO_2). The immunofluorescent staining of EF5-30A4 (EF-5), which targets oxygen-deficient cells, showed that EF-5-positive cells were abundant in agglomerate-type patches under the normoxic condition. The hypoxic condition induced slightly increased EF-5-positive expression, but it was observed to be similar with the normoxic condition.
***Conclusion/Significance:** In conclusion, we established the optimum ratio of bio-ink and confirmed hypoxia-resistant cPatch with sufficient oxygen and nutrition supply compared to aPatch. Our study could be a fundamental data for clinical application of cell patch transplantation.

148 - 3D Bioprinting Of Bioglass Incorporated Methacrylated Collagen Scaffolds For Bone Tissue Engineering

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***Purpose/Objectives:** Biomimetic scaffolds using collagen and Bioglass (BG) are promising candidates for bone tissue engineering (BTE) applications [1]. However, most existing methods employed thus far to produce collagen-BG scaffolds are not conducive to cell encapsulation during the fabrication process [2]. Recent studies have shown that BG incorporated scaffolds can be fabricated with precise control of pore microstructure and cellular distribution using 3D bioprinting techniques, allowing them to exhibit far more favorable morphological, mechanical and biological properties [3]. The current study is the first attempt to employ 3D bioprinting for the generation of finely controlled, biomimetic collagen-BG scaffolds for BTE applications.
***Methodology:** 3D printed collagen-BG scaffolds were fabricated via Freeform Reversible Embedding of Suspended Hydrogels (FRESH) gelatin method [4] by employing a bioink composed of BG (70% w/w BG:collagen), VA-086 (1% w/v in ultrapure water) and chilled neutralized methacrylated collagen (CMA) solution (6 mg/ml). The printed scaffolds were crosslinked by UV light (11 mW/cm^2) for 1 min and incubated at 37 ^oC for 45 min to melt the gelatin media and recover the scaffolds. CMA scaffolds with no BG were used as control. Alizarin Red S (ARS) staining was performed to confirm the presence of BG in 3D printed CMA scaffolds. The effect of BG incorporation on the compressive modulus of CMA scaffolds was assessed by uniaxial compression tests. SEM and Raman spectroscopy were used to assess the bone bioactivity of BG incorporated CMA scaffolds by incubating them in simulated body fluid (SBF) at 37 °C for 7 days [5]. The print fidelity (i.e., line width and pore size) of 3D printed CMA scaffolds was evaluated using ImageJ analyses.
***Results:** BG incorporated scaffolds stained dark red upon ARS staining confirming the uniform distribution of BG in CMA scaffolds, while non-BG containing scaffolds did not stain. Uniaxial compression tests showed a 2-fold increase in the compressive modulus of the BG incorporated CMA scaffolds over that of non-BG scaffolds. BG incorporation improved the bone bioactivity of 3D printed CMA scaffolds confirmed by the formation of hydroxyapatite layer via SEM and crystallization of the phosphate peak around 960 cm^{-1} with Raman spectral mapping. Finally, the print fidelity of CMA scaffolds in maintained upon BG incorporation.
***Conclusion/Significance:** In conclusion, 3D bioprinting is a promising approach for the generation of biomimetic BG incorporated collagen scaffolds for BTE applications. Future work will employ 3D bioprinting of human MSCs laden CMA scaffolds and investigate BG mediated material-directed osteogenic differentiation of human MSCs as a growth-factor free approach. Further, the 3D bioprinting approach developed in this work can be extended to generate continuous BG gradient incorporated collagen constructs for bone-tendon and bone-ligament interface tissue engineering applications.
Acknowledgements: This work was funded by a grant from NIH (1R15AR071102).
References: [1] B. Sarker *et al.*, *Adv. Healthc. Mater.*, 4(2), 176-194, 2015. [2] J. Glowacki and S. Mizuno, Biopolymers, 89(5), 338-344, 2008. [3] Ojansive et al., Biofabrication, 2019. [4] T.J. Hinton et al., Sci Adv, 1(9), e1500758, 2015. [5] T. Kokubo and H. Takadama, Biomaterials, 27(15) 2907-15, 2006.

149 - Automation And Scale Up Of Tissue Biomanufacturing And Perfusion

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***Purpose/Objectives:** At present, fabricated tissues, whether for clinical or industry use, are manufactured one at a time by hand in a laboratory setting. Commercial-level production of even simple tissues (e.g. cells on a scaffold), much less tissues with more complex architectures and multiple components (e.g. with a vasculature), require more effective manufacturing solutions which will ultimately depend on automated, flexible fabrication solutions. While solutions are emerging for the large-scale expansion of cells and their incorporation into tissue constructs, no solution is yet in place to manipulate these fabricated tissue constructs through a production line or assembly into larger tissue systems/organs at a manufacturing scale. Four key challenges of automated tissue assembly relate to the physical gripping of tissue components, guidance of the assembly process, formation and incubation of the tissue assemblies, and quality assessment of such. With these goals in mind, we have brought forth a robotic-based biomanufacturing platform, the BioAssembly® Workcell, which is uniquely suited to automate the fabrication of living tissues. We are developing and integrating task-specific controls within our existing modeling and manufacturing software, in combination with new manufacturing tool sets and microfluidic capabilities, to seamlessly control and execute a robotic workflow to create prototypical vascularized tissues and tissue models. This includes implementing an automated tissue assembly whereby tissue modules are assembled together to manufacture larger tissues and organs.
***Methodology:** Implementation involves developing and integrating task-specific controls within our existing tissue design and make software in combination with new manufacturing tool sets and microfluidic capabilities to seamlessly control and validate a robotic biofabrication workflow. Importantly, our workflow solution integrates an off-the-shelf analysis platform, General Electric's INCell 6500 high-content scanner, and custom algorithms to provide in-line and post-fabrication tissue product quality assessments and validation. This complete solution, called the Agile Biofabrication Workcell, is a flexible, intuitive, automated tissue fabrication platform for building perfused and non-perfused tissues suitable for use in clinical and non-clinical commercial activities. The automated, integrated workflow involving the fabrication and detection of angiogenesis was developed and evaluated on a vascularized tissue construct
***Results:** We've implemented discrete phases of the automated workflow: 1) loading and transfer of well plates containing construct to and from the GE INCell scanner and the incubation platform, 2) two-stage fabrication of microfluidic channels within a 3D matrix environment, and 3) detection and measurement of angiogenesis in growing human isolated microvessel tissue constructs. With this biofabrication platform, we are developing a variety of fabrication approaches and applications related to bone, liver, microfluidic biosystems, tissue vascularization, and others. Each tissue fabrication strategy will utilize an automated, single-run process with the robotic arm performing the sequence of required, varied, tasks as instructed by the user console.
***Conclusion/Significance:** With this platform, we envision technicians and clinicians utilizing patient-specific data, in combination with patient-specific cells and materials, to fabricate bespoke tissues with sufficient rigor and at a scale to meet the needs of numerous patients.

150 - Development Of A Decellularized Extracellular Matrix Based Bio-ink Having An Enhanced 3d Printability

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***Purpose/Objectives:** Recently, hydrogel form of decellularized extracellular matrix based bio-ink (dECM hbio-ink) derived from animal organ has been attracting many researchers because of its excellent cytocompatibility. In 3D bioprinting system, bio-inks should have proper mechanical properties for precise patterning to develop tissues constructs. However, its low printability and weakness are still big difficulties. Here in, we introduced novel method to prepare dECM bio-ink for high printability. The new bio-ink, dECM hydrogel-gelatin bio-ink (dECM hgBio-ink), was prepared by combining a gelatin bio-ink as composite materials. The dECM hgBio-ink competency, in this research, was evaluated by assessment of mechanical properties, 3D printability and liver tissue specific functionality test.
***Methodology:** Chopped porcine liver tissue was immersed in detergents such as sodium dodecyl sulfate (SDS), sodium deoxycholate (SDC) and Triton X-100 with ammonia. Liver dECM were characterized in histology and biochemicals to optimize decellularization protocol. When detergent groups had DNA below 50 ng/mg, remaining biochemicals were quantified. The liver dECM was digested using pepsin to produce dECM hBio-ink. In addition, dECM hgBio-ink was combined with dECM hBio-ink and gelatin bio-ink composed of hyaluronic acid, gelatin and fibrinogen. On the research, gelatin bio-ink, dECM hydrogel, dECM hgBio-ink were analyzed in mechanical properties. Furthermore, using 3D bioprinter, 2D patterning and 3D stacking tests were conducted to evaluate improved printability. Lastly, cytocompatibility and hepatic functionality of primary hepatocyte were evaluated.
***Results:** Among various detergent conditions, 1%v/v Triton X-100 with 0.1%v/v ammonia solution removed DNA contents below 50 ng/mg in 48 hours. This condition well preserved highest GAGs, collagen, elastin contents. After optimization of liver decellularization, mechanical property and 3D printability of the dECM hgBio-ink were greatly improved compared with conventional dECM hBio-ink. Especially, developed dECM hgBio-ink showed 41.37 times improved viscosity and 6.22 times higher compressive modulus comparing with dECM hBio-ink. Besides, the developed bio-ink had 3.31 times accurate pore fidelity(%) after 3D pattern printing. Above all, the dECM hgBio-ink showed superior performance in stacking layers for 3D printing as designed, whereas the dECM hBio-ink collapsed. Lastly, the dECM hgBio-ink possessed improved functionality of primary hepatocytes in albumin, urea secretion and CYP expression. As a result, it was successfully confirmed that the hgBio-ink strengthened printability with composite materials and supported hepatic functionality as dECM hBio-ink.
***Conclusion/Significance:** Decellularized extracellular matrix based bio-ink material suggests new possibility in successful fabrication of functional tissue or organ. in this research, liver dECM hgBio-ink overcame 3D printability and mechanical properties. Accurate micro-patterning of dECM hgBio-ink was available in bioprinting system. The strategy to combine composite bio-inks can be widely applied to various organ derived dECM bio-ink in 3D bioprinting technology.

152 - Retinal Pigment Epithelial Cell Sheet Fabrication Derived From Human Induced Pluripotent Stem Cells In An Automated Closed Cell Culture System For Regenerative Medicine

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Cell production is a fundamental technology for regenerative medicine. Conventionally, it requires numerous skilled experimental operators performing manual cell culture. Major obstacles of manual culture are the variable quality of products based on the skills of operators and costs. Technological development including automated cell culture has been made to overcome these issues. Age-related macular degeneration is a refractory ocular disease that causes severe deterioration in central vision due to senescence in the retinal pigment epithelium (RPE). We previously reported the results of an autologous transplantation of induced pluripotent stem (iPS) cell-derived RPE cell sheets that the transplanted sheet remained intact and functioned for one year after surgery. In this study, human iPS cell-derived RPE (hiPS-RPE) was grown using our latest automated cell culture equipment in a closed system, to determine the feasibility of the machine culture of RPE cell sheets. Immunohistochemical analysis using specific antibodies against tight junction, basement protein and RPE markers showed no significant difference between hiPS-RPE cell sheets fabricated by machine and manual culture. The amounts of secreted pigment epithelium derived factor and vascular endothelial growth factor in the culture media determined by ELISA suggested that hiPS-RPE cultured by both machine and manual methods exhibits polarity. Transepithelial electrical resistance value of the machine-cultured hiPS-RPE cell sheets was higher than that of the manually cultured . These results are suggesting that hiPS-RPE cell sheets were successfully cultured by a closed automated cell-culture system, thus removing the need for operational skills and facilities. **References:** *PLoS One*. Mar 13;14(3):e0212369, 2019

153 - Hepatocyte Spheroids With Directly Reprogrammed Human Hepatocytes-like Cells For Personalized Drug Evaluation

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Drug-induced liver injury it is the most common cause of acute liver failure in the United States, and is also the most frequently cited reason for withdrawal of medications from the marketplace. Primary hepatocyte 3D spheroids are useful for predicting drug-induced liver injury. However, there is a shortage of primary human hepatocytes. Directly reprogramming fibroblasts into human hepatocyte-like cells will address the shortage of primary hepatocytes and make personalized drug prediction possible. We aim to study the possibility of making hepatocyte 3D spheroids with directly reprogrammed human hepatocyte-like cells. In order to improve the reprogramming efficiency from adult fibroblasts, we used six lineage-specific transcription factors, HNF4, FOXA2, FOXA3, ATF5, PROX1 and HNF1 for direct reprogramming. We used two lentiviral vectors, each expressing three factors, to deliver the 6 factors. The factors can efficiently reprogram fibroblasts into hepatocyte-like cells expressing albumin and alpha-1-antitrypsin. The reprogrammed hepatocyte-like cells can form viable 3D spheroids with primary liver-derived supporting cells. This method will make it possible to generate patient-specific hepatocyte 3D spheroids for personalized drug evaluation.

154 - Chitosan/dextran-based 3D Bioprinted Core/shell Regenerative Constructs

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Cell-based tissue engineering is an encouraging methodology for treating damaged tissues, yet challenges remain regarding the development of fully functional biomimetic wound healing constructs. To address these concerns 3D bioprinting has emerged with the goal of fabricating vascularized networks within bioengineered constructs. A promising strategy using core shell (c/s), extrusion 3D bioprinting technology that employs biomaterials to biofabricate regenerative, vascular constructs is reported. A custom designed cell-responsive bioink consisting of human bone marrow derived mesenchymal stem cell (hBMSC)-laden gelatin methacrylate (GelMA) shell surrounding a dual peptide, succinylated chitosan (C)/dextran aldehyde (D)[1-2] human umbilical vein endothelial cell (HUVEC)-laden core was successfully printed resulting in organized microdesigns. Physical, mechanical, and biological characterizations of the c/s constructs showed the constructs to exhibit an appropriate mechanical integrity, biocompatibility, and served as guides or conduits to direct subsequent vessel formation. In all, c/s 3D bioprinted, bicellular, composite constructs provided a suitable microenvironment for *in vitro* stem cell viability, delivery, and differentiation. We foresee these c/s regenerative constructs as representing a fundamental step toward engineering larger scale regenerative, vascularized tissues.

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155 - Efficacy Evaluation Of Transplantation Of Adipose-derived Stem Cell-based Multi-cell Sheet Into Cardiovascular Disease

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***Purpose/Objectives:** The integration of cell sheet engineering technology has received much interest in the regenerative medicine. Recently, the development of human adipose-derived stem cell (hASC) sheets has been proposed to improve the engraftment, cardiac function, and vascularization in cardiovascular disease animal models.

***Methodology:** In this study, the methods of producing cell sheets using human cardiomyocytes (AC16) and cardiac fibroblasts (NHCF) which are component of hearts were established. Their therapeutic efficacy was also examined using cardiovascular disease animal models.

***Results:** First of all, ASC sheets were formed using thermo-responsive plates. In addition, cardiomyocytes and cardiac fibroblasts were seeded to produce ASC sheets covered with two cell types (ASC-based multi-cell sheets). After 48 hrs of incubation, the cross-section of ASC-based multi-cell sheets revealed the mixed and multi-layered sheets of AC16, NHCF and ASC. Cytokine arrays of secretome revealed that ASC-based multi-cell sheets showed increased secretion of various growth factors and cytokines compared to ASC sheet. Significantly increased expression of PHH3 and decreased expression of cleaved caspase-3 were detected in ASC-based multi-cell sheets compared to only ASC sheet. Furthermore, four groups of acute myocardial infarction (AMI) rat models were assigned: AMI induction only (Sham), ASC sheets, ASC-based multi-cell sheets, and ASC-based multi-cell sheets with substance P (SP) transplantation. After 6 weeks of transplantation, echocardiography revealed that significantly improved ejection fraction and fraction shortening in the ASC-based multi-cell sheets and ASC-based

multi-cell sheets with SP group compared to sham and ASC sheet groups. Moreover, transplantation of ASC-based multi-cell sheets with SP showed enhanced neovascularization in AMI rat models.
***Conclusion/Significance:** In conclusion, these results indicated that the use of ASC-based multi-cell sheets as a novel tissue engineering approach to improve the cardiac function and vascularization.

156 - Homing Of Adult Stem Cell Derived Smooth Muscle Cells To Aortic Aneurysms For Augmented Ecm Repair

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***Purpose/Objectives:** Impaired elastic matrix homeostasis in the wall of the abdominal aorta is a major determinant for growth of rupture prone aortal expansions called abdominal aortic aneurysms (AAAs). Reversing pathophysiology of AAAs requires an external stimulus to elastic matrix regenerative repair since adult vascular smooth muscle cells (SMCs) and more so aneurysmal SMCs lack this ability. In this context, we are investigating a matrix-regenerative therapy for AAAs based on delivery of bone-marrow mesenchymal stem cells (BM-MSCs)-derived SMCs (cBM-SMCs). We have previously shown cBM-SMCs to exhibit significantly higher elastogenic potential than do aneurysmal SMCs and to secrete biological factors that provide significant pro-elastogenic and anti-proteolytic stimuli to the diseased cells, outcomes not seen with undifferentiated BM-MSCs. BM-MSCs are known to home in to SDF-1α overexpressing injured tissues via chemokine receptors CCR3 and CXCR4. In this study, we investigated if our derived SMCs also express these homing receptors and if so, if priming with the inflammatory cytokine TNF-α can promote their migration to diseased cells/tissue.
***Methodology:** cBM-SMCs were differentiated from rat BM-MSCs on an Fn substrate, in presence of growth factors TGF-β1 and PDGF-ββ as we have published. For the expression of homing receptors, the cells were seeded at a density of 30k and 15k per well in a 6 well plate for western blots and PCR respectively and 10k cells/well in a 24 well plate for IF. After 2 weeks of culture, the cells were serum starved for 3 hours and treated with 3 doses (0.1, 1 and 10 ng/ml) of TNF-α for 24 hours for RT-PCR and western blots. The cells were then harvested and experiments were performed following the standard protocol. For IF, the cells were cultured for a week and treated similarly with TNF- α as described above and IF was performed according to standard protocol. Migration assay was performed in a microfluidic device and the cells both unprimed and primed with TNF-α were observed under time-lapse microscope for 48 hours. Ongoing studies are investigating homing abilities of the BM-SMCs in an elastase-injury rat AAA model.
***Results:** No significant differences in the mRNA expression of CXCR4 and CCR3 was seen with TNF-α treatment in both BM-MSC and cBM-SMC however, mRNA expression of both the receptors were significantly higher in cBM-SMCs vs BM-MSCs at all the TNF- α doses except 10 ng/ml. Western blots and IF results were consistent with the mRNA expression. With the time lapse instrument, cells were seen to have obvious migration towards SDF-1α compared to control. Our initial in vivo experiments suggest that intravenously infused BM-SMCs are capable of homing in to the injured AAA wall.
***Conclusion/Significance:** The results so far supports our hypothesis that cBM-SMCs have homing potential as indicated by the expression of homing receptors.

157 - Design Of Exosome Mimics For Vascular Regeneration

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***Purpose/Objectives:** Exosomes are nanovesicles (50-150 nm) derived from the invagination of the plasma membrane (PM) of cells and released to mediate critical cell paracrine functions, including cell proliferation, angiogenesis, and immunomodulation. Exosomes derived from endothelial progenitor cells (EPC) have been shown to facilitate vascularization *via* delivery of miRNA-126. However, their therapeutic translation has been greatly hindered by the inherent disadvantages in exosome isolation, purification, and standardization. Here, we sought to overcome these shortcomings by engineering a biomimetic synthetic exosome that can recapitulate the proangiogenic, targeting, and cell recruiting properties of native EPC-derived exosomes. We propose that exosome mimics (EM) can be synthesized by coating an miRNA-126-loaded poly(lactic-co-glycolic acid) (PLGA) core with SILY (a collagen-targeting ligand)-functionalized EPC-membrane shell in order to mimic the functional miRNA cargo, targeting potential, and physical characteristics of native EPC exosomes.
***Methodology:** Loaded PLGA cores were synthesized using a water/oil/water double emulsion method and mechanically coated with plasma membrane fractions isolated from EPCs. Fluorescence microscopy and transmission electron microscopy (TEM) were used to visualize morphology to confirm coating while dynamic light scattering (DLS) was used to characterize the size and homogeneity of the particles over 15 days. Next, a proof-of-concept model was established to simulate SILY ligand conjugation to the EPC membrane shell by using a DBCO-sulfo-NHS molecule to link azide-Cy5, a proof-of-concept dye, to the PM with Click chemistry. Fluorescence microscopy was used to confirm successful ligand conjugation.
***Results:** PLGA nanoparticle cores were successfully synthesized and demonstrated high stability in water, remaining a homogeneous and consistent size of ~100 nm over 15 days at 4°C. Molecules were also able to be loaded within the PLGA core using DiI as a proof-of-concept cargo. SDS-PAGE and Western blot analysis confirmed plasma membrane isolation with exosome surface marker retention while fluorescence microscopy and TEM showed successful coating onto the PLGA cores. The size and stability of the EMs were seen to be dependent on the PM:PLGA ratio, with higher PM: PLGA ratios resulting in smaller diameters and increased stability over 2 weeks. Surface modification of the PM was also shown to be possible via Click chemistry. Fluorescence microscopy showed the successful proof-of-concept conjugation of the ligand to the PM in the presence of the DBCO-sulfo-NHS.
***Conclusion/Significance:** This engineered system shows potential in mimicking native exosomes. Thus far, a relatively stable biomimetic exosome structure has been synthesized to broadly recapitulate the physical structure of EPC-derived exosomes. Future work will focus on optimizing the EM structure and assessing its angiogenic properties *in vitro* and *in vivo.*

158 - Blended Electrospun Meshes With Low Amounts Of Collagen Type 1 Are Hemocompatible

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***Purpose/Objectives:** Blended scaffolds made with natural and synthetic biomaterials are often used in vascular applications to provide benefits of both components. For example, we previously showed that 10% collagen incorporation with poly(ε-caprolactone) (PCL) within tissue engineered vascular grafts (TEVGs) reduced macrophage marker expression 6 weeks post-grafting, including the *Cd68* gene and the CD80 protein, yet the grafts still exhibited mechanical integrity [1]. Additionally, there were no concerns with thrombosis even though collagen type 1 is pro-thrombogenic. Thus, the goals of this study were to systematically investigate the impact of collagen ratio on *in vitro* hemocompatibility and to characterize the material to investigate the biomaterial mechanism for the observed results.
***Methodology:** For hemocompatibility, meshes were prepared by electrospinning 0, 10, 25, and 50% (w/w) blends of collagen/PCL dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol. Thick sheets were incubated in rat platelet-rich plasma, dried, and analyzed with SEM to semi-quantitatively characterize platelet adhesion and activation. Testing of the meshes and controls also included hemolysis. For characterization, blends of collagen type 1 and PCL were electrospun on glass coverslips to clearly observe individual fibers. Sample were analyzed with polarized light both with and without a lambda plate to semi-quantitatively characterize the material crystallinity, including the degree of crystallinity and crystal orientation. Samples were also stained with picrosirius red to assess the presence of collagen.
***Results:** Interestingly, our hemocompatibility testing revealed that 10% and 25% collagen electrospun meshes had significantly lower platelet adherence than 50% collagen and comparable or lower levels than pure PCL (n = 3; one-way ANOVA with Tukey). Additionally, 10% and 25% collagen samples had more activated platelets (e.g., dendritic) than 50% collagen or pure PCL. Only 50% collagen exhibited large platelet aggregates, indicating a difference on these surfaces. Our picrosirius red staining demonstrated increased birefringence in fibers with low collagen percentages indicating that collagen was present on the fiber surface for platelet interaction. However, the results suggest that percent collagen within the fibers impacts PCL crystallinity as a systematic decrease in birefringence and an increase in changes in crystal direction was observed when collagen concentration was increased from 0% to 50%. This indicated a more random orientation of individual PCL crystals. We are also investigating different macroscale fiber orientations and fiber diameters to determine their impact on electrospun mesh crystallinity. To further investigate the biomaterials mechanism, we are currently investigating the distribution of collagen with TEM.
***Conclusion/Significance:** The aforementioned hemocompatibility results combined with our previous results [1], suggest that the balance between collagen and synthetic polymers can have an important impact on TEVG viability. This impact cannot be explained by the lack of collagen on the surface since it is still present with lower collagen ratios. It is clear that different collagen ratios impacted the PCL crystallinity, but also potentially the collagen structure and its distribution. These results are also likely relevant for other natural / synthetic blend meshes used as biomaterials. **References:** [1] Shojaee et. al., Acta Biomaterialia, 2017, 64:p.80; [2] Motlagh et. al., Biomaterials, 2006, 27:p.4315. **Acknowledgments**: American Heart Association support (18AIREA33960390)

159 - A Biofunctional Electrospun Vascular Scaffold For Replacing Small-diameter Blood Vessels

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***Purpose/Objectives:** Diseases of the vascular system affect a large number of patients and have resulted in a clear clinical need for the development of functional vascular substitutes. We have previously fabricated a vascular scaffold by electrospinning of poly(ε-caprolactone) (PCL) combined with collagen [1]. In this study, we aimed to develop a biofunctional electrospun vascular scaffold by chemically conjugating antibody and anti-thrombogenic agent for small-diameter blood vessels. We hypothesized that multiple bioconjugation of endothelial progenitor cells (EPC)/endothelial cell (EC) specific antibodies and anti-thrombogenic agents onto the vascular scaffold could facilitate in situ endothelialization while preventing blood clotting.
***Methodology:** We optimized bioconjugation parameters on a vascular scaffold to provide proper biological properties and structural configuration that enhanced cellular interactions of ECs on the lumen. To examine the functionalized vascular scaffold, the scaffolds were examined by flow chamber study using a parallel rectangular flow chamber system for cell capturing capability and platelet adhesion assay for anti-thrombogenic effect. To test the functionalized bioengineered vessels, the heparin-conjugated vascular constructs were implanted in a sheep carotid artery. The implanted vascular constructs were followed by ultrasound to evaluate the vessel diameter as well as patency quantitatively.
***Results:** The biofunctionalized scaffolds conjugated with EPC/EC-specific antibodies and heparin were able to achieve effective EPC/EC capturing and anti-thrombogenesis. *In vivo* experiment showed that these vascular scaffolds maintained a high degree of patency and structural integrity without eliciting a histologic inflammatory response over the course of the 6-month period in sheep. Moreover, the matured EC coverage on the lumen and smooth muscle layer were observed at 6 months after implantation.
***Conclusion/Significance:** We demonstrated that the generation of the biofunctional vascular scaffold, along with existing tools for selection and directed immobilization of EPC/EC-specific antibodies and anti-thrombogenic agent, could provide the necessary components for successful tissue engineering of small diameter blood vessels.
Acknowledgments: This work was supported by the Army, Navy, NIH, Air Force, VA, and Health Affairs to support the AFIRM II effort under Award No. W81XWH-14-2-0003. The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.
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160 - Stem Cell-loaded Coaxially Electrospun Cardiac Patches For Regeneration Of Infarcted Myocardium

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***Purpose/Objectives:** Myocardial infarction (MI) is a leading cause of death and disability throughout the world. MI results in the irreversible loss of cardiomyocytes and triggers an immune response which leads to inflammation and cytokine activation resulting in fibrotic scar deposition. The damage to the infarcted myocardium leads to remodeling of left ventricle and reduced left ventricular function.

***Methodology:** Polymeric biocompatible cardiac patches are promising biomaterials to counter left ventricular dilation and reduce remodeling after MI by inducing stem cell function in the heart. Electrospinning is a versatile technique to develop extracellular matrix mimicking structures for various regenerative applications.
***Results:** Core-sheath electrospun polycaprolactone (PCL) (core) and gelatin/gelatin methacyrlate (sheath) fibers were attained by coaxial electrospinning and its role as cardiac patches was tested with electrospun polycaprolactone fibers as control.
***Conclusion/Significance:** The appropriate patch will allow increased survival of mesenchymal stem cells, enabling increased efficiency in the generation of damaged myocardium in a mouse model of myocardial infarction.

161 - Vascular Grafts Functionalized With Magnetic Particles For Superior Endothelialization With Autologous Cells

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***Purpose/Objectives:** Cardiovascular disease is the leading cause of death worldwide and commonly associated with occlusion of blood vessels and stenosis. Treatment methods involve revascularization utilizing autologous vessels and synthetic grafts. However, the limited availability and donor site morbidity of autologous vessels; and failure of small-caliber synthetic grafts due to thrombosis, restenosis, intimal hyperplasia around the anastomosis site and graft infection suggests a critical need for synthetic vascular grafts that could serve as a potential solution for the future of vascular surgery (*Pashneh-Tala et al., Tissue Engineering: B. 2016*). Synthetic vascular grafts are a promising choice for patients requiring long term revascularization. To achieve this and limit graft failure, we present an approach that involves the design of magnetically endothelialized vascular grafts for small diameter applications under high pressure arterial flow.
***Methodology:** Magnetic grafts with wall thickness ranging from 1.05-1.8 mm were fabricated by electrospinning biostable polyurethane and stainless-steel magnetic powders using a layer by layer technique. As preliminary studies, magnetic properties of the graft were tested using a magnetometer and its thrombogenicity analyzed with a thrombin generation assay (TGA) kit. Dynamic radial compliance of the grafts was measured using a piezoresistive pressure transducer with accuracy to +/- 2% of the reading (at maximum pressure 150 mmHg, +/- 3 mmHg). Grafts were held under isotonic conditions with a longitudinal preload of 50 g and the change in external diameter was measured by a Laser-Scan Micrometer at three pressure ranges: 50-90, 80-120, and 110-150 mmHg.
***Results:** The magnetic field strength of magnetized grafts represented as mean +/- SD (n=8) was 227 +/- 2.4 mG. Coagulation potential of the graft was assessed according to ISO 109934:2002 and maximum thrombin generation patterns for the magnetic graft and intermediary reactive reference material, PDMS, was found to be 485 and 1602 mU/mL/min/cm^2 (n=1), respectively. Dynamic radial compliance given as mean +/- SD (n=3) of the magnetic grafts was 2.248 +/- 1.638 % per 100 mmHg at physiological conditions (80-120 mmHg) and 5.885 +/- 2.181 % per 100 mmHg at 110-150 mmHg which is similar to previously reported values for synthetic vascular grafts (*Konig et al., Biomaterials. 2009*).
***Conclusion/Significance:** The range of magnetic strength has shown to capture and retain magnetically labeled endothelial cells in our previous studies (*Tefft et al., IEEE T.Magn. 2013*). Thrombin generation

started between 2-3 min and reached its maximum after 4 min of plasma-incubation in magnetic graft. This was well within the range for a valid test provided by the certificate of analysis in TGA kit. TGA assay needs to be repeated for all groups to confirm thrombin generation potential prior to in vivo implantation. Preliminary results indicate that our grafts are within the benchmark based on ISO-7198 standards. Experiments including cell capture studies (for porcine endothelial cells labelled with super paramagnetic iron oxide nanoparticles; *Tefft et al., J Vis Exp. 2015*) and burst pressure will be repeated/performed with at least n=3 sample size and statistical analysis with one-way ANOVA and post-hoc analysis will be performed to determine significant difference among groups.

163 - Engineering A Novel Microphysiological System To Recapitulate Biologic Barrier Functions

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***Purpose/Objectives:** This abstract reports the design and validation of a novel microphysiological system (MPS) capable of recapitulating various in-vivo microenvironments and enables the functional assessment of multicellular constructs engineered to model biologic barriers. Specifically, we demonstrate the filtration function of the kidney that occurs in the glomerulus and the air-liquid interface (ALI) of the lung alveolus. Despite the widespread use of polydimethylsiloxane (PDMS) for microfluidic cell culture applications, its absorption of small molecules has created a hinderance to the translation of MPS. Previously, our group has demonstrated the feasibility of subtractive rapid prototyping (SRP) to develop resealable, optically-clear fluidic chips from biocompatible poly(methyl methacrylate) (PMMA). Here, we have expanded our use of SRP of PMMA to develop a novel microfluidic chip with an apical and basal flow channel and incorporated a pressure-driven microfluidic pumps to recapitulate in-vivo microenvironments.
***Methodology:** Using 3D computer-aided design (CAD) software, a two-piece fluidic chip comprised of two fluidic channels and a culture well was developed in a resealable form-factor. Chips were fabricated using a micromilling machine from optically-clear PMMA workpieces. The final form factor of the chip was laser cut from the workpiece using a 30W CO_2 Laser Engraver. Silicone gaskets, fabricated in-house, create a fluidic seal in the upper channels of the chip when clamped in a commercial microfluidic chip holder. Cells were cultured on a porous membrane which was bonded into the culture well of the chip. The glomerular pressure gradient was modeled using two pressure-driven microfluidic pumps which independently perfuse the apical and basal channels. To mimic breathing forces, mechanical loading of the alveolar model was achieved using a cyclic application of air pressure in the apical channel. Tight junction formation, in static culture, for both models assessed by measuring the trans-endothelial/epithelial electrical resistance (TEER). GFB function was assessed in the MPS by calculating albumin filtration using the equation for renal clearance.
***Results:** The MPS was validated by demonstrating leak-free perfusion and maintaining viable HUVECs after 24hrs of culture. Recapitulation of the glomerular filtration barrier (GFB) was achieved by co-culturing conditionally immortalized human podocytes and human glomerular microvascular endothelial cells on opposite sides of a permeable culture insert with 3µm pores. The lung alveolus was modeled by an air-liquid co-culture of human alveolar epithelial cells and human lung microvascular endothelial cells, developed using previously described methods. Both models achieved confluence in 1 week as measured by TEER. The ALI model was improved by the inclusion of dexamethasone in the culture

media. The GFB co-culture model significantly reduced urinary clearance of albumin.
***Conclusion/Significance:** We have engineered a novel MPS that can recapitulate physical, cellular, and functional features of both the GFB and lung alveolus. The fabrication of devices from PMMA, which is less absorptive to small molecules than PDMS and amenable to large scale manufacturing (i.e. injection molding), addresses some of the translational challenges that have hindered widespread adoption of MPS. Future development will focus on developing and characterizing models of drug induced injury and disease states within our MPS.

164 - A High-throughput Approach To Compare The Biocompatibility Of Candidate Bioink Formulations

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***Purpose/Objectives:** When investigating bioprinting as a technique for fabricating physiologically relevant tissue constructs, modern bioengineers have an abundance of natural and synthetic biomaterials at their disposal. However, there is a scarcity of clearly-defined methods and minimum standards that outline biocompatibility and what makes a bioink worthy of further investigation, and this impedes the development of future clinically-translatable bioprinting therapies. This work establishes a high-throughput method to quantify and compare the biocompatibility of any bioink formulation, where the effect of composition as well as encapsulated cell density can be observed from the resulting data.
***Methodology:** Three straightforward laboratory assessments of cellular viability and metabolism were compiled in coordination with the use of microscopy-suited multi-well plates. Through viability staining, assessment of cell population proliferation via adenosine triphosphate (ATP) sensing, and observing phenotypic surface marker expression via confocal laser-scanning microscopy, this approach concurrently observed cellular behavior in multiple hydrogel formulations and cell densities. Cell handling, sample transfer, and the completion of experiments on separate groups at separate times were eliminated to prevent the introduction of any potential confounding factors present in certain biocompatibility assessments seen in the published literature. Our high-throughput method was validated by encapsulating human mesenchymal stem cells (hMSCs) in four common bioink materials composed of on collagen-, hyaluronic acid-, gelatin-, and alginate-based formulations at two density levels, $1x10^6$ and $1x10^5$ cells/mL.
***Results:** Clear live/dead staining microscopy was attainable for all samples, and methacrylated collagen (CollMa) and methacrylated gelatin (GelMa) bioinks indicated the best cell viability within the cultured bioink droplets after seven-days. A proliferation assay applied directly in-plate revealed significant differences between the bioinks, with GelMa groups possessing the largest cell populations by day 7 of the culturing period. Immunofluorescent staining, also performed directly in-plate, was able to distinguish between the nuances of cellular morphology observed in each bioink group via expression of phenotypic surface markers. The cell-adhesive bioink materials best preserved the adherent, CD90-positive, CD34-negative phenotype of these hMSCs while alginate-encapsulated cells did not spread within their matrix and expressed the hematopoietic CD34 surface marker, complementing alginate

groups' low viability and proliferation results from the other assessments.
***Conclusion/Significance:** Among the evaluated bioink groups, results from this method conclude that GelMa-based bioinks should be evaluated in further bioprinting studies using hMSCs for tissue engineering applications, particularly if maintenance of the undifferentiated hMSC phenotype is crucial. This is among the first works demonstrating a way to standardize the direct comparison of bioink formulation effects on cell biocompatibility, and to also challenge commercially available biomaterial claims of universal cell compatibility. With the proof-of-concept results given here, a replicable platform is created which aims to aid researchers in the selection of the most appropriate biomaterials for future bioink studies. We believe that the distribution of broadly applicable methods and standards will support the biomanufacturing of tissue products that reach clinical translation.

165 - Evaluation Of Dental Pulp Stem Cells Proliferation And Differentiation On Three Dimensional Scaffolds

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***Purpose/Objectives:** • An In Vitro study to evaluate the odontoblastic differentiation and Dental Pulp Stem Cells (DPSC) cells proliferation and survival in three dimensional scaffolds.
***Methodology:** • Normal human impacted third molars were collected from adults (19-29 years of age) and stem cells were isolated at the Faculty of Dental Sciences, King George's Medical University under approved guidelines set by the ethics committee of the university. • The crowns of immature human premolars extracted for orthodontic reasons in 12- to 14-year-old patients were sectioned, and the dental pulps were carefully removed without touching the predentin. Decoronation of tooth was done to standardize the length to 12 mm. Teeth were cleaned and disinfected with ethylic alcohol and thoroughly washed with PBS. Roots were stabilized vertically on inverted Transwell inserts in such a way that only the apical third of the root was immersed in cell culture medium. Dental pulp stem cells (DPSC's) were suspended in 50 µL ofHyStem® Cell Culture Scaffold, in PRF scaffold and injected into the roots of human premolars (n = 24 teeth/experimental condition). After 7 to 28 days, DPSC's were removed from the root canals, and RNA purification, amplification, and RT-PCR for DMP-I, DSPP, and MEPE were performed.
***Results:** Pre-liminary results obtained so far are quite promising with identification of DMP-1, DSPP and MEPE growth factors on different scaffolds used in this study. to tr
***Conclusion/Significance: Data indicate the role PRF in the induction of soft and hard tissue healing, which could be utilized further, to understand the regenerative procedures to treat the periodontal diseases in future**

166 - Aerogel Scaffolds For Bone Regeneration: In Vitro Assessment

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***Purpose/Objectives:** Temporary scaffolds are to conduct bone regeneration. Several techniques have been proposed for scaffolds fabrication but with various limitations. Three-dimensional (3D) printed scaffolds have rough surfaces and slow degradation rate and is difficult to control the pore interconnection, pore size and overall porosity of the scaffolds. Other techniques are challenged by balancing the macro and microstructural characteristics for clinical application. We aim to develop and evaluate a new synthetic scaffold, aerogel, for its biocompatibility of osteoblast adhesion and proliferation, as a possibility for a scaffold for improved bone regeneration.
***Methodology:** We tested a new supercritical fluid assisted technique for the formation of 3D aerogel scaffolds. This technique involved three subprocesses: the formation of a polymeric gel loaded with dioxane as porogen, the drying of the gel using -20°C freezer, followed by drying the sample in high vacuum and vented the vacuum chamber with nitrogen gas to eliminate the porogen. We obtained poly(l-lactic acid) (PLLA) aerogel scaffolds and PLLA/hydroxyapatite (HAP) aerogel scaffolds with high level of porosity (>90%), interconnectivity, and mechanical properties (compressive modulus up to 2kPa). The fibrous nanostructure of these scaffolds were joined to micronic cells of controllable size. The scaffolds were then fabricated with predetermined shape and size within a short time (<30 h) and without an appreciable solvent residue (<5 ppm). Attachment and proliferation of osteoblasts on the scaffolds of the two modifications were analyzed.
***Results:** In the pure PLLA aerogel scaffolds, the osteoblasts attached primarily on the outer surface of the polymer. In the PLLA/HAP aerogel scaffolds, the osteoblasts rooted deep into the scaffolds and were regularly distributed. There were more osteoblasts surviving in the PLLA/HAP aerogel scaffolds than in the PLLA aerogel scaffolds. Osteoblastic proliferation were observed in both scaffolds, but higher cell number was observed in the PLLA/HAP group after several weeks of in vitro cultivation. The PLLA/HAP aerogel scaffolds documented more abundantly bone-specific markers (mRNAs encoding bone sialoprotein and osteocalcin) than the pure PLLA aerogel scaffolds counterpart.
***Conclusion/Significance:** These results demonstrated the highly porous PLLA/HAP aerogel scaffolds were superior to pure PLLA scaffolds for osteoblasts attachment and regeneration.

167 - Human Bone Marrow-derived Mesenchymal Stromal Cell-seeded Bone Biomaterial Directs Fast And Superior Mandibular Bone Augmentation In Rats

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***Purpose/Objectives:** Atrophic maxillary ridges present a challenge in the field of oral implantology. Autologous bone is still considered the gold standard grafting material, but the increased morbidity and surgical complications represent a major drawback for its use. The aim of this study was to assess the efficacy of an off-the-shelf cell-seeded bone biomaterial for mandibular bone augmentation, compared to its acellular counterpart.
***Methodology:** We used a rat model to test the osteogenic properties of bone marrow-derived mesenchymal stromal cells (MSCs)-seeded bone microparticles compared to acellular bone microparticles alone. Rats were euthanized at 4 and 8 weeks, and results analyzed using micro-CT

imaging, histology (H&E, Masson's Trichrome), histomorphometry and immunohistology (Tartrate-Resistant Acid Phosphatase-TRAP, Osteocalcin and human specific anti-mitochondria antibodies).
***Results:** Micro-CT analysis demonstrated that the cell-seeded biomaterial achieved significantly more bone volume formation at 4 weeks (22.75±2.25mm^3 vs 12.34±2.91mm^3, p=.016) and at 8 weeks (64.95±5.41mm^3 vs 42.73±10.58mm^3, p=.029), compared to the acellular bone microparticles. Histology confirmed that the cell-seeded biomaterial was almost completely substituted at 8 weeks, in opposition to the acellular biomaterial group. Immunohistochemical analysis showed a significantly higher number of TRAP and Osteocalcin positive cells at 4 weeks in the cell-seeded group compared to the acellular group, thereby demonstrating a higher rate of bone remodeling in the presence of MSCs. The grafted human cells remained viable and were detected up to at least 8 weeks, as observed using the human specific anti-mitochondria antibody.
***Conclusion/Significance:** This off-the-shelf material available in unlimited quantities could therefore represent a significant advance in the field of mandibular bone augmentation by providing a larger volume of new bone formation in a shorter time.

168 - Low-level Laser Therapy Using 635nm Diode Laser On Oral Ulcer Animal Model

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***Purpose/Objectives:** Several treatment protocols have been introduced to manage oral ulcer which may be caused by trauma, immunological factors, infection, and chemo/radiotherapy. Recently, the effect of low-level laser therapy (LLLT) on oral ulcer or stomatitis has been introduced. However, previous studies applied small spot size irradiation which should be delivered multiple times in multiple spots. In this study we sought to evaluate the impact of LLLT using 635 nm diode laser applied via a cylindrical probe as a wide-field irradiation.
***Methodology:** Oral ulcer was developed at the buccal mucosa of Sprague Dawley Rats (200-220 g, male) using a 6 mm skin punch. LLLT was delivered via a cylindrical probe with a diameter of 1.6 cm (Area = 2.01 cm^2). Treatment began 3 days after initial tissue injury and was conducted once a day for 5 days. Twenty eight rats were divided into four groups as group A (LLLT, 75 J/ cm^2, 500mW, irradiation time : 300 sec), group B (LLLT, 20 J/ cm^2, 200mW, irradiation time : 200 sec), group C (LLLT, 5 J/ cm^2, 200mW, irradiation time : 50 sec), and group D (control group with no LLLT). Rats were sacrificed 10 days after initial injury and histologic analysis was conducted.
***Results:** Area of ulcer at 10 days after initial injury was smaller in group B (20 J/ cm^2) compared to those of other groups and control group. Histologic analysis showed that group B (20J/ cm^2) had better outcomes regarding reepithelization, inflammation and granulation.
***Conclusion/Significance:** LLLT using 635 nm diode laser via a cylindrical probe as a wide-field irradiation improved the wound healing process in an animal model of oral ulcer. LLLT with 20 J/ cm^2 showed more organized and rapid wound healing compared to LLLT with 5 J/ cm^2 and 75 J/ cm^2

169 - Cell Morphology Monitoring During Culture For Daily Cell Quality Control

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***Purpose/Objectives:** In regenerative medicine fields, cell quality control is critical for ensuring reproducibility. Cell proliferation and differentiation potentials are closely related to its donor origin and/or passage number [1,2]. One of the methods to check the cell quality is monitoring cell morphology. It has been indicated that the osteogenic differentiation potential can be predicted by the cell morphology [3], and a more recent report has shown that the morphological changes during early osteogenic induction of MSCs were corelated to their mineralization [4].
***Methodology:** Here, we propose an *epi* relief-contrast cellular monitoring system (eRC-CMS) which is noninvasive and can monitor a large culture area simultaneously and continuously [5]. The system can be fitted into a typical CO_2 incubator and consists of a flat stage which takes a well plate (13 cm×8 cm). The imaging unit includes a CMOS camera, a lens, and LEDs that were integrated on a small board, which was precisely driven in x-y directions by automated stepping motors. Using an image analysis algorithm (CKX-CCSW, Olympus), during recording the system automatically adjusts the image contrast to improve the data quality and recognizes cell regions/non-cell regions, cell numbers, and locations based on luminance values. The acquired images were analyzed by softwares (IMARIS (Bitplane) and cellSens (Olympus)).
***Results:** Using this system, we cultured and monitored HUVECs and human bone-marrow MSCs. HUVECs (Cambrex bio company) were cultured in 2 of 6 well plates (Corning) each time and after 24 hours of culture, images of cells at 5 different regions/well were taken with eRC-CMS every 60 min for the following 48 hours. The cells were passaged every 3 days and at each passage 3.0×10^4 cells/well were re-seeded while the others were cultured in collagen for further analysis. In total, approximately 5760 images were stored, and 9 million individual cells from P4 to P15 were analyzed to generate the data of cell number, cell area and circularity for each passage. Then, the angiogenic activity was evaluated from the HUVECs cultured in collagen. As a result, it is found that the doubling time and cell area and circularity in subcultures were closely correlated with the passage number their angiogenic activity.
hBMSCs from three donors (Lonza, Riken and Stanford University) were also cultured and monitored following the same set up as HUVECs. It is found that the cell morphology was different among 3 donors. The osteogenic and adipogenic differentiation potentials were evaluated by gene expression of Osteocalcin and Adipocyte protein 2. It is shown that there were differences in the gene expression level among 3 donors and it is related to the cell morphology.
***Conclusion/Significance:** In summary, this system may provide a robust and versatile approach for daily cell monitoring to facilitate reliable and reproducible cell-based studies.
1. Marin, et al. Journal of immunological methods 254, 1-2, 183-190.2. Kim, et al. Mechanisms of ageing and development 133.5, 215-225.3. Matsuoka, et al. PloS one 8.2, e55082.4. Marklein, et al. Stem Cells 34.4, 935-947. 5. Osaki, et al. Scientific reports 7,1, 1897.

170 - Oxygen Imaging Of Common Biomaterials

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***Purpose/Objectives:** The purpose of this study is to probe the compatibility of electron paramagnetic resonance oxygen imaging (EPROI) with commonly used biomaterials. EPROI is an emerging absolute oxygen mapping technology based on magnetic resonance principles. EPROI measures partial oxygen pressure (pO_2) of tissues/animal with the precision of 1-3 torr within 1-10 minutes with 0.5-1 mm spatial resolution. The oxygen maps obtained using EPROI can provide valuable information to improve the therapeutic outcome in regenerative medicine. EPROI has been tested extensively in animal models for tumor drug assessment, chemotherapy, immunotherapy assessment, and radiation treatment. However, its use in artificial tissues and cell encapsulation devices is relatively new.
***Methodology:** EPROI experiments were performed on commonly used acellular biomaterials, such as agar, vitro-gel, collagen, chitosan, PLGA and gelatin etc. Trityl, OX063-D24 was used as a spin probe to obtain oxygen maps of these biomaterials during the cycle of deoxygenation with N2 bubbling. Calibration of the spin probe relaxation in the biomaterials allows to derive quantitative oxygen maps.
***Results:** We demonstrate that EPROI is compatible with commonly used biomaterials. We demonstrate the possibility of obtaining high resolution (0.5 mm) pO_2 maps of these biomaterials at different pO2 and physiologic temperatures.
***Conclusion/Significance:** Oxygen is an important physiological parameter and oxygenation may carry diagnostic and prognostic information for artificial tissue development. The pO_2 maps of the regenerative medicine devices may act as an essential feedback to control the implantation and performance of therapeutic devices.

171 - Optical Coherence Tomography Analysis Of Articular Cartilage Under Dynamic Biaxial Loads

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***Purpose/Objectives:** The complex articular cartilage structure gives rise to its unique depth-dependent mechanical behavior. Depth-dependent mechanical behavior has been evaluated using optical methods to track tissue features. Although several standardized mechanical assessment methods have been developed to measure the depth-dependent mechanical properties of AC, the growing appreciation of non-intrusive/label-free (or minimally intrusive) methods has led to the development of new mechanical evaluation modalities. Optical coherence tomography (OCT) is a promising elastography imaging technique to measure the displacement field of a sample under load.
***Methodology:** We designed and built a biaxial loading apparatus for applying compressive and shear displacements. A load cell (ATI Nano 17) measured the corresponding shear and compressive forces. Cylindrical 6mm calf Osteochondral samples were cut across their diameter and glued between the loading device arms. Three-dimensional images of the tissue were obtained using a custom spectral-domain optical coherence tomography (SD-OCT) system (λ_{center}=1310nm, $\lambda_{Bandwidth}$=100nm, A-line rate= 47kHz, axial resolution= 6µm, lateral resolution= 15µm, phase stability = 5nm). One hundred cross sectional images (512 x 1000 pixels) were acquired at 47 fps while steps of biaxial displacements

were applied at a rate of 25 μm/s (three compressive steps with 15 minutes relaxation time between steps, and then three shear displacement steps with 5 minutes relaxation time). OCT images were obtained as the sample was loaded. The total acquisition time was 2 seconds per displacement step. In compression, speckle tracking was used to calculate the displacement field. For shear measurements, displacement was calculated along each row of the cross-sectional images by complex phase-shift analysis of the OCT signal followed by a phase-unwrapping algorithm. Displacements were averaged over 100 frames to generate final displacement and strain curves for each row of the cross-sectional image. All calculations were done with a custom Matlab code.
***Results:** Combining speckle-tracking and phase-resolved displacement (for compressive and shear loads, respectively) enables us to analyze the dynamic biaxial displacements in the samples using one set of images. It provides distinctive advantages in imaging the specimens without repositioning the sample during loading steps, and eliminating all the complications caused by repositioning the apparatus and disturbing the measured loads. Under both shear and compressive loading, clear differences in local strain distribution were observed as a function of distance to articular surface.
***Conclusion/Significance:** This agrees with known mechanical properties distributions throughout the cartilage, such as a compliant middle layer for both, shear and compression. This technique enables us to measure and monitor the real-time displacements of the samples during dynamic biaxial loading. It could be readily extended to volume scanning or high-throughput screening, and may be useful for quickly and nondestructively determining the functional properties of engineered tissue.

172 - A Tissue Engineering Approach to Repair Volumetric Muscle Loss in the Ovine Peroneus Tertius Following a 3-Month Recovery

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***Purpose/Objectives:** In an effort to combat the limitations of current treatment options for volumetric muscle loss (VML), our lab has developed scaffold-free tissue-engineered skeletal muscle units (SMUs). To address the nerve injuries that often accompany VML, our lab has also developed an engineered neural conduit (ENC) composed primarily of collagen to bridge gaps between native nerve and the injury site. The goal of this study was to test the efficacy of our SMUs and ENCs in restoring muscle function in a clinically relevant large animal model: sheep receiving a 30% VML injury in a load-bearing hindlimb muscle, specifically the peroneus tertius (PT), following a 3-month recovery period.
***Methodology:** To test the efficacy of our SMUs and ENCs in repairing an acute VML injury, the animals were divided into three experimental groups: VML only, VML+SMU, and VML+SMU+ENC. In all groups, a full-thickness longitudinal portion of the PT constituting 30% of the total muscle volume was dissected. The VML only animals (negative control) received the injury without a repair. In the VML+SMU group, the injury was immediately repaired by suturing an SMU within the defect. Additionally, the distal branch of the peroneal nerve was transected and re-routed to the SMU. In the VML+SMU+ENC group, the VML injury was also repaired with an SMU; however, in this group, 1cm of the re-routed peroneal nerve was dissected to simulate a nerve injury. The gap between the SMU and the peroneal nerve was then bridged with an ENC. Following a 3-month recovery, we conducted *in situ* biomechanical testing in which measurements of maximum isometric tetanic force were taken. Immediately after biomechanical testing, both the contralateral and surgical PTs were dissected, weighed, and prepared for histology.

***Results:** Animals gained weight normally during the recovery period and there was no significant difference in body weight between experimental groups (P=0.2528, n=45). We assessed the ability of our grafts to restore lost muscle mass by comparing the weights of the contralateral and surgical PTs. The VML only group exhibited a significant difference in muscle mass between the contralateral and surgical sides (P=0.0003, n=15), indicating a lack of mass recovery. Regarding biomechanical outcomes, the maximum force production of the PT muscle with VML only was significantly lower than the contralateral PT (P<0.0001, n=11), but there was no significant difference in force production in the VML+SMU group (P=0.0613, n=9) or the VML+SMU+ENC group (P=0.5755, n=11). Direct stimulation of the re-routed nerve was possible in multiple animals within both the VML+SMU and the VML+SMU+ENC groups.
***Conclusion/Significance:** Overall, these results demonstrate the ability of our SMUs to restore both muscle mass and force production to a level that is statistically indistinguishable from the uninjured contralateral muscle after only 3 months. Furthermore, these results show that the ENCs have been able to effectively bridge the gap between re-routed nerve and the repair site. Future histological analysis will provide a better understanding of the repair process and the mechanism by which our SMUs and ENCs are aiding the restoration of muscle mass and force production.

173 - Engineered Skeletal Muscle Constructs Fabricated from Human Satellite Cells and Fibroblasts

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***Purpose/Objectives:** Volumetric muscle loss (VML) is a loss of skeletal muscle tissue that impairs muscle self-repair mechanisms and function. Tissue engineering technologies can potentially address current VML treatment shortcomings by growing exogenous tissue that promotes appropriate muscle regeneration *in vivo*. Key limitations inhibiting the translation of such methodologies into medical therapies include challenges in fabricating human skeletal muscle with contractile functions. Our lab has developed scaffold-less skeletal muscle units (SMUs) for VML treatment in sheep models. The next steps are to advance our fabrication methodologies to develop human cell-sourced SMUs. Thus, this study evaluated the structure and function of human cell-sourced SMUs engineered using current lab methodologies.
***Methodology:** To investigate the efficacy of our SMU fabrication protocol using human cells, cells isolated from human skeletal muscle surgical discards were plated on tissue culture plastic at a density of 10,000 cells/cm^2. Additionally, a subset of isolated human cells underwent cryopreservation prior to plating. For comparison, cells isolated from sheep semimembranosus muscle were plated at the same density. All three experimental groups (i.e. human fresh cells, human frozen cells, and fresh sheep cells) underwent the SMU fabrication protocol. Light microscopy was used to visualize myotube size and density in developing monolayers. After monolayers delaminated to form 3D SMUs, constructs underwent biomechanical testing to measure SMU tetanic force production. Afterwards, constructs underwent immunohistochemical analysis (IHC) to identify myosin heavy chain and laminin and Masson's trichrome staining to evaluate SMU structure.
***Results:** At 8 days post-seeding, each experimental group's cell plates had monolayers with extensive myotube fusion and networking. All plates formed robust monolayers that could be rolled into 3D SMUs, indicating cohesive and advanced cell and extracellular matrix (ECM) networks. Biomechanical testing

indicated SMUs in all groups that were capable of producing tetanic contractions. Average isometric tetanic forces were 78.2 ± 68.8 μN, 29.5 ± 37.2 μN, and 16.0 ± 34.3 μN for sheep, fresh human, and cryopreserved human cell SMUs respectively. There was no observed significant difference in force production between fresh sheep cell and fresh human cell SMUs, but there was a significant difference between sheep cell and frozen human cell SMUs ($p=0.0390$). Trichrome stains revealed muscle fibers and collagen in all three experiment groups. In all groups, IHC indicated cell viability through the SMU's entire thickness, with human SMUs showing an increase in cell number per unit area compared to sheep SMUs. Sheep SMUs showed greater muscle fiber alignment. In all groups, MF20 and laminin staining showed muscle fibers surrounded by laminin, similar to native skeletal muscle's structural organization. Qualitatively, sheep SMUs displayed more muscle fibers and ECM deposition than human SMUs.
***Conclusion/Significance:** This study evaluated human cell-sourced SMUs fabricated using lab methodologies previously successful in sheep models. Freshly-derived and cryopreserved human cells resulted in robust monolayers with dense myotube networks and SMUs capable of isometric tetanic force production Overall, the lab's SMU protocol can successfully create engineered human tissue with skeletal muscle characteristics, but optimization needs to occur to improve SMU muscle fiber density, ECM deposition, and contractile function.

174 - In Vivo Structural And Cellular Remodeling Of Engineered Skeletal Muscle Used For Volumetric Muscle Loss Repair In Sheep

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***Purpose/Objectives:** Volumetric muscle loss (VML) is the loss of skeletal muscle by trauma or surgery which causes impairment in muscle function. In order to develop an effective treatment for VML injuries, our laboratory has fabricated scaffold-free tissue-engineered skeletal muscle units (SMUs) and engineered nerve conduits (ENCs) for implantation into a VML. The goal of this study was to histologically evaluate the repair capabilities of our SMUs and ENCs in an ovine peroneus tertius (PT) muscle VML model following a 3-month recovery. We evaluated muscle architecture, tissue integration, and vascularization in the repair site between three experimental groups: VML-Only (negative control), VML repaired with an SMU (VML+SMU), and VML repaired with an SMU and an ENC used to bridge the gap between re-routed native nerve and repair site (VML+SMU+ENC).
***Methodology:** Following a 3-month recovery, the animals were euthanized, and both contralateral and surgical PTs were dissected and prepared for histology. A midbelly portion fixed in formalin and embedded in paraffin was stained with hematoxylin and eosin (H&E) and Masson's Trichrome to observe gross morphology. Select muscle sections were snap-frozen in tissue freezing medium with chilled isopentane. These samples were immunofluorescently stained for myosin heavy chain (MF20) and laminin to identify muscle fibers and basal lamina, respectively. All animal procedures were conducted in accordance with The Guide for Care and Use of Laboratory Animals (Public Health Service, 2011 NIH Publication No. 85-23).
***Results:** Following a 3-month recovery, gross observations of tissues stained with H&E show that the repair site is characterized by an increase in vasculature in all experimental groups compared to the uninjured contralateral muscle. In tissues stained with Masson's trichrome, there are large fibrotic regions, evidenced by positive collagen staining, that integrate with the muscle. We also observed the presence of intramuscular fat in the experimental groups, but not in the uninjured contralateral muscle.

Immunohistochemical staining for myosin heavy chain (MF20) and laminin show an increase in small muscle fibers within the repair site in the VML+SMU and VML+SMU+ENC groups compared to the VML only group.
***Conclusion/Significance:** After a 3-month recovery, we observed integration of the engineered SMUs and ENCs with the host muscle and newly formed muscle fibers within the repair site of injuries repaired with our SMUs. This histological evidence further elucidates the ability of our constructs to recover force production following a VML injury. This demonstrates that our engineered skeletal muscle has potential to be a viable treatment for VML, and constitutes a significant step towards a treatment that restores native muscle structure on a scale that is clinically relevant to humans.

175 - A 30% Volumetric Muscle Loss Does Not Result In Sustained Functional Deficits After A 90-day Recovery In Rats

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***Purpose/Objectives:** Currently, there exists a lack of a standardized animal model and consistency of Volumetric Muscle Loss (VML) type that involves a severity of injury exceeding the body's self-regenerative capacities. Previous work has examined the use of a 20% and 30% VML model using the tibialis anterior (TA) muscle of rats throughout a recovery period of 28-days. The purpose of this study was to evaluate different percentages of VML - 30%, 40%, and 50% - in rats TA to establish which percentage results in a permanent functional deficit after a 90-day recovery period.
***Methodology:** An incision was made along the lower left limb of rats to expose the TA. A longitudinal portion of muscle comprising 30%, 40%, or 50% of the total muscle volume was dissected. Animals recovered for 90 days. Following the recovery period, TA force production was measure *in situ*. The distal tendon was severed and attached to a force transducer. After obtaining the optimal length (L_o), isometric tetanic force (P_o) was measured in control group (n=5) and VML groups (30% n=3, 40% n=5, 50% n=6). Specific force of each group was calculated by dividing the P_o by the physiological cross-sectional area of each muscle. TA muscles were dissected and frozen for histology. Muscles were cryosectioned and stained with hematoxylin & eosin (H&E), and Masson's trichrome. Significance was established at p<0.05 with a one-way ANOVA and a Tukey's multiple comparison test.
***Results:** All VML groups had significant losses of TA mass (p<0.05) compared to their respective control. The 30% VML group resulted in a muscle loss of 0.043g (p=0.0029) and a decreased force of 13.4%. For the 40% and 50% VML groups, the muscle loss was of 0.057g (p=0.0013) and 0.073g (p<0.0001) with decreased forces of 24.6% and 23.4%, respectively. P_o measurements revealed that maximum force produced by 30% VML (5432.03µN) rats was not significantly different (p=0.3674) from uninjured muscles. In contrast, maximum force of 40% VML (P_o of 4669.93µN, p=0.0136) and 50% VML (P_o of 4741.78µN, p=0.0141) groups was significantly lower in comparison to uninjured muscles (P_o=6193.03µN). Calculations of the specific force showed no significant differences between groups (p=0.2010).
***Conclusion/Significance:** Overall, this study further supported our observation that in rats, a 30% VML is not severe enough to create a functional deficit that is sustained after a 90-day recovery. The 30% VML group only loss 13.4% of force capabilities while the 40% VML and 50% VML lost over 20% of force production, suggesting that a 30% VML rat model is not ideal in mimicking the functional effects of the VML injuries long-term. A VML injury of at least 40% will be used in our future rat studies to better

mimic functional effects of VML. A standardized and clinically relevant model for VML producing a long-term deficit in muscle self-regeneration and force production provides a strong base for future tissue engineering techniques in regenerative medicine. In addition, it will provide the ability to develop treatments for more drastic muscle loss injuries and better outcomes for patients.

176 - Infrapatellar Fat Pad (IFP)-derived MSC Possess Boosted Immunomodulatory And Trophic Profiles Upon CD146 Selection And Inflammatory Priming In 3-D Spheroids, Emerging As A Cellular Alternative For Osteoarthritis Treatment

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***Purpose/Objectives:** Osteoarthritis (OA) is a chronic degenerative disease characterized by progressive articular cartilage (AC) loss with contributing local immune/inflammatory responses. Mesenchymal Stem Cells (MSC) exhibit immunomodulatory and trophic properties, making them an alternative to treat inflammation-related musculoskeletal conditions including OA. Infrapatellar fat pad (IFP) constitutes a promising alternative source of MSC to other adult/fetal tissues such as bone marrow (BM), given its anatomical relationship with intra-articular structures and its pivotal role in OA and joint homeostasis in general. We hypothesize that CD146-selected IFP-MSC 3-D spheroids possess increased immunomodulatory effects whereas their anti-inflammatory/anti-fibrotic properties are enhanced with prior exposure (*i.e.* MSC priming/licensing) to environments rich in IFNγ, TNFα, CTGF and/or immune cells. Therefore, we assessed unfractionated and CD146-selected IFP-MSC secretory response to inflammation (priming) in 2-D and 3-D spheroids and functional immunomodulatory effects.
***Methodology:** Human IFP-MSC chondrogenic potential, immunophenotype, growth kinetics and transcriptional profile pre- and post- inflammatory/fibrotic priming (TNFα/IFNγ or TNFα/IFNγ/CTGF) were assessed in 2-D. Inflammation-related multiplex secretome was interrogated in 2-D and 3-D spheroids. CD146-selected MSC were generated and naïve and primed cells immunomodulatory transcripts and functional immunopotency assay (IPA) with human PBMCs interrogated *in vitro*. IFP-MSC in 2-D and 3-D spheroids were tested for their immunomodulatory properties in an acute OA rat model *in vivo*.
***Results:** Naïve unfractionated IFP-MSC showed MSC-related immunophenotypic profile (with low CD146 protein expression) whereas inflammatory/fibrotic priming resulted in sharp increase of CD146, CXCR4, CD10, and CD200 proteins, significant up-regulation of immunomodulatory genes and alterations in MSC chondrogenic differentiation capacities. Upon inflammatory/fibrotic priming, the overall "2-D secretome signature" in IFP-MSC involves the upregulation of key immunomodulatory molecules including the T leukocyte recruitment chemokines MCP-2 and RANTES and the immunosuppressive proteins AR, ICAM-1, IP-10. CD146-selection of naïve IFP-MSC further increased IDO expression while reduced pro-inflammatory IL-6 and IL-8 in primed $CD146^{Pos}$ cells. Primed $CD146^{Pos}$ cells strongly abrogated proliferation of activated human PBMCs in a dose-dependent manner, outperforming unfractionated cells at large PBMCs:MSC ratios and $CD146^{Neg}$ cells at all doses, suggesting $CD146^{Pos}$ as the actual immunomodulatory subset. Both unfractionated and CD146-selected IFP-MSC form 3-D spheroid cultures that show similar secretory profile upon TI and TIC priming. Importantly, 3D spheroid cultures resulted in a sustained increase in CD146 expression and protein presence (IHC), with

enhanced inflammation-related secretome in primed IFP-MSC, especially the immunosuppressor IDO. Moreover, upon inflammatory/fibrotic priming, the "3-D secretome signature" in IFP-MSC involves the upregulation of key immunomodulatory proteins such as IP-10, MCP-1, MCP-2, TIMP-2. Transcriptional assessment of naïve and primed IFP-MSC showed that primed $CD146^{Pos}$ IFP-MSC express significantly higher levels of ICAM-1, HLA-G , and IDO immunomodulatory genes. Functionally, the enhanced secretory and transcriptional profiles of primed IFP-MSC 3-D spheroids were reflected in the strong inhibition of activated human PBMCs proliferation observed *in vitro*. These findings were reproduced *in vivo* in a rat model of acute OA, where transiently engrafted human IFP-MSC induced local immunomodulation.
***Conclusion/Significance:** Inflammatory priming and CD146-selection of IFP-MSC 3-D spheroid cultures result in enhanced immunomodulatory profile, thus evolving in a viable alternative for cell-based therapy protocols in Osteoarthritis.

178 - Bone Tissue Engineering From Human Adipose Stem/stromal Cells Spheroids Seeded Into PLA/CHA 3D Printed Scaffolds

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***Purpose/Objectives:** Adipose derived stem/stromal cells (ASCs) spheroids are considered as modular units to engineer biological tissues. The aim of this study was to associate osteogenic induced ASC spheroids with a 3D printed poly(lactic acid)/carbonate apatite (PLA/CHA) scaffold for bone tissue engineering.
***Methodology:** The micromolded nonadhesive hydrogel (agarose 2% in NaCl 0.9%) with 800 mm diameter in each 81 circular recesses (3D Petri Dish; MicroTissues, Inc.) was produced according to the manufacturer's protocol. For cell seeding, a suspension of $2x\ 10^6$ cells was prepared in 190 uL of DMEM supplemented with 50 mg/mL ascorbic acid, 1.25 mg/mL human albumin , 100 U/ mL penicillin, 100 mg/mL streptomycin, and ITS comprising the medium for noninduced ASC spheroids.Osteogenic induction of ASC spheroids was carried out in two steps: (1) spheroids were maintained under chondrogenic medium for two weeks; (2) and for additional three weeks under osteogenic medium. Pre-clinical experiments (*Wistar* rats) are being conducted in a calvaria bone critical-size defect model, according to the following experimental groups: (1) PLA/CHA scaffold; (2) PLA/CHA scaffold with spheroids; (3) clot, corresponding to the negative control.
***Results:** Induced ASC spheroids showed a mean of 430 µm diameter and high cell viability. At week 2, induced spheroids presented upregulation of collagen type X ($p < 0.001$) and MMP-13 ($p < 0.0001$) genes when compared to non-induced spheroids. Induced ASC spheroids showed strong *in situ* immunostaining for collagen type X and low immunostaining for the anti-angiogenic protein TSP-1 at week 2. Positivity for collagen type I, osteocalcin, biglycan and tenascin C was found at week 5 evaluated by immunohistochemistry, such as the presence of calcium deposits revealed by Alizarin red O staining. Young's modulus values of induced ASC spheroids were higher than 100kPa at week 3 and more than 10 times higher than non-induced spheroids ($p < 0.0005$). Induced ASC spheroid quartets do not present the same fusion kinetics of non-induced. After fusion, induced ASC spheroids showed an increase of *in*

situ immunostaining for collagen type I and osteocalcin. Non-induced ASC spheroids seeded into 3D printed PLA/CHA scaffold with a spacing value of 300 µM and four layers showed greater adhesion, spreading and cell migration.
***Conclusion/Significance:** In this study, induced-ASC spheroids showed a hypertrophic cartilage phenotype at week 2, and a osteogenic commitment at week 5 of spheroids culture. Pre-clinical experiments are in progress. Our study opens a new perspective for bone tissue engineering.

179 - Biological Differences At The Early Stage Of Bone Healing Between Fractures And Large Bone Defects

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***Purpose/Objectives:** Treatment of segmental bone defects remains a major clinical problem, and innovative strategies are often necessary to successfully reconstruct large volumes of bone. When fractures occur, the resulting hematoma serves as a reservoir for growth factors and a space for cell infiltration, both crucial to the initiation of bone healing. Our previous studies have demonstrated very clear ultrastructural differences between fracture hematomas formed in normally healing fractures and those formed in segmental bone defects. However, there is little information available regarding potential differences in the underlying gene expression between hematomas formed in normal fractures, which usually heal by themselves, and segmental bone defects, which do not. Therefore, the aim of this study was to identify differences in gene expression within hematomas collected from 0.5 mm (normally healing defect) and 5.0 mm (segmental bone defect) fracture sites during the earliest stages of bone healing.
***Methodology:** Osteotomies of 0.5 and 5.0 mm in the femur of Fisher 344 rats were stabilized with external fixators (RISystem AG). After 3 days the rats were sacrificed, and the fracture hematomas were collected for RNA-sequencing. Ingenuity pathway analysis (IPA) was used to identify upstream regulators and biological functions that were significantly enriched with differentially expressed genes from the RNA-sequencing analysis. Animal procedures were conducted following the IACUC protocol of the UT Health Science Center San Antonio.
***Results:** Key upstream regulators of bone formation were less active (e.g. TGFB1, FGF2, SMAD3) or even inhibited (e.g. WNT3A, RUNX2, BMP2) in non-healing defects when compared to normally healing fractures. Many upstream regulators that were uniquely enriched in healing defects were molecules recently discovered to have osteogenic effects during fracture healing (e.g. GLI1, EZH2). Upstream regulators uniquely enriched in non-healing defects were mainly involved in an abnormal modulation of hematopoiesis, revealing evidence of impaired maturation of functional macrophages and cytokines (e.g. IL3, CEBPE), both essential for successful bone healing. In addition, the enrichment pattern suggested a dysregulation of megakaryopoiesis (e.g. MRTFA, MRTFB, GATA2), which directly affects platelet production, and therefore fracture hematoma formation. Remarkably, the organization of the ECM was the most significantly enriched biological function in the normally healing fractures, and implies that the defect size directly affected the structural properties within the fracture hematoma. Conversely, genes encoding important ECM components (e.g. BGN, various collagens, IBSP, TNC), cell adhesion molecules, MMPs (MMP2), and TIMPs were all significantly downregulated in non-healing

defects.
***Conclusion/Significance:** Our most recent findings reveal new important key molecules that regulate defect size-dependent fracture healing. Combined with our previous results, which identified structural differences in fracture hematomas from both types of defects, current findings indicate that differential expression of genes is dictated by the structural properties of the hematomas formed during early fracture healing. Consequently, creating a bioscaffold that mimics the structure of normal fracture hematomas could be the first step towards developing new orthoregenerative treatment strategies that potentiate healing of large bone defects and non-healing fractures.

180 - CRISPRa-Driven Nascent Collagen Type II Deposition Drives Chondrogenesis Without Exogenous Growth Factors

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***Purpose/Objectives:** Human adipose-derived stem cells (hADSCs) have gained traction in recent years for their multipotency, ease of use, and their vast number of potential applications in musculoskeletal tissue engineering and cell therapies. Traditionally, the regulation of stem cell differentiation and phenotype has been accomplished by the addition of specific exogenous growth factors. Here, we utilize CRISPR-activation to upregulate type II collagen (COL2A1) and aggrecan (ACAN) in order to promote hADSC tissue deposition and demonstrate that nascent collagen type II deposition drives chondrogenesis without exogenous growth factors.
***Methodology:** Guide RNAs (gRNAs) targeting the promoter regions for genes of interest (ACAN, COL2A1), and a scrambled nontarget gRNA control were designed and cloned into lentiviral vectors. Subsequently, each gRNA vector and a dCas9-VPR vector were used to transduce hADSCs to deliver a complete CRISPR activation system targeting genes of interest. To test the ability of this system to upregulate the target genes, target gene expression, 7 days post transduction, was quantified by qRT-PCR (n=3, one-way ANOVA, Tukey post hoc). Cells containing the best-performing gRNAs were then cultured in chondrogenic pellet cultures without exogenous growth factors for 7 or 21 days, to investigate the ability of this gene regulation to enhance the production of these extracellular matrix (ECM) proteins and subsequent chondrogenesis. Pellets cultured for 21 days were qualitatively analyzed by Alcian Blue staining (n=3) and COL2A1 immunohistochemistry (n=3), and quantitatively analyzed for sulfated glycosaminoglycan (sGAG) content using the dimethyl methylene blue assay ($n \geq 5$, one-way ANOVA, Tukey post-hoc). Pellets cultured for 7 days were analyzed by RNA-seq to discern differential gene expression driving the phenotypes induced by ACAN and COL2A1 upregulation.
***Results:** Using our CRISPR activation systems, we were able to robustly upregulate both targeted genes with up to a 40,000-fold increase in COL2A1, and a 27-fold increase in ACAN mRNA expression (relative to nontarget group). Pellet cultures of both the COL2A1- and ACAN-targeted cells revealed a substantial increase in pellet size as compared to nontarget control cultures (nontarget: 1.27 mm^3, ACAN: 7.76 mm^3, col2: 2.68 mm^3). Alcian blue staining and DMMB assays of ACAN-and COL2A1-edited cultures demonstrate 4.1 and 7.9 fold increase respectively in sGAG production and enhanced collagen type II staining in COL2A1-edited groups. However, the highest levels of GAG deposition were in the COL2A1-edited groups, and not the ACAN-edited groups. RNA-seq data reflects how the COL2A1 edit more strongly changes phenotype as it indicates broader changes in gene expression with more genes

differentially expressed (COL2A1: 2,532, ACAN: 38). Furthermore, gene ontology analysis using Enrichr indicated differentially expressed genes in COL2A1 edited cells are associated with ECM organization and the IRE1-mediated unfolded protein response, which has been indicated to occur with chondrogenesis. Additionally, proteoglycans Lumican and Biglycan and glycosyltransferases associated with sGAG synthesis were upregulated explaining the increased sGAG in COL2A1 edited samples.
***Conclusion/Significance:** Overall this indicates that both of these edits can regulate tissue deposition without the addition of exogenous growth factors, but that collagen type II deposition drives broader chondrogenesis through control of nascent ECM deposition.

181 - In Vitroassessment Of Scaffoldless Tissue-engineered Skeletal Muscle For Volumetric Muscle Loss Repair

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***Purpose/Objectives:** Volumetric muscle loss (VML) is the traumatic loss of skeletal muscle resulting in damage that overwhelms the body's capacity for self-repair, leading to functional impairment. Thus, the need for technologies that promote regeneration of skeletal muscle fibers to integrate with remaining muscle architecture is imperative. Our lab has developed scaffoldless, tissue-engineered skeletal muscle units (SMUs) for VML treatment in sheep. Additionally, to ensure proper innervation of the SMU myofibers, we developed an engineered neural conduit (ENC) to bridge the SMU and the damaged nerve. This study aims to develop a fabrication method for SMUs and ENCs that restore function following an acute VML injury in a more clinically relevant, load-bearing model in sheep.
***Methodology:** Ovine bone marrow stromal cells (BMSCs) were harvested and used to fabricate ENCs. Before 3-D formation, silicone tubing was pinned in the tissue culture dish to allow the delaminating monolayer to roll around the tubing, creating a lumen. For SMU fabrication, semimembranosus muscle was harvested from female lambs and the cell isolation mixture was plated onto tissue culture plastic. When elongating myotubes began to form a network, the plates were shifted to differentiation media until spontaneous delamination of the monolayer occurred. The monolayers were then pinned into 3-D cylindrical constructs and 2-3 single constructs were placed side-by-side and allowed to fuse. 2-3 fused SMUs were then sutured together, just prior to implantation. A subset of SMUs, fabricated on 60mm dishes, were used to measure contractile and structural properties. All animal procedures were conducted in accordance with The Guide for Care and Use of Laboratory Animals.
***Results:** The biomechanical properties of the SMUs indicated that on average the isometric tetanic force was 657 ± 667 μN. Structural maturation of the constructs was evaluated histologically with H&E, myosin heavy chain, and laminin. Images of developing monolayers were taken 10 days after initial plating and showed a highly aligned and dense myotube network without fibroblast overgrowth. Immunohistochemistry for myosin heavy chain (MF-20) and laminin showed that the construct is largely composed of aligned muscle. Picrosirius red staining revealed that the ENC's are mostly composed of collagen.
***Conclusion/Significance:** We evaluated the development, structure, and function of our SMUs and ENCs throughout the fabrication process. We were successfully able to fabricate and implant 60 SMUs, all 13cm long and 5-10mm in diameter. The biomechanical data showed that we were able to consistently fabricate constructs that met our release criteria for force production. It is important to note that improper alignment of the fibers along the longitudinal axis may have reduced potential force

production of the constructs and account for the variability in force data. Histology revealed that the overall structure of the constructs is linear and that the core is not necrotic, indicated by the presence of positively stained-DAPI cells throughout the construct.

182 - Enhancing Regenerative Capacity Of In Vitro-expanded Chondrocytes Via Selectively Removing Senescent Cells

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***Purpose/Objectives:** Autologous chondrocyte implantation (ACI) is a clinical procedure for the repair of cartilage damage in joint. However, the outcome of ACI is still variable, and the quality of chondrocytes may account for one of the major reasons. In particular, in order to get enough cell number, isolated primary chondrocytes usually undergo an extensive *in vitro* expansion (e.g. from 0.1 million to more than 40 million), which may lead to the generation of senescent cells, adversely affecting the quality and quantity of newly formed cartilage. Therefore, selectively removing senescent cells would be necessary in order to enhance the reparative outcome of ACI. Recently, FOXO4-DRI, a FOXO4 peptide, was reported to selectively kill the senescent cells through triggering apoptosis [1]. In this study, we for the first time examined the effect of FOXO4-DRI on chondrocytes that have been expanded to a number ready for ACI. We hypothesized that the number of senescent chondrocyte increased with the expansion time, and FOXO-DRI could selectively remove these cells and lead to a superior articular cartilage regeneration.
***Methodology:** Chondrocytes were isolated from healthy young donors with IRB approval. To simulate *in vitro* culture process in ACI, cells were expanded to a population doubling level (PDL) at 9 (PDL9, representing cells ready for implantation), with PDL3 as the control (representing primary chondrocytes). Cells were then treated with FOXO4-DRI (at 25μM) for 5 days. Afterwards, cell phenotype was extensively assessed using different methods, included cell morphology observation, cell proliferation (CCK-8 assay), cell senescence assessment (SA-β-gal staining, Real time-PCR), and *in vitro* cartilage and co-culture test with cartilage explant from bovine. Each experiment was repeated three times with three experimental replicates, and the results expressed as the mean ± SD. Significant differences among different groups were determined by two-tailed Student's t-test for two-group comparisons or ANOVA followed by post hoc analysis for multiple-group comparisons ($p < 0.05$).
***Results:** The ratio of SA-β-gal staining-positive cells was significantly higher in PDL9 (~40%) than PDL3 (~5%), indicating that there were more senescent cells in PDL9 chondrocytes. This result was further confirmed by real time PCR analysis of senescence-associated secretory phenotype (SASP)-associated genes. Interestingly, the treatment of FOXO4-DRI did not significantly change the cell number of PDL3 chondrocytes, but removed more than half of the cells in PDL9 chondrocytes, mainly through the apoptosis. In particular, comparing to untreated control (~40%), the ratio of senescent cells in FOXO4-DRI treated PDL9 dropped to <5%, which was accompanied by significantly reduced expression of SASP genes and the increased expression of proliferation marker genes. Finally, compared to the untreated control, FOXO-DRI treated PDL9 chondrocytes not only displayed enhanced cartilage formation, revealed by higher chondrogenic gene expression and more cartilage matrix deposition, but also maintained the phenotype of explants in co-culture study.
***Conclusion/Significance:** Our study suggested a robust way to enhance the reparative capacity of

chondrocytes, through selectively removing senescent cells, which will finally lead to a stable and enhanced clinical outcome after ACI.**Reference:** [1] Peter L.J. et al. Cell, 2017, 169(1): 132-147

185 - Enhancing The Chondrogenic Abilities Of Interleukin 1-beta Induced Chondrocytes And Reducing The Inflammatory Environment With The Use Of Alpha-tocopherol

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***Purpose/Objectives:** Articular cartilage (AC) is an avascular, anerual tissue that lines articulating joints and has a limited ability to regenerate upon injury. Osteoarthritis (OA) is a degenerative disease that marks the degradation of AC. OA affects millions of adults worldwide. Current treatments of OA rely on treating the symptoms and not the degenerated AC. Thus, there is a need for alternative treatments that will aid in maintaining AC and alleviating the inflammation associated with OA. Inflammation is characterized by the presence of reactive oxygen species (ROS) that can cause a series of inflammatory reactions that lead to degradation of AC. Nutraceuticals are nutritional chemicals capable of scavenging ROS. We hypothesize that the use of alpha-tocopherol (Alpha) as a nutraceutical will improve interleukin 1-beta (IL1B) induced chondrocytes' (AChs) chondrogenic ability and reduce the inflammatory environment. This will be assessed through improvements in the production of biomarkers collagen type II (COL2), and glycosaminoglycans (GAGs), and a reduction in inflammatory markers nitric oxide synthase 2 (NOS2) and tumor necrosis factor alpha (TNF-a).
***Methodology:** Inflammation to AChs will be induced using IL1B on day zero. Cells supplemented with Alpha in media will be cultured in micromass cultures in triplicates. At day 21, cultures produced using the IL1β induced AChs and healthy AChs with or without Alpha in medium will be assayed biochemically and histologically to characterize for total collagen and GAG, as well as for viability. Relative gene expression will be quantified for chondrogenic markers such as COL2 and for inflammatory markers such as NOS2, and for matrix metallopeptidase 13 (MMP13) responsible for COL2 degradation. Griess assay will be used to quantify Nitric Oxide (NO) as a marker for inflammation. Mechanical properties of the tissue, including the Young's Modulus, will be determined using atomic force microscopy.
***Results:** In our preliminary study, the effects of nutraceuticals on osteoarthritic human AChs based on gender, we found that the use of alpha decreased TNF-a expression significantly versus gallic acid (GA) and ascorbic acid (AA) in both genders. Furthermore, the use of Alpha significantly decreased the NOS2 expression compared to the negative control in both genders. The use of Alpha resulted in significantly higher COL2 and COL9 (chondrogenic marker) expression in comparison to GA and AA in both genders. NO content decreased significantly from day 1 to day 21 for all nutraceuticals.
***Conclusion/Significance:** As observed in the results above alpha shows promise in its use to reduce the inflammatory environment. Alpha had a significant effect in reducing inflammatory markers compared to other nutraceuticals and increasing the expression of chondrogenic markers. Our ongoing work expands on the finings above and explores the effects of Alpha on the inflammatory microenvironment of IL1B induced inflammation on AChs as well as on the mechanical properties of the engineered tissues. At the conclusion of our study, our expectation is that an improvement in mechanical properties of the

treated group and a significant decrease in the inflammatory environment will be observed with the use of Alpha.

186 - Laminin-111 Enriched Fibrin Hydrogels Support Myogenic Activity In A Rodent Model Of Volumetric Muscle Loss

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***Purpose/Objectives:** Skeletal muscle has remarkable regenerative capabilities following mild physical or chemical injury, but when challenged with a major traumatic injury resulting in volumetric muscle loss (VML), the regenerative process is impaired. A high prevalence of VML injuries is seen among military personnel. In military conflicts, many of the injuries sustained were extremity injuries involving severe musculoskeletal defects which result in long-term disability. Current clinical therapies, such as muscle grafts, used in the treatment of VML are ineffective at promoting muscle regeneration and function recovery. Tissue engineering strategies for skeletal muscle repair have been investigated with the purpose of providing the appropriate cellular cues and structural support and to restore muscle function. However, these therapies often fail to promote muscle resident stem cell (i.e. satellite cell) activity often resulting in impaired muscle regeneration and fibrotic tissue deposition.
***Methodology:** Laminin-111 (LM111), an embryonic isoform, has been associated with a variety of biological activities and supplementation has demonstrated remarkable regenerative capacity in several models of disease and injury [1]. In this study, we fabricated fibrin hydrogels enriched with LM111 at varying concentrations (50 - 450 µg/mL) as described previously [2,3]. These hydrogels were implanted into an established rodent model of VML injury (male Lewis rats, n=6-8/group/time-point). Muscle regeneration and functional recovery was assessed at days 7 and 14 post-implantation.
***Results:** Histological analysis showed that hydrogel implantation promoted cellular infiltration in the VML defect site. At days 7 and 14, the expression of myogenic markers associated with satellite cell activation and proliferation (i.e. MyoD, Pax7) were higher in the hydrogels containing 450 µg/mL of LM111, while myogenic markers associated with differentiation (i.e. Myogenin, desmin, alpha-actinin) were lower. A marker associated with cellular stress, heat shock protein-70, also showed lower expression in injured muscles that were implanted with fibrin hydrogels containing 450 µg/mL of LM111.
***Conclusion/Significance:** Therefore, higher concentrations of LM111 are more likely to maintain a satellite cell pool at the site of VML injury. Future studies will include identification of immune and stem cells infiltrating the defect site and assessment of peak isometric force.
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187 - Extracellular Matrix Sponges Support Functional Recovery Of Volumetric Muscle Loss In A Composite Musculoskeletal Trauma Model

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***Purpose/Objectives:** Combat veterans often present with debilitating fractures and these injuries account for nearly two million cases of delayed or non-union fractures in the United States annually. Most of these injuries involve volumetric muscle loss (VML), defined as the as the surgical or traumatic loss of muscle tissue with resultant functional impairment. While skeletal muscle is remarkably regenerative, VML injuries are irrecoverable in humans and animal models due to the complete loss of indispensable regenerative elements such as basal lamina and resident satellite cells. Currently, there are no approved therapies for the treatment of bone fracture with a concomitant VML injury.
***Methodology:** To improve regeneration of skeletal muscle and bone, we have developed biomimetic sponges composed of collagen, gelatin, and laminin (LM)-111 that were crosslinked with 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC). Collagen and LM-111 are crucial components of the muscle extracellular matrix and were chosen to impart bioactivity whereas gelatin and EDC were used to provide mechanical strength to the scaffold. Previous studies have shown that VML injury to the tibialis anterior (TA) results in delayed tibia fracture healing in a composite trauma model. This delay is elicited by the prolonged inflammatory state induced by the concomitant injury to both the muscle and bone tissues. To combat this, sponges were loaded with FK-506, a clinically relevant immunosuppressant that can moderate inflammation and accelerate fracture healing. To determine the effect of FK-506 loaded sponges on muscle regeneration and fracture repair, 13 week old male Lewis rats (n=4/group/time-point) were subjected to bilateral full thickness muscle biopsy (6 mm diameter) of the TA and osteotomy (1 mm) of the tibia. The fractured tibias were stabilized by a 0.7 mm intermedullary K-wire. FK-506 loaded biomimetic sponges were cut to fit and implanted in both the VML site and fracture site. Untreated controls received no sponge treatment. Animals were left to recover for 7, 14 and 28 days. Peak isometric torque production was measured to evaluate muscle recovery at 28 days.
***Results:** Histological analysis that sponge treatment showed increased presence of regenerating myofibers (myosin heavy chain+) in the defect and significantly higher myosin: collagen ratio at 7 days post-trauma. Additionally, protein markers associated with pro-inflammatory macrophages (iNOS) were reduced. Gene expression of CCR7 was significantly lower and TNFα trended lower (p=.0769) with sponge treatment. The serum concentration of interleukin (IL-2) trended lower (p=0.08) with sponge treatment at 7 days post-trauma. MicroCT analysis indicates implantation at the fracture site increases callus volume two weeks post-injury leading to increased bone volume by one month.
***Conclusion/Significance:** At the time of submission, FK-506 loaded sponge treatment significantly enhanced TA muscle mass and function at 28 days post-trauma. These results could be attributed to increased regeneration and reduced inflammation observed at 7 days post-trauma. Further experiments identifying key factors of recovery at each time points (7, 14, and 28 days post-trauma) are ongoing, including fracture stiffness, gene expression, growth factor and histological analysis. **Acknowledgments**: This work was supported by a grant from the National Institute of Health (NIGMS) 1R15GM129731.

188 - Neural Cell Integration Into 3d Bioprinted Skeletal Muscle Constructs For Restoration Of Muscle Function

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***Purpose/Objectives:** Bioengineered skeletal muscle tissue can be a promising solution to achieve functional recovery of volumetric muscle loss (VML) injuries. However, the conventional fabrication methods are limited to building volumetric tissues with the functional cellular organization. More importantly, bioengineered muscle tissues need to be integrated with the host nervous system following implantation, as a failure of innervation results in muscle tissue atrophy. In this study, we fabricated 3-dimensional (3D) human neural muscle constructs with pre-formed neuromuscular junctions (NMJs) and investigated the feasibility of improving the structural and functional recovery of VML injuries.
***Methodology:** We utilized the 3D bioprinting strategy to fabricate volumetric skeletal muscle constructs that mimic native skeletal muscle organization. To facilitate long-term tissue survival and accelerate neural integration, human neural stem cells (hNSCs) were combined with human muscle progenitor cells (hMPCs) in the 3D bioprinted muscle constructs. To determine the feasibility of treating critical-sized VML injuries, we applied the bioprinted human neural skeletal muscle constructs in a rat model of VML and evaluated the functional outcomes of muscle tissue reconstruction and innervation.
***Results:** Neural input on the bioprinted skeletal muscle construct showed improved muscle differentiation, long-term survival, and formation of NMJs *in vitro*. Implantation of the bioprinted neural muscle constructs in a rat tibialis anterior (TA) muscle excisional model facilitated rapid innervation and matured into organized muscle tissue that restored normal muscle weight and function.
***Conclusion/Significance:** Our results demonstrate that creation of innervated bioengineered skeletal muscle tissue constructs using the 3D bioprinting system is feasible, and that the muscle construct can contribute to restoration of muscle functions.

189 - Rehabilitative Exercise And Engineered Skeletal Muscle Enhance Regeneration For Treatment Of Volumetric Muscle Loss

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***Purpose/Objectives:** Muscle regeneration can be permanently impaired by traumatic injuries, despite the high regenerative capacity of skeletal muscle. Implantation of engineered biomimetic scaffolds to the site of muscle ablation may serve as an attractive therapeutic approach. The objective of the study was test the efficacy of a three-dimensional engineered skeletal muscle construct, in conjunction with rehabilitative exercise, for the treatment of volumetric muscle loss.
***Methodology:** Collagen scaffolds were fabricated by extruding high concentration rat-tail collagen-Type I (30 mg/mL) from 22G blunt tip needles into pH neutral buffer to initiate fibrillogenesis. To create a 3D scaffold bundle, 8 scaffold strips were aggregated in parallel with dimensions that were 9mm x 2mm x 3mm. Engineered skeletal muscle was generated by sequentially growing and differentiating approximately 500,000 GFP+ mouse myoblasts with 500,000 human microvascular endothelial cells per

scaffold and cultured in 3% horse serum in DMEM media at 37°C and 5% CO2. For in vivo studies, constructs were cultured for 9 days followed by transplantation into a mouse model of volumetric muscle loss (VML) that was created by surgical excision of 20% of the anterior tibealis (TA) muscle. Constructs were sutured at the distal and proximal ends of the defect followed by suture closure of the muscle and skin flaps. Following transplantation, animals were allowed to recover in traditional housing cages for 7 days, after which, animals were either transferred to individual cages containing cage wheels or remained in their original housing for 14 days. On day 21, the tail veins were injected with isolectin, a fluorescently labeled endothelial binding protein and the TA muscle was extracted and processed for histological analysis.
***Results:** To mimic the physiologic composition of skeletal muscle, endothelialized engineered skeletal muscle constructs were fabricated. When implanted into the ablated murine tibialis anterior muscle, the engineered muscle in conjunction with voluntary caged wheel exercise could significantly improve the density of transplanted GFP+ regenerating myofibers by >25% and the density of isolectin+/CD31+ perfused microvessels by 2-fold in comparison to treatment of constructs without exercise. Furthermore, the abundance of neuromuscular junctions was greater when treated with engineered constructs in conjunction with exercise, in comparison to treatment without exercise.
***Conclusion/Significance:** These findings demonstrate that voluntary exercise improved the regenerative effect of endothelialized engineered skeletal muscle by augmenting vascular regeneration and myogenesis, and has important translational implications in the therapeutic design of engineered biomimetic scaffolds for the treatment of traumatic muscle injury.

190 - Use Of Uniformly Sized Muscle Fiber Fragments For Restoration Of Muscle Tissue Function

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***Purpose/Objectives:** Treatment of extensive muscle loss due to traumatic injury, congenital defects, or tumor ablations is clinically challenging. The current treatment standard is grafting of autologous muscle flaps, however significant donor site morbidity and graft tissue availability remain a problem. Alternatively, muscle fiber therapy has been attempted to treat muscle injury by transplanting single fibers into the defect site. However, irregularly organized long fibers resulted in low survivability due to delay in vascular and neural integration, thus limiting the therapeutic efficacy. Therefore, no effective method is available to permanently restore extensive muscle injuries. To address the current limitations, we developed a novel method that produces uniformly sized native muscle fiber fragments (MFFs) for muscle transplantation. We hypothesized that fragmentation of muscle fibers into small and uniformly sized fragments would allow for rapid reassembly and efficient engraftment within the defect site, resulting in accelerated recovery of muscle function.
***Methodology:** We developed an MFF processing method that produces uniformly sized fragments (approximately 100 µm in width and length) with intact muscle cells on the fiber surface. To test the therapeutic effects of the MFF technology, we created several rodent muscle injury models, including 1) a muscle atrophy model using toxin treatment such as barium chloride (BaCl2), 2) a volumetric muscle defect model by surgical ablation, and 3) urinary incontinence (UI) model by damaging the external sphincter of urethra. The effectiveness of the MFF therapy was determined by structural and functional recovery of muscle tissues in these models.
***Results:** The processed MFFs have a dimension of approximately 100 µm and contain living muscle

cells on extracellular matrices (ECM). In preclinical animal studies using muscle atrophy, volumetric defect, and urinary incontinence models, histological and functional analyses confirmed that the transplanted MFFs into the injury sites were able to effectively integrate with host muscle tissue, vascular, and neural systems, which resulted in significant improvement of muscle function and mass.
***Conclusion/Significance:** These results indicate that the MFF technology platform is a promising therapeutic option for the restoration of muscle function, and can be applied to various muscle defect and injury cases.

192 - Differentiation And Characterization Of Wild-type And SOD1 Mutant HiPSC-astrocytes And Their Application To NMJ Models Of ALS

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***Purpose/Objectives:** Astrocytes are the most abundant non-neuronal cell type in the central nervous system (CNS) and serve numerous critical functions. However, under disease or injury conditions, astrocytes can be activated and become toxic or even detrimental to neurons. Increasing evidence indicates that astrocytes play active roles in neurodegenerative pathologies and represent an important therapeutic target. The goal of this study was to develop an in vitro astrocyte model for ALS.
***Methodology:** ALS patient-derived induced pluripotent stem cells (iPSCs) will be utilized as the source to generate astrocytes. The differentiated ALS-astrocytes were characterized for their pathology by phase and immunocytochemistry, and will be investigated for their effects on motoneurons and their synaptic target, neuromuscular junctions.
***Results:** First, a protocol was established to differentiate astrocytes from human iPSCs. Cells in different differentiation stages were characterized by immunocytochemistry and flow cytometry. This protocol was then optimized by establishing cell stocks at different differentiation stages so that the cell preparation period could be significantly shortened if needed. The protocol was further modified so that the generated astrocytes are easily adapted into our established human motoneuron and functional NMJ systems. Next, astrocytes were generated from iPSCs of normal subjects (WT) and ALS patients (carrying the SOD1 mutation), respectively, by the protocol application. Preliminary characterization by phase microscopy and immunocytochemistry has revealed pathological changes in SOD1-astrocytes. Compared with WT-astrocytes, SOD1-astrocytes have a high proliferation rate and demonstrate strong expression of GFAP, a hallmark for astrocyte activation. Subsequently, their interactions with MNs will be dissected by mix-match coculture experiments and their effect on NMJs will be investigated by integrating these astrocytes into a functional NMJ system.
***Conclusion/Significance:** The outcomes from this study will not only shed light on the etiology of ALS disease, but also develop invaluable platforms for drug evaluations for their effect and toxicity.

193 - Production Of Galantamine Releasing PLGA Microparticles And In Vitro Evaluation Of Their Biocompatibility In Primary Astrocytes Culture

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***Purpose/Objectives:** Galantamine, an acetylcholinesterase inhibitor, is shown to have neuroprotective and anti-oxidant effects, as well as increasing neurogenesis. Previous studies from our group have indicated that galantamine improves recovery after spinal cord injury (SCI). However, the need of repeated dosing and the cholinergic side effects of galantamine are the major hurdles for the optimum usage of this drug. Hence, the aim of this study is to produce and characterize microparticles of the biodegradable polymer poly (lactic-co-glycolic acid) (PLGA) containing galantamine as a strategy for sustained release of the drug after its local administration.
***Methodology:** The microparticles were produced by electrospraying, with 2.5% of galantamine mixed in a 4% PLGA solution and 4% PLGA alone used as a control (vehicle only). The solutions were electrosprayed with a flow rate of 0.1 ml/h, voltage of 26kV and 9 cm of distance from the needle to the collector plate. The morphology of the particles was evaluated by Scanning Electron Microscopy (SEM) and the diameter, zeta potential and polydispersion index of the particle suspension was measured by the Zetasizer. The galantamine release from the particles was evaluated by suspending the particles in PBS and collecting the supernatant after 1, 3, 6, 12 and 24 hours. The galantamine concentration in each sample was evaluated by HPLC. Astrocytes isolated from the cortex of 3-5 day Wistar rat pups were incubated with the microparticles and the cellular viability was analyzed by WST8. Nuclear morphology was evaluated by Nuclear Morphometric Analysis after DAPI staining.
***Results:** The average particle diameter was 434.73 ± 49.67 for the 4% PLGA particles and 568.3 ± 172.5 nm for the PLGA particles with 2.5% of galantamine. The zeta potential of the particles was of -41.5 ± 4.95mV for the 4% PLGA particles and -23.6 ± 4.6 mV for particles containing 2.5% of galantamine. The polydispersion index was 0.543 ± 0.04 for the 4% PLGA particles and 0.6 ± 0.09 for PLGA with 2.5% galantamine. The HPLC analysis of galantamine release revealed that the drug was continuously released during the period analyzed, with a peak of 18.8µg of galantamine after 12 hours. The WST8 assay showed that the PLGA microparticles increased astrocyte viability on days 1, 3 and 7 when compared to the controls. However, the PLGA particles containing galantamine did not present this effect, showing that galantamine reversed the proliferative effect of PLGA. Nuclear morphometric analysis showed no significant differences regarding the percentage of senescent, irregular and apoptotic nuclei after the treatments.
***Conclusion/Significance:** In conclusion, the preset study shows the successful production of PLGA-galantamine microparticles, with a controlled drug release and no cytotoxic effects on the astrocytes.
Financial support: MCTI, FINEP, CNPq and Stem Cell Research Institute.

195 - Chemical Substances Affect Cell Behavior

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In regenerative medicine production, preparing desired numbers of cells in scheduled time is very important. We are studying key factors to stabilize cell proliferation. Especially, factors that inhibit cell growth. We have analyzed air in cell culture environment and found there are chemicals released to cell cultured air that are soluble to medium. These dissolved chemical components are found in cell culture

environment, but their effects have not been investigated since they have never been eliminated from air. We have developed a novel device that removes these components from air. Human umbilical cord vein endothelial cell (HUVEC) is cultured in this container for 48 hours and compared with cells cultured in original air environment. The result shows that there is no significant change observed morphologically and cell proliferation. However, significant change in gene expression which indicates that chemical components originated in air may affect cell behavior when cells are cultured for longer period or cultured more sensitive cells. Future work will be culturing cell for longer period, and using stem cells to see if chemical components are one of the factors to destabilize stem cell culturing, and which substances needs to be controlled.

196 - The Use Of Electronic Laboratory Notebooks (ELN's) In A Required Cellular Engineering Laboratory Course For Undergraduate Biomedical Engineering

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***Purpose/Objectives:** In this study we discuss the implementation of Lab Archives Electronic Notebook (ELN) in a lab-based course (junior level) at the University of Florida. The transition from paperbound laboratory notebooks to electronic notebooks is motivated by industry needs for improved documentation skills and the prevalence of electronic formats in research laboratories and clinical environments; all of which biomedical engineers are active. Although paperbound notebooks allow users to quickly take notes and sketch designs, it is difficult to appendage and share the vast types and amounts of digital media being generated.
***Methodology:** Instructors provided documentation scaffolding language for each ELN page which mapped with rubric performance indicators used for assessment. Compared to previous semesters, lab documentation became a formalized output of the class and accounted for 15% of the student's final grade in the course. Digital submission of lab documentation required students to publish their ELN page to PDF and upload in the learning management system Canvas. Transition to ELN began in Fall 2017 with a course enrollment of n=8 and has continued through Spring 2019; with n=65, n=56, and n=55 for Spring 2018, Fall 2018, and Spring 2019, respectively.
***Results:** The implementation of ELNs into the laboratory course has been a critical aid in the scalability of the course as our undergraduate program continues to grow. Students accessed their ELN an average of 420 unique instances over the course of the semester (15 weeks). With the ELN ease-of-use and instructor management features, we are also able to investigate how often students access their entries and how this relates to class engagement, and the quality of their documentation entries and how they relate to scored rubrics, and overall student performance in the course. We have found a positive correlation between student's total lab documentation score and final score for the course [r=0.79, n=182].
***Conclusion/Significance:** Sharing these study results with students has the potential to reinforce the concept of laboratory notebook-keeping as a foundational skill. The integrated use of electronic laboratory notebooks and learning management systems can be translated to other lab-based courses in biomedical engineering with the ultimate goal of introducing and reinforcing proper lab documentation across the undergraduate curriculum.

197 - Innovation And Entrepreneurship In Undergraduate Curriculum

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***Purpose/Objectives:** Innovation is interdisciplinary in nature, and students with interests in Biology, Business, Law, Psychology, and Engineering can work together to solve problems. The interdisciplinary aspects of planning, designing, testing, and promoting biological projects makes it ideal for increasing entrepreneurship in the classroom. The rationale underlying the success of the students rests on evidence that cooperative, active, and entrepreneurial learning activities improve student performance by increasing student engagement, retention, and achievement. MS&T's BioDesign & Innovation classroom model includes active and cooperative learning activities that are interdisciplinary in nature. The approach is also consistent with the MS&T culture and environment which provides and requires experiential learning.
***Methodology:** Evaluated learning outcomes included: entrepreneurial mindset, innovation, creative problem solving, design thinking, communication skills, and applied biology. It has also been observed increased confidence and enthusiasm of students at the end of the course.
***Results:** This course provided students a realistic understanding of the process of innovation, product development, regulation, and business aptitude. In the two semesters this course has been offered, nine teams (60%) have competed at regional entrepreneurship competitions, with 6 wins. Ten students (34%) have received funding to develop their ideas. Multiple groups have filed patent disclosures and formed LLC's. Additionally, this course has increased interdisciplinary interaction of undergrads. The first semester, the class attracted only biological sciences majors. The second course offering, over four departments were represented, including Biology, Psychology, Business, and Mechanical Engineering.
***Conclusion/Significance:** Utilizing an interdisciplinary, problem-based learning approach to biodesign and innovation allows students to foster an understanding of the process used in biological entrepreneurship. The tools and information gained in this class are valuable in creating a well-rounded education that allows students to drive products towards the market and create innovation.

198 - Biotechnician Assistant Credentialing Exam

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***Purpose/Objectives:** Biotility, the education and training arm of the University of Florida's Center of Excellence for Regenerative Health Biotechnology (UF CERHB) was established to enhance Florida's competitive ability to attract and grow a world class biotechnology industry; building on the availability of responsive and relevant education and training programs which provide a multi-tiered source of workforce talent.
***Methodology:** The *Biotechnician Assistant Credentialing Exam* (BACE) was established in 2012 at Biotility to support economic development and provide skilled workers for Florida's expanding Biotechnology Industry. The opportunity to earn industry-recognized credentials is becoming increasingly important in programs designed to prepare students for careers, and as result the BACE is now utilized nationwide.
***Results:** We invite you to learn about the development of the credentialing exam, the involvement of the bioscience industry for its recognition, outcomes, and the industry positions to which it aligns.

***Conclusion/Significance:** Emphasis will be on bioscience industry credentialing and careers, national efforts on transportability of credentials, secondary-to-postsecondary credentialing, and articulated credit.

199 - Efficient In Vivo Direct Conversion Of Fibroblasts Into Cardiomyocytes Using A Nanoparticle-based Gene Carrier

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The reprogramming of induced cardiomyocytes (iCMs) has shown potential in regenerative medicine. However, in vivo reprogramming of iCMs is significantly inefficient, and novel gene delivery systems are required to more efficiently and safely induced in vivo reprogramming of iCMs for therapeutic applications in heart injury. In this study, we show that cationic gold nanoparticles (AuNPs) loaded with Gata4, Mef2c, Tbx5 function as nanocarriers for cardiac reprogramming. The AuNP/GMT/PEI nanocomplexs show high reprogramming efficiency in human and mouse somatic cells with low cytotoxicity and direct conversion into iCMs without integrating factors into the genome. Importantly, AuNP/GMT/PEI nanocomplexes led to efficient in vivo conversion into cardiomyocytes after myocardial infarction (MI), resulting in the effective recovery of cardiac function and scar area. Taken together, these results show that the AuNP/GMT/PEI nanocarrier can be used to develop effective therapeutics for heart regeneration in cardiac disease patients.

200 - Ecklonia Cava Phlorotannin Prevents Vocal Fold Fibrosis: In Vitro And In Vivo Study

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***Purpose/Objectives:** Vocal fold fibrosis is most commonly caused by trauma related to prolonged intubation, tracheostomy, inhalational burns, irradiation, infection, and surgical defect. There are diverse treatments for vocal fold fibrosis such as invasive procedures, laser treatment, stent placement, cryosurgery, treatment of corticosteroids, and treatment of mitomycin C. In particular, mitomycin C is used primarily for the treatment of vocal fold fibrosis, but it has several side effects including lung fibrosis, bone marrow depression, and edema. Therefore, we investigate the *Ecklonia cava* phlorotannin could prevent vocal fold fibrosis by *in vitro* experiments and *in vivo* animal model.
***Methodology:** Phlorotannin was extracted from *E. cava*. We investigated that *E. cava* phlorotannin has an inhibitory effect on vocal fold fibrosis and mechanisms of action through MTT assay and western

blotting. Moreover, we conducted an in vivo experiment in a rabbit disease model to evaluate the fibrosis preventive effect on a vocal fold.
***Results:** We evaluated *E. cava* phlorotannin has vocal fold fibrosis inhibitory effect in vivo and in vitro. The in vivo results indicated that *E. cava* phlorotannin inhibited the acute inflammatory reaction and suppressed fibrosis or ulceration. Additionally, *E. cava* phlorotannin repressed cell proliferation of vocal fold fibroblasts and inhibited type I collagen protein expression through suppressing phosphorylation of mothers against decapentaplegic homolog 2/3 (Smad 2/3), p38 mitogen-activated protein kinase (p38 MAPK) and extracellular signal-regulated kinase (ERK).
***Conclusion/Significance:** In the present study, we demonstrated that *E. cava* phlorotannin inhibited vocal fold fibrosis in the rabbit model and showed inhibitory effects on cell migration and TGFβ1-induced type I collagen in human vocal fold fibroblasts. Additionally, *E. cava* phlorotannin suppressed phosphorylation of p38 MAPK and ERK. Based on these finding, we suggest that that *E. cava* phlorotannin may be used in the prevention or treatment of vocal fold fibrosis

201 - Characterization Of Growth, Proliferation And Biomechanics In The Developing Murine Larynx: Implications For Tissue Engineering

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***Purpose/Objectives:** Vocal fold (VF) scarring is the greatest cause of voice disorders and remains resistant to treatment, due to permanent VF physiological changes. Current research employs tissue engineering strategies to promote vocal fold tissue regeneration. Novel approaches seek to use principles of developmental biology to guide tissue regeneration through mimicking native developmental cues, thereby causing the mature tissue or injected progenitor cells to undergo the generation process. We are characterizing structure-proliferation relationships and tissue elastic moduli over embryonic development to provide parameters with which to design scaffolds to investigate mechanoregulation of candidate cell differentiation and tissue formation.
***Methodology:** Laryngeal growth was measured as laryngeal length, maximum transverse diameter, and outer dorsoventral diameter; VF growth was measured as internal VF length and thickness. Growth measurements, cell proliferation (EdU) and tissue stiffness (measured using atomic force microscopy) were taken at E13.5, E15.5, E16.5, E18.5, P0, and adult time points.
***Results:** All growth profiles were found to be linear, except VF thickness, which underwent quadratic growth. Proliferation measured with EdU in the coronal and transverse planes was found to decrease with increasing development and age. Mean elastic moduli were measured with significant changes in modulus found between E15.5 and E18.5, and E18.5, P0 and adult time points.
***Conclusion/Significance:** Taken together, our results indicate that as the VF mature and develop linearly, there is a concomitant increase in stiffness. Greater gains in mechanical stiffness at later pre-natal stages, correlating with reduced cell proliferation suggest that changes in tissue extracellular matrix deposition could be responsible for VF thickening and increased mechanical function. Further, the abrupt change from embryonic to postnatal environments appear to have an impact on tissue mechanics. It is our intent that these data can provide a profile of VF mechanical and growth properties that can guide the development of mechanically-relevant scaffolds and progenitor cell differentiation for VF tissue regeneration. Acknowledgements: NIDCD R01 04336

202 - Treatment Of Pediatric Laryngotracheal Stenosis By Targeting Smad Signalling

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***Purpose/Objectives:** Laryngotracheal stenosis (LTS) is the buildup of pathologic fibrotic tissue in the lower larynx, which results in a life threatening narrowing of the airway and breathing impairment. About 8% of the infant in the intensive care unit then develop stenosis because of intubation. Current therapies are not successful in stopping scar tissue buildup and as LTS becomes more severe, it frequently requires laryngotracheal reconstruction by open surgery. The ensuing fibrosis is characterized by excessive production and accumulation of collagen mainly by laryngeal fibroblasts driven by higher local levels of TGF-β1. The aim of this research is to prevent scar tissue formation in the airway by halting TGF-β1signal transduction, namely by modulating the Smad 2/3 intracellular signal mediator using delivery of a small molecule inhibitor.
***Methodology:** We explored the targeted delivery of 6-[2-tert-butyl-5-(6-methyl-pyridin-2-yl)-1H-imidazol-4-yl]-quinoxaline), known as SB525334, to block TGF-β1 induced scar collagen accumulation and its application to prevent and treat LTS. The inhibitory effect of SB525334 on Smad2/3 transcription after TGF-β1 stimulation of fibroblast cells (NIH/3T3) were investigated *in vitro* and the effective concentration of the drug were determined using immunofluorescence staining (anti-Smad 2/3), quantitative Real Time PCR qRT-PCR (Collagen I, Collagen III, and fibronectin), and fibroblast proliferation assays. Then, Poly (lactic-co-glycolic acid) (PLGA) porous microparticles were fabricated and loaded by SB525334 using a single emulsion method. Then *in vitro* release profile of the SB525334 were measured and the inhibitory effect of PLGA/SB525334 on TGF-β1 stimulated fibroblast were explored using the same assays. The optimized *in vitro* system will be used for application on our LTS mouse model.
***Results:** The results showed that the effective concentration of the SB525334 to induce inhibitory effect without toxic responses is around 1μM. Due to the hydrophobic nature of both PLGA and SB525334 high loading can be achieved while optimized porosity of PLGA microparticle can be used to control the rate of release of the inhibitory molecule to maintain its local concentration within the therapeutic window. Anti Smad 2/3 fluorescence staining, expression of fibrosis markers, (Collagen I, Collagen III, and fibronectin), and the proliferation of fibroblast using Live/Dead and Quant-iT PicoGreen reveal the efficiency of the SB525334 to inhibit the fibrosis development *in vitro*.
***Conclusion/Significance:** Since the current method is cost effective, and more targeted compared to broad spectrum immunosuppressant, it is a promising approach to prevent and/or treat pediatric laryngotracheal stenosis.

203 - Increasing The Post-transplant Viability Of Kidney By Means Of Ex-vivo Normothermic Perfusion In A Porcine Model

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***Purpose/Objectives:** A short period of isolated normothermic perfusion (NP) can be used to improve the condition of the kidney after periods of warm and cold ischemic injury [1]. We have assessed the capability of NP to increase the viability of kidney after a long cold ischemia storage period using the ARK system.
***Methodology:** Female pigs were selected for both control and NP groups. Left kidneys were extracted and flushed with Ringer's lactate at 4°C. Kidneys were stored in ice for 20 hours with Custodiol HTK. In control group, preserved kidneys were reimplanted and right kidney nephrectomies were performed. In NP group, kidneys were connected to the ARK system for a 3-hour NP before reimplantation. ARK system is formed by a portable preservation unit, which features peristaltic and infusion pumps, heating and oxygenation systems, sensors and a control unit; and a disposable closed circuit, where organ and perfusate are contained in sterile conditions. Kidneys were perfused with a solution composed by Ringer lactate, 20% human albumin, red blood cells, creatinine and sodium bicarbonate. Perfusate was continuously supplemented with a nutrients solution, insulin and a vasodilator. Secreted urine was collected for analysis and corresponding lost volume of perfusate was replaced. Renal flow rate, mean arterial pressure, urine output, temperature, glucose concentration, hematocrit and oxygen saturation were recorded continuously. A blood gas analyzer was used to record parameters for acid-base homeostasis. Serum and urine samples were obtained hourly for biochemical analysis. After the 3-hour NP, kidneys were reimplanted and right kidney nephrectomies were performed. Pigs' evolution was monitored for 7 days after reimplantation.
***Results:** ARK NP system was able to maintain physiological levels of temperature, mean arterial pressure and arterial oxygen saturation in all cases throughout the 3-hour NP. Biochemical analyses in the transplanted animals show clear difference between groups: control group urea increases to values greater than 500 mg/dl; the same as creatinine, which exceeds 17 mg/dl. NP group shows increase of these values reaching the maximum (400-450 mg/dl and 10-11 mg/dl respectively) in post-surgery days 3 and 4, decreasing in the subsequent days to healthy levels. Similar trend is observed for BUN (control values >140 mg/dl vs NP peaks <140 mg/dl) and creatinine (control values >19 mg/dl vs NP peaks <13 mg/dl). Survival rate in the control group was 0% and in the NP group 100%.
***Conclusion/Significance:** Collectively, data illustrate the capacity of NP applied with the ARK system to increase the viability of kidneys previously subjected to long periods of cold ischemia.
Acknowledgments: Financial support was received from the Spanish Ministry of Economy and Industry (RTC-2016-5108-1), the Spanish Ministry of Energy, Tourism and Digital Agenda (TSI-100700-2015-13) and H2020 SME Instrument Ph.2 (804252).
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204 - Airway Smooth Muscle Cell Phenotype Is Modulated By Substrate Stiffness

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***Purpose/Objectives:** Airway smooth muscle, capable of modulating the bronchomotor tone, has been associated with several life-threatening diseases such as asthma and COPD. These conditions exhibit hyperreactivity in terms of mechanical transduction within the airways. In this study, we have developed

a hydrogel-based model to assess the effect of mechanical stiffness to the morphology, proliferation, and expression of α-smooth muscle actin of the primary human airway smooth muscle cells.
***Methodology:** Primary ASMCs (n=3) were expanded with 10% serum on hydrogels of increasing stiffness (1x, 6x, and 12x physiological stiffness) and assessed for proliferation by metabolic assay. Following serum starvation to induce a contractile phenotype the cell size was assessed using morphometric analysis and flow cytometry. Additionally, cells were immunostained for alpha-smooth muscle actin (a-SMA) expression. Finally, propidium iodide staining was used to determine cell cycle stage.
***Results:** After 24h of serum starvation the morphology of ASMCs changed significantly as cell area, cell perimeter, and nuclei area increased with increased matrix stiffness ($p<0.0001$). The aspect ratio and nuclei eccentricity decreased denoting a morphological change from "spindle-like" to a "spread-out" morphology ($p<0.0001$). The increase in cell size was confirmed using flow cytometry, with increasing forward scatter as the matrix stiffened. α-SMA expression increased four-fold with increased matrix stiffness. Furthermore, increased matrix stiffness led to an increase in ASMC proliferation and an increased in the percentage of cells in S phase of cell cycle.
***Conclusion/Significance:** The stiffness of the matrix affects the morphology, proliferation and the α-SMA expression of ASMCs. Together, the results suggest that the airway remodelling/stiffening could contribute to a phenotypic change on resident ASMCs, which occurs in chronic conditions such as asthma

205 - Between Air And Liquid: A Physiologically Relevant Pulmonary Organoid

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***Purpose/Objectives:** The airways of the human respiratory system are complex structures with multiple cell types and cell-cell interactions. Presently, two-dimensional (2D) culture methods fail to provide this complexity with monocultures of airway epithelial cells and no three-dimensional (3D) architecture, while animal models differ significantly due to species-specific physiologies and metabolisms. We have developed a novel multicellular bronchial airway organoid within a Transwell® culture system at air-to-liquid interface (ALI) for modeling pulmonary disease and infection. Initial data demonstrate that this organoid could provide an improved *in vitro* model for more closely modeling the physiology of human airways.
***Methodology:** 3D airway organoids were fabricated in a layered fashion in a Transwell® culture system with a basal hydrogel layer of lung fibroblasts, and an apical layer of differentiated primary bronchial epithelial cells. For an additional physiological component, a monolayer of endothelial cells can be seeded on the basal side of the membrane. The hydrogel contains solubilized human lung extracellular matrix (ECM) to provide native ECM components and growth factors that are expected to improve differentiation. The lung ECM was analyzed with colorimetric assays to quantify ECM components including collagen, elastin, glycosaminoglycans, and hyaluronic acid. Histology and multispectral analysis was completed to quantify similarities and differences in key bronchial differentiation markers between 2D culture, organoids, and native lung, while transepithelial electrical resistance (TEER) measurements was measured using an Ussing chamber and EVOM2 device. TEER can verify epithelial integrity and demonstrate pharmacological blocking and rescue of CFTR.

***Results:** After 21 days, the airway organoids are viable, and the epithelial cell layer has completely differentiated according to histology and multispectral analysis. Further imaging analysis indicates the presence of epithelial markers similar to that of native lung including cilia, mucins, and the cystic fibrosis transmembrane regulator (CFTR). The analysis of the solubilized lung ECM illustrated the presence of several key ECM components that could play a role in improved cell functionality including the high presence of collagen and glycosaminoglycans. Tenfold increases in TEER measurements indicated an increase in epithelial integrity characteristic of a barrier function once the cultures reached a complete monolayer and were transferred to ALI culture. Ussing chamber analysis further demonstrated epithelial integrity and functionality of the CFTR protein with characteristic curves and peaks.
***Conclusion/Significance:** We have developed a bronchial airway organoid model that more accurately recapitulates the human airway environment compared to current culture standards. The resident cells express markers similar to native lung and are expected to behave correspondingly when subjected to external stimuli. The bronchial epithelial cells behave differently than in standard 2D culture showing the importance of the 3D architecture, cell-cell interactions, and ALI culture conditions. Future directions for this model include incorporating more resident pulmonary cells and optimization of the hydrogel properties for improved physiological responses.

206 - Chemically Induced Hepatocytes Like Cells And Hepatic Tissue For Liver Failure Rescue And Regeneration

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Mesenchymal Stem Cells (MSC) differentiation strategy employed in deriving functional hepatocytes like cells (HLC) is growth factor based 28 days protocol, making it expensive and time consuming. Therefore, we have devised small molecules based stage specific differentiation strategy to derive functional HLC in 14 days using MSC. Our results confirmed the development of HLC in 14 days by undergoing differentiation in a stage specific manner mimicking embryological liver development *in vivo*. To achieve this objective, we divided the differentiation strategy into four stages, S1-Definitive Endoderm, S2-Hepatic Competence, S3-Hepatic Specification and S4-Hepatic differentiation and maturation. We employed small molecules for activating different stages of liver development. Development of stage specific cells were confirmed by RT PCR, ICC and the efficiency was confirmed by flow cytometry. Functionality of HLC was confirmed by CYP450 enzyme activity, PAS staining, albumin and urea synthesis. To confirm the therapeutic activity of the derived HLC, we transplanted SPION loaded cells by tail vein injection, under the influence of external magnet, in acute liver failure model created by hepatic devascularization technique. We further studied the differentiation protocol to derive hepatic tissue in decellularized liver ECM and transplanted it in a rat chronic liver failure model. In both models, significant improvement in liver function compared to the liver failure model was observed. Our work displays a novel method to derive biologically relevant and functional HLC from MSC which can improve liver function in acute and chronic liver failure, making it potentially viable for clinical application in hepatic failure patients.

207 - Cell Migration In Carbon Nanotube-glycol Chitosan Hydrogels For Vocal Fold Tissue Regeneration

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***Purpose/Objectives:** The vocal folds are heavily solicited during phonation, and they are prone to inflammation. Injectable hydrogels are under investigation as implants for injured vocal folds. Cell migration is an essential step of vocal folds wound healing process. Carbon nanotubes (CNTs) can play the role of fibrous proteins in the extracellular matrix and accelerate cell migration and tissue regeneration. The objective of this study was to investigate the influence of carboxylic-functionalized CNTs on three-dimensional cell migration.
***Methodology:** Composite hydrogels were prepared in triplicates using 2% glycol chitosan solution as the precursor, 0.005% glyoxal as the crosslinker, and different concentrations of CNTs (0, 250, 500, and 750 µg/ml). Human vocal folds fibroblasts (HVFFs) were cultured in completed Dulbecco's Modified Eagle Medium (DMEM)+10% fetal bovine serum (FBS). Before adding the crosslinker, the cells were passed and encapsulated in the hydrogels. A volume of 200 µl of hydrogels was then added into Delta-treated polycarbonate cell culture inserts with a pore size of 8 µm. After gelation, 200 µl of serum-free DMEM was added on the hydrogels' surface, and the inserts were suspended over the 12-wells companion plate, which contained 400 µl of completed DMEM +10% FBS. A gradient of chemoattractants toward the bottom of the hydrogel was therefore achieved. The samples were then incubated at 37°C in a humidified incubator with 5% CO_2. On day 7, the migrated cells were dissociated from the bottom of the inserts and were collected for cell counting. Counting beads were added to the cell solutions to do the volumetric cell counting using a FACSCanto™II flow cytometer.
***Results:** The chemoattractants gradient toward the lower compartment encouraged HVFFs to move across the porous polycarbonate membrane. The forward scattered area (FSC-A) and side scattered area (SSC-A) signals of the flow cytometer were the only signals used to discriminate the migrated cells and the counting beads. Since the size and the internal complexity of the counting beads were different from those of the HVVFs, there was no need for staining the cells. The cell migration index (CMI) is defined as the ratio of the number of cells migrated through the porous membrane and the total number of encapsulated cells. The addition of CNT to the hydrogels caused a significant increase in CMI. The CNTs that were firmly positioned in the hydrogel network served as potential focal adhesion sites for the HVFFs. The CNT-free hydrogels, which were used as the control samples, were lacking adhesion sites, with consequently lower CMI.
***Conclusion/Significance:** The 3D chemoattractant cell migration results confirmed that the presence of CNT in glycol chitosan hydrogel enhances cell migration in the hydrogel network. The greatest CMI was for CNT250 (the hydrogel with 250 µg/ml of CNT), which reveals that although the addition of CNT promotes cell migration, a greater concentration of CNT does not necessarily encourage the cells to migrate. In general, CNTs were found to be helpful in promoting cell migration in hydrogels, and ultimately increase tissue regeneration rate.

208 - Neonatal Vs. Adult Fibrin Matrix Properties In Wound Healing

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***Purpose/Objectives:** Fibrinogen is the primary clotting protein involved in hemostasis and forms an insoluble fibrin matrix following injury. In addition to its significant role in coagulation, fibrin provides a scaffold for cellular infiltration during tissue repair and thus, is often used as a biomaterial in tissue engineering. To date, differences have been identified in fibrin network properties between adults and neonates including structural and fibrinolytic properties. The outcome of wound healing has been linked to fibrin matrix structure, such as porosity and permeability, as it can affect the binding and migration of hemostasis proteins. Additionally, fibrin matrix functional properties such as stiffness and degradation rate has been shown to affect cellular proliferation and migration. The age-dependent differences in fibrin properties may be a factor in fetal scarless wound healing, a phenomenon in which in utero full thickness wounds heal through complete regeneration rather than repair. In this study, we aimed to fully characterize differences in fibrin matrix properties between adults and neonates and the resulting implications in wound healing.
***Methodology:** For all assays, fibrinogen was isolated from platelet poor plasma from adults or neonates (infants less than 30 days of age) via an ethanol precipitation reaction and fibrin clots were formed in the presence of thrombin. Structural analysis of fibrin matrices was conducted with cryogenic scanning electron microscopy (cryoSEM) and confocal microscopy, mechanical properties were explored using atomic force microscopy, and clot polymerization and degradation properties were analyzed with absorbance based assays. Cell based assays were conducted with human neonatal dermal fibroblasts on fully polymerized fibrin matrices. Additionally, validation of age related differences in porcine fibrin clot properties was conducted.
***Results:** Structural results indicate neonatal fibrin matrices are thin, sheet-like, and have a low degree of cross branching compared to adults. Functional characterization revealed neonatal fibrin networks are significantly less stiff than adults with slower rates of polymerization and rapid network degradation. Cell assays indicated significantly higher fibroblast attachment to neonatal fibrin matrices compared to adults. In our characterization of porcine specimens, we identified similar age-dependent distinctions in fibrin clot structure, stiffness, degradation, and functionality between porcine and human samples
***Conclusion/Significance:** Neonatal fibrin clots have distinct structural, mechanical, and functional properties compared to adults. On-going experiments include investigations into the impact of age-specific distinctions in fibrin matrix properties on wound healing, cell attachment and migration. Preliminary cell experiments indicate differential cellular behavior on neonatal and adult fibrin matrices. Additionally, we found neonatal porcine fibrin matrix properties mirrored those of neonatal humans. Therefore, it is possible that subsequent studies and materials may utilize neonatal porcine fibrin in place of human derived protein.
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209 - Understanding Contact Guidance In Oriented Fibrin Gels

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***Purpose/Objectives:** Contact guidance, a phenomenon in which cells orient in response to the aligned extracellular matrix, is known to play a critical role in many physiological processes and tissue engineering. However, the underlying signal that drives cell contact guidance in an aligned matrix

remains elusive, mainly due to the simultaneous presentation of multiple interdependent cues by the complex 3D fibrillar networks, including anisotropy of stiffness, adhesion, and porosity.
***Methodology:** In order to dissect the underlying signal of contact guidance, we developed a 3D guidance field comprising of highly oriented fibrin matrices entrapping human dermal fibroblasts by employing a high strength magnetic field (9.4T). Subsequently, to test the anisotropic stiffness hypothesis of contact guidance, we systematically modulated the stiffness of these aligned matrices via Ruthenium-based photocrosslinking technique which induces dityrosine bond formation both within and between the fibrils. Further, to probe the role of adhesion anisotropy, we modulated adhesion by incubating the cells with varying concentrations of integrin blocking antibodies before entrapment in the magnetically-aligned fibrin gel.
***Results:** Preliminary active microrheology assessment revealed an increase in the anisotropic stiffness with increasing extent of crosslinking. However, photocrosslinking did not induce any detectable change in network microstructure or the adhesion strength, as confirmed by 3D morphometric analysis of confocal reflectance images and centrifugation assay, respectively. Thus, by utilizing magnetic alignment and the photocrosslinking strategy, we were able to modulate the anisotropic stiffness of 3D matrices, without affecting the adhesion and porosity anisotropies. Accordingly, fibroblasts exhibited stronger contact guidance in the aligned and crosslinked gels, suggesting the role of stiffness anisotropy in dictating the contact guidance response. On the other hand, varying the adhesion was found to have much less impact on the cell contact guidance response.
***Conclusion/Significance:** Overall, these results could improve our current understanding of contact guidance mechanism and shed light on engineering clinically relevant tissue equivalents with better control of cell alignment.

211 - High Hydrostatic Pressure Therapy Kills Squamous Cell Carcinoma Cells

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***Purpose/Objectives:** Cutaneous squamous cell carcinoma (cSCC) is one of the most common skin cancers. In the treatment of cSCC tumor, it is important to completely extirpate the tumor. Depending on the size of the tumor, reconstructive surgery—such as a skin graft or a local or free flap—may be required. When we reconstruct skin defects after tumor removal surgery, donor site morbidity is an important issue for the patient, so aggression at the donor site must be managed. We therefore considered how to perform excision and reconstruction of a malignant tumor with limited aggression at the donor site. High hydrostatic pressure (HHP) was used in an attempt to decellularize various tissues. We previously reported that HHP at 200 MPa for 10 min was able to inactivate all cells in giant congenital melanocytic nevus (GCMN), and we have already started a clinical trial using this technique. In our clinical trial for GCMN, a nevus inactivated by HHP was used as autologous dermis to cover full-thickness skin defects after the tumor removal instead of autologous skin grafting. We suspected that cSCC tissue treated with HHP might be able to be transplanted as an autograft.
***Methodology:** In vitro, we purchased and prepared cutaneous cell carcinoma A431 cells. We set 5 kinds of pressurization conditions in this study: pressurization at 150, 160, 170, 180 and 190 MPa for 10

min respectively. Live/dead staining of cells, morphological observation and a proliferation assay of the cells were performed. In vivo, a suspension of 10^6 cells/mL cSCC cells was prepared in the culture medium. Cell suspension was treated with HHP at 200 MPa for 10min. 200 microliters of suspension including 10^6 cells/mL cells were injected into the intradermal space on the back of 8-weeks-old male mice in pressurized group and non-pressurized group. Each specimen was taken at 9 weeks after injection. The formed cSCC tumors were devided into small peaces. Half of them were preserved at room temperature without HHP, and the other half were pressurized at 200 MPa for 10min. The tumors were implanted into subcutis on backs of 8-weeks-old male mice. Specimens were observed, taken and weighted at 5 weeks after implantation. The hematoxylin and eosin (HE) staining, p63 immunohistochemical staining, and Ki67 immunohistochemical staining of the cSCC tumors were conducted 9weeks after cell injection and 5 weeks after tumor implantation.
***Results:** cSCC cells were inactivated by ≥160 MPa for 10 min, and these inactivated cells did not grow *in vitro*. *In vivo*, the cSCC cells inactivated by HHP at 200 MPa for 10 min were unable to proliferate in mice. In addition, the transplanted cSCC tissue inactivated by HHP decreased the weight of the transplanted cSCC at 5 weeks after implantation.
***Conclusion/Significance:** HHP at ≥160 MPa for 10 min can inactivate cSCC cells and tissue. HHP at 200 MPa for 10 min was able to inactivate malignant tumor. HHP can become one application of new cancer treatment.

212 - Application Of A Porcine Small Intestine Submucosa Nerve Cap For Prevention Of Neuromas And Associated Pain

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***Purpose/Objectives:** Painful neuroma formation is a common and debilitating sequela of traumatic or oncologic nerve transection during amputation surgeries. Studies suggest that isolating the nerve-end within a protective cap either at the time of amputation or during revision procedures may assist in the prevention of symptomatic or painful nerve-end neuromas. This study evaluated the local effects including objective behavioral pain stimulus assessments that novel porcine small intestine submucosa (pSIS) nerve caps with internal chambering had on neuroma formation at a terminal nerve end in an animal model.
***Methodology:** The tibial nerves of forty-eight Sprague Dawley rats were transected, trans-positioned, and secured in a subcutaneous pocket of the lateral hindleg. The nerves were treated with either a Nerve Cap with spiral chambering termed Spiral Nerve Cap (SNC), a Nerve Cap with bifurcated chambers termed Chambered Nerve Cap (CNC), or were non-treated Surgical Controls (SC). Weekly pain response testing was performed by observing animals after mechanically stimulating transposed nerve ends. Samples were explanted at 8 and 12 weeks and stained with Hematoxylin and Eosin, Masson's Trichrome, or Neurofilament-200. Sample analysis included axonal swirling, axon optical density (OD), and nerve width.
***Results:** The SC group had significantly higher axonal swirling and pain response scores compared to the Nerve Cap groups. Nerve width was notably greater in the SC group compared to the Nerve Cap groups. The SC group also showed significantly lower axon optical density compared to all other groups. A lower optical density suggests a higher concentration of collagenous tissue, which is a characteristic of neuromas.

***Conclusion/Significance:** Application of pSIS nerve caps in this animal model demonstrated increased axon optical density and decreased axonal swirling, distal nerve stump width and behavioral pain response. This suggests that nerve caps with internal chambering may support axonal fibrin cable formation and facilitate axonal alignment thereby reducing the likelihood of painful neuroma formation.

214 - Reinforced Biomaterial Scaffolds For Tissue Engineering

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***Purpose/Objectives:** Collagen and gelatin are commonly used natural polymers for tissue engineering. Compared to synthetic polymers, natural polymers have better performance in mimicking extracellular matrices. Despite the advantages, single-component natural polymer-based templates do not always meet the necessary requirements of a viable biomaterial scaffold. Disadvantages of natural polymers include high degradation and catabolization rates, low stability, and lack proper biomechanical properties for different tissue types. Chemical modification may improve the functional characteristics of natural polymer-based constructs.
***Methodology:** Collagen and gelatin-based scaffolds were structurally reinforced via chemical crosslinking using carbodiimide/N-hydroxysuccinimide (EDC/NHS) and physical crosslinking with dehydrothermal (DHT) treatment. Polymer blends were synthesized with polycaprolactone (PCL) and hydroxyapatite (HA). Biologically active three-dimensional (3D) constructs were created. Physical properties including degradation, porosity, chemical composition and mechanical properties of the reinforced biomaterials were analyzed using microCT, Fourier transform infrared (FTIR) microscopy, micro-mechanical tester and atomic force microscopy (AFM). Biocompatibility including cell viability and cell proliferation were assessed with *in vitro* dermal fibroblast and myoblast culture.
***Results:** Low-high carbodiimide concentrations of 25mg/ml-100mg/ml used to crosslink collagenous scaffolds improved the mechanical integrity of scaffolds and stability against dissolution in a proportional manner. DHT crosslinking was found to modify the mechanical properties of scaffolds proportionally based on treatment time. Cell attachment tests using myoblasts showed high metabolic activity and good cell morphology across the scaffold.
Elastic porous Collagen+HA scaffolds were fabricated from the self-organized HA/Collagen nanofibers by lyophilization and tested to compare against collagen alone. The scaffolds demonstrated sponge-like elastic properties when wetted and higher compressive strength compared to collagen alone. They had high cell attachment and proliferation compared to more compact scaffolds. These mechanical and biological properties provided easier handling and better cell invasiveness in vitro than those of porous ceramics (HA alone) and collagen alone.
PCL-collagen copolymer sheet was designed and tested. IR spectroscopy revealed the presence of amide I and II peaks for the conjugated material. After 3 days of culture, fibroblasts exhibited a spindle-like morphology, spreading homogeneously along the PCL-collagen film surface. Good metabolic activity was obtained on PCL-collagen films compared to PCL controls.
Porosity obtained from microCT was correlated with mechanical behavior from microscale testing to determine relationship between the two physical properties. Higher porosity resulted in higher indentation for collagen scaffolds while lower porosity portrayed more rigidity at micro-scale in the crosslinked-collagen and DHT collagen samples.

The reinforced biomaterial scaffolds demonstrated high tunability. The 3D scaffolds maintained high viability and enabled the proliferation of different cell types without the use of specialized growth mediums. The reinforced scaffolds exhibited significant enhancement in mechanical properties and slower degradation rates as compared to controls.
***Conclusion/Significance:** Enhanced natural polymer-based scaffolds were created that were compatible with skin, cartilage and muscle cell types with possible utility for dermal, myo, and cartilage applications. The scaffolds were characterized with non-destructive techniques to better understand how different types of modification strategies altered their performance for tissue engineering utilization. The novelty and significance of this research offers a major step forward in tuning the biological performance of engineered scaffolds.

215 - Network Formation Of Endothelial Cells On Hydrogels Derived From Decellularized Matrices

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***Purpose/Objectives:** The decellularized tissues have been widely used as biomaterials to reconstruct and repair the various tissue. It can be easily processed in many forms such as powders and hydrogels. Recent reports demonstrated that decellularized extracellular matrix (dECM) based hydrogels, which are prepared by solubilizing the decellularized tissues, have composition similar to that of native tissue, and useful as scaffolds. We have previously reported that high-hydrostatic pressure (HHP) method shows little damage to tissues even after decellularization process compared with detergent method. So, we hypothesize that the HHP-dECM hydrogels have better effects on tissue regeneration. In this study, we prepared various types dECM hydrogels using various decellularization methods, and investigated the characteristics of dECM hydrogels. Also, the network formation of endothelial cells on their dECM-hydrogels was investigated.
***Methodology:** Porcine urinary bladder matrix (UBM) and small intestinal submucosa (SIS) were decellularized by two kinds of methods; detergent method and HHP method, respectively. The decellularization was confirmed by H-E staining and residual DNA quantification. The dECM hydrogels were prepared by solubilizing dECM by pepsin and following adjusting pH and salt concentration to physiological conditions. The gelation kinetics, hydrogel structures and mechanical properties were evaluated by turbidity measurement, SEM observation and compression test, respectively. The cell behavior on the dECM hydrogels was evaluated using endothelial cells derived from rat brain. The cells were seeded at 1.0×10^5 cells/cm2 on dECM hydrogels, and cultured for 3 days. The network formation was analyzed by ImageJ software.
***Results:** The gelation of HHP-dECM solution was much faster than that of detergents-dECM solution. SEM observation revealed that all hydrogels show fibrous morphologies with randomly oriented collagen fibrils. The mechanical properties of dECM hydrogels were different depending on the origin tissue and decellularization methods. It was suggested that HHP-dECM hydrogels have high mechanical properties compared to SDS-dECM hydrogels. The endothelial cells effectively formed their network on the HHP-dECM hydrogel compared to on a collagen hydrogel. Also, the network formation depended on the mechanical property of the HHP-dECM hydrogel.
***Conclusion/Significance:** The hydrogel formation mechanism is different by the decellularized methods, and the mechanical properties of hydrogels affect cellular behaviors.

216 - Pre-infiltration And In-tissue Polymerization Of Photocrosslinkable Hydrogel For Effective Fixation Of Implants Into Host Cartilage

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***Purpose/Objectives:** Cartilage defects represent a challenging clinic problem which can cause pain, swelling, and eventual onset of osteoarthritis (OA). There are some surgical treatments available, none of which however can fully restore the structure and function of injured cartilage. In particular, the effective fixation of grafts/implants into host tissue has been poorly achieved. This study aimed to develop a robust strategy to fix grafts into host tissue. We have recently developed a biodegradable and photocrosslinkable [Poly-D,L-lactic acid/polyethyleneglycol/poly-D,L-lactic acid] (PDLLA-PEG, monomer as DLLA-EG) hydrogel scaffold[1]. Interestingly, DLLA-EG and photoinitiator LAP can infiltrate cartilage before polymerization, and then form a hydrogel within the cartilage upon visible light illumination. With this unique property, we hypothesize that *pre-infiltration of DLLA-EG/LAP into the graft and host cartilage can form an interconnected hydrogel network between them upon light illumination, thus resulting in a tight fixation.*
***Methodology:** We employed a ring-disk cartilage repair model, in which a bovine cartilage ring with a 6mm outer diameter and a 4mm inner diameter was used as the host tissue, and a cartilage disk with a 3mm diameter served as the implant. Both the ring and the disk were first soaked in a PDLLA-PEG/LAP solution for 5 minutes (PreI-5min). The group lacking pre-infiltration (PreI-0min), and the group fixed by fibrin served as the controls. A standard push-out test was conducted to evaluate the binding strength (peak force, PF, in the best) between the graft and host tissue.
***Results:** PreI-5min group had a significant higher PF (1504.75±313.33kPa, n=3) than the PreI-0min group (475.77±78.04kPa, n=3) and fibrin group (73.86±27.27kPa, n=3), suggesting a significantly stronger fixation. To test whether pre-infiltration would adversely affect the phenotype of cartilage, we evaluated the gene expression by q-PCR. Results showed that the gene expression level of SRY-Box 9(*SOX9*), collagen type I (*COL1*), matrix metalloproteinase 13(*MMP13*), and thrombospondin motifs 5(*ADAMTS5*) had no difference. Interestingly, PreI-5min group had a higher gene expression level of aggrecan (*ACAN*) and collagen type II (*COL2*). Live/dead assays showed that cell viability was not affected by the pre-infiltration process. After 4 weeks of culture, implants from PreI-5min group were still tightly fixed in position, which were not observed in other two groups. Finally, we measured the Sulfated glycosaminoglycan (sGAG) in cartilage. Interestingly, cartilage in PreI-5min group maintained highest sGAG, suggesting the protective effect from hydrogel within the cartilage.
***Conclusion/Significance:** With the pre-infiltration of monomer, we achieved a strong and long-term fixation between the implant and host cartilage without affecting the cell viability, phenotype, and the gene expression of cartilage. Since PDLLA-PEG is biodegradable, it is eventually replaced by newly formed cartilage matrix.
References: [1] Sun AX, Lin H, Tuan RS, et al. (2015). Front.Bioeng.Biotechnol.

219 - BEHAVOIR OF WJ-MCSS SEEDED ON EXTRACELLULAR MATRIX-SCAFFOLD.

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***Purpose/Objectives:** Although the bones have the capacity for repair and regeneration, these conditions are limited to the size of the lesion, so that critical defects (with a diameter greater than 1 cm) require the addition of grafts or bone substitutes, where a technique that can be considered as a gold standard has not yet been found and therefore can be used routinely clinically. We propose a temporal bioscaffold (extracellular matrix-based) obtained from bovine cancellous bone, in combination with human mesenchymal stem cells of Wharton´s jelly (WJ-MSC), which we will evaluate, physics, chemistry, and biology to identify the in vitro behavior of the WJ-MSC.
***Methodology:** We obtain an acellular, 3-D interconnected porous scaffold derived from cancellous bovine bone, which is processed by decellularization, demineralization, and hydrolysis of the collagen protein, we cut horizontal slices of the bovine femur, then decellularized sequentially with use of sodium dodecyl sulfate (SDS) and TritonX-100. To carry out the demineralization we use the standard method, which is achieved at a concentration of 0.5 M HCl, we make variations to the concentration to determine if it causes damage to the structure and/or alterations to the architecture and porosity, and the alkaline hydrolysis of the collagen, we carried out it by immersing the cancellous bovine bone in NaOH. The histological sections allowed to evaluate the structure and decellularization of the scaffolds on the other hand by scanning electron microscopy (SEM) we evaluate the 3D structure and diameter of pores, the FTIR and XDR techniques were used to determine the chemical composition and demineralization respectively of the scaffold. Finally, we evaluate the biocompatibility of the scaffold.
***Results:** We obtain extracellular matrix-based bioscalds wich shows sponge-like properties. On histology sections and SEM scaffolds showed the conservation of the structure of the ECM and cell removal, and by FTIR we determined that the main component is collagen type I with different degrees of denaturation depending on the concentration of hydrochloric acid (HCl). XDR allowed determining that the mineral phase was eliminated according to the guidelines established for the FDA for demineralized scaffolds The scaffolds also showed excellent swelling and mechanical stability.
***Conclusion/Significance:** In vitro cell culture study showed that the scaffold has no cytotoxic effect in the first 28 days.

220 - Tunable Bioink For Support Of Multiple Type 2 Diabetes Specific Tissue Constructs

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***Purpose/Objectives:** The interactions between glucose and fatty acids, specifically competition between the two when in abundance, may play a role in insulin sensitivity and diabetes. Major organs/tissues participating in this cycle are the islet (source of insulin), the adipose tissue (source of fatty acids in lipid abundance), the muscle (consumer of glucose and fatty acids), and the liver (source of glucose). Considering these components, we have developed tissues to study the Type 2 Diabetes micro-physiological system for disease modeling and potential drug treatment screening.

***Methodology:** Islet, adipose, liver, and muscle models were created using methacrylated collagen type-1 and thiolated hyaluronan acid with laminin, fibronectin, both, or neither. Each tissue system was created independently and quantitative measures in relation to response to glucose, culture viability, metabolism, and tissue specific markers were observed overtime.
***Results:** Islet models created within the universal bioink showed appropriate response to hyperglycemic glucose stress testing and maintained viability across 7 days. Data suggest that when normalized to initial insulin production, the universal bioink with supplemental laminin allowed islets to have the greatest insulin output. Adipose models were also found to differentiate at a greater rate when cultured with laminin, and when tested using glucose (high, low) adiponectin response was decreased, as in the case of human patients. Liver and muscle tissues were each developed within the bioink to create healthy baseline models. Current studies include integration of all 4 tissue construct types in a closed microfluidic platform and integrated hyperglycemia insult studies.
***Conclusion/Significance:** Using a universal collagen type-1 and hyaluronan based bioink we have been able to create four normal tissues of which two have been tested and responsive to glucose. Combined we will be able to model diabetes through modulation of the islet model and study the systemic effect and responses from supporting models (adipose, liver, muscle).

221 - Enzyme Assisted Peptide Folding, Self-assembly And Bioactivity

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***Purpose/Objectives:** Most of peptide segments in proteins are constrained into certain conformations, especially for bioactive peptides. However, when these peptides are separated from proteins, they failed to fold into their inherent conformations and therefore lost their functionalities.
***Methodology:** The generation of α-helical peptide and α-helix mimetic is very important to achieve biological functions of peptides or inhibit protein-protein interactions. Several strategies have been developed to induce α-helix conformation of short peptides including constrained peptides and foldamers. We recently found that enzyme instructed self-assembly (EISA) provided a unique pathway to trigger the formation of α-helix of short peptides, which was unable to be achieved by other conventional methods.
***Results:** The nanomaterials formed by EISA held several advantages including more stable nanostructures, better stability against enzyme digestion, enhanced cellular uptake, bigger fluorescence signal to noise ratio, and higher efficiency to inhibit cancer cells and tumors. Using different pathways to trigger peptide self-assembly, we could fold bioactive peptides into α-helix and β-sheet and we developed a self-assembling peptides with superior bioactivity to the growth factor of IGF-1.
***Conclusion/Significance:** We envisioned that EISA, in combine with kinetic control of enzymatic reaction, would lead to nanomaterials with well controlled nanostructures and biofunctions.

222 - Evaluation Of A Red Propolis Bioproducts For Regenerative Medicine

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***Purpose/Objectives:** The anti-inflammatory, antimicrobial and regenerative properties of red propolis (RP) are interesting for regenerative medicine, especially skin healing, in which treatment of burns and wounds are in high demand.
***Methodology:** With this purpose, the RP properties were added to nanocapsules and GelMA hydrogels, which are 3D polymeric structures with high aqueous content, used with the aim of mimicking ECM. Therefore, hydrogels with RP lipid core nanocapsules were constructed. Two groups of nanocapsule suspensions were developed: NC PCL, a Poly-ε-Caprolactone nanocapsule without RP, and NC Prop, a PCL nanocapsule with 5 mg/mL of RP. A RP hydroalcoholic solution (HA) (50:50)(5mg/mL) was also produced. Following this, nanocapsules and HA solution were incorporated in GelMA hydrogels (15% with 0.5% Irgacure). They were evaluated for cytocompatibility by WST-8, lactate dehydrogenase (LDH) dosage and confocal microscopy, on days 1, 4 and 7 after seeding the adipose-derived stem cells. The tested groups were: 1) Control, which corresponds to cells seeded on wells; 2) NC PCL, a PCL nanocapsule added to the wells in the same volume of NC Prop; 3) NC Prop; 4) HA; 5) GelMA, a pure hydrogel; 6) G PCL, GelMA plus NC PCL; 7) G Prop, GelMA plus NC Prop; and, 8) G HA, GelMA plus HA solution. All nanocapsules and HA solution were added to obtain a final concentration of 50µg/mL in the same quantity. For LDH dosage a positive control was also analyzed, which corresponds to a maximum release of the enzyme. 30,000 cells were used in all the groups.
***Results:** As preliminary results for WST-8, there is no significant difference between Control, NC PCL and NC Prop groups. NC PCL and NC Prop did not show statistical significance to each other. Control group presented statistical difference in comparison with all other groups. NC Prop and HA had no difference, although NC Prop showed improved results with cells. All hydrogels groups expressed similar behavior, with a decrease in viability over the days of analysis. They did not present statistical difference in comparison with HA group. When compared to control group (100% viability), hydrogels presented viability between 7 to 40% depending on the group and day of analysis. In LDH dosage, all the groups had significant difference when compared to the positive control. There was no statistical difference between all the other groups. In relation to confocal images, the cells showed typical morphology in the groups tested; however, when cultivated on the hydrogels, there were fewer cells and they presented a cluster behavior.
***Conclusion/Significance:** In conclusion, although several evaluations are still required, hydrogels with or without NC PCL, NC Prop and HA showed an unexpected result, with low stem cell viability when compared to control group and all the groups without hydrogels. Moreover, NC Prop was shown to be biocompatible with the cells. None of the groups showed a cytotoxicity profile, as observed in LDH dosage.

223 - Production Of A Decellularized Spinal Cord Hydrogel As A Strategy For Nervous System Injuries

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***Purpose/Objectives:** Spinal cord injury (SCI) is a debilitating neurological syndrome that compromises the lives of patients, causing loss in motor function and sensibility around the lesion site and for which there is no efficient therapy. New and innovative approaches for regenerative medicine have been proposed, including biomaterials able to carry and deliver cells and/or drugs to the damaged spinal cord. Hydrogels are typically soft and elastic due to their thermodynamic compatibility with water and they represent a possible strategy for improving the healing process and functional recovery. The aim of this study has been to produce a hydrogel using decellularized spinal cord tissue of rats as a possible biomaterial for the treatment of SCI.
***Methodology:** The spinal cord tissue of the animals was collected, cut in 1 cm length segments and submitted to the decellularization process with varying sodium dodecyl sulfate (SDS) concentrations (0.5%, 1% e 5%) and different time frames (9 hours, 12 hours and 18 hours). To assess the efficiency of the descellularization, the total DNA content was quantified in each sample. Histological sections of the samples were stained with DAPI, which binds to double strand DNA or with hematoxylin and eosin. After determining the optimal decellularization time and the SDS concentration, the tissue samples were digested by the proteolytic enzyme pepsin in 0.1 M chloridric acid to produce the hydrogel. The cytocompatibility of the hydrogel was analyzed by MTT assay using PC 12 cells cultivated on top of the hydrogel. The PC12 cell line is a neuronal cell model, derived from a rat pheochromocytoma.
***Results:** The DNA quantification of the samples showed that the most efficent SDS concentration to promote decellularization was of 1% and the best time was 9 hours, presenting 19,026.40 ng DNA/mg of tissue, while the control spinal cord tissue presented 194,734.38 ng of DNA/mg of tissue. The histological sections stained with DAPI presented evident nuclei only with the lowest SDS concentration (0.5%). The histological analysis with eosin and hematoxylin revealed only a few cells at the end of the process. After the tissue decellularization, the treatment with pepsin led to a hydrogel with high viscosity. However, the MTT test revealed that the PC12 cells cultivated on the hydrogel presented lower adherence and viability. The results indicate that the ideal SDS concentration to realize decellularization of the spinal cord tissue is 1% and the best time is 9 hours.
***Conclusion/Significance:** To conclude, it was possible to produce a hydrogel with the decellularized tissue and this hydrogel may be an easy-available biomaterial alternative as a cell carrier for SCI treatment. **Financial support:** MCTI, FINEP, CNPq and Instituto de Pesquisa com Células-tronco.

227 - Preparation And In Vitro Evaluation Of A Chitosan-based Photocrosslinkable Bioadhesive For Comminuted Bone Fractures

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***Purpose/Objectives:** The development of biocompatible bone adhesives with significant adhesion performance under wet conditions is currently considered one of the main challenges in bone tissue regeneration. Currently, commercially available materials, such as cyanoacrylates and polymethyl methacrylate (PMMA), fail to provide a cell-friendly environment on the surroundings of the fracture, which in turn limits tissue regeneration [1]. Chitosan, however, has shown a positive effect on cell adhesion and osteoblast spreading [2], thereby turning into a promising material for bone regeneration. Consequently, here we aim to develop a chitosan-based bioadhesive with adhesion properties similar to those of PMMA-based bone cements but with enhanced biocompatibility. Furthermore, we propose the

conjugation of methacrylic acid on the main backbone of chitosan to yield a photocrosslinkable bioadhesive with controlled degradation rate and enhanced mechanical stability.
***Methodology:** Rheological flow and time sweep tests were performed to assess the viscous behavior of the bioadhesive when varying shear rate and to determine changes in stiffness before and after exposure to irradiation for photocrosslinking. Tensile strength tests were performed on bovine bone probes to assess the maximum adhesive strength on a wet environment simulating in vivo temperature and ionic concentration conditions. Additionally, compression tests were conducted to validate mechanical strength in response to loads inherent to the bone fracture surface. Similarly, a rigid double cantilever beam test (RDCBT) was performed to measure fracture toughness and cohesive strength. The bioadhesive degradation rate as a function of irradiation time was studied by recording weight change over time while being submerged in culture medium at physiological conditions. Finally, hemolysis and LDH cytotoxicity assays were performed to determine the biocompatibility of the bioadhesive, when exposed to varying irradiation times.
***Results:** Results demonstrated increased mechanical stiffness of the bioadhesive as a function of irradiation time. Mechanical and physicochemical experiments suggest the ability of the bioadhesive to support the bone loads and to hold local adhesion while local regeneration processes occur. Similarly, the bioadhesive showed exceptional adhesive properties even under wet conditions, as it could maintain two bovine bone probes glued together for over 20 days. Furthermore, cell survival appeared to remain unaffected despite methacrylation of chitosan and the subsequent exposure of the material to blue light. This was also the case for red blood cell lysis, which remained below 5%.
***Conclusion/Significance:** The bioadhesive developed here exhibited high biocompatibility as well as exceptional adhesive properties even under wet conditions. Future work must evaluate the performance of the bioadhesive on an in vivo model of bone fracture and explore the possibility of extending its usage to wound closure and epidermal grafting applications.
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228 - Preparation And Evaluation Of Magnetite-doxorubicin And Magnetite-paclitaxel Nanoconjugates As Thrombogenic Agents For Anticancer Therapies

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***Purpose/Objectives:** Cancer is the second leading cause of death worldwide, killing over 8 million people every year [1]. The uncontrolled vascularization of solid tumors, known as cancerous angiogenesis, permits that these malignant tissues have almost unlimited access to oxygen and nutrients, which ultimately leads to high local interstitial pressures [2]. As a consequence, tumors can rapidly grow to difficult-to-treat sizes, which therapeutic agents can mistarget and end up severely attacking healthy tissues. Targeted thrombogenic therapies for the obstruction of cancerous vasculature have been devised as promising treatments for cancer due to their ability to reduce the growth rate of tumors and provide a more convenient environment for the efficient administration of anticancer therapies [3]. Moreover, due to the high genetic stability of tumoral endothelial cells, tumor-developed resistance to these therapies is not a concern [4]. Here, we aimed at developing thrombogenic nanoconjugates that can be intravenously administered and precisely directed inside the body by the

application of magnetic fields.
***Methodology:** To accomplish this, two widely used chemotherapy medications, namely doxorubicin (DOX) and paclitaxel (PAC), were independently immobilized on the surface of magnetic nanoparticles (Mag) to produce the nanoconjugates Mag-DOX and Mag-PAC. Successful immobilization was qualitatively confirmed via Fourier-transformed infrared (FTIR) spectroscopy and subsequently quantified via thermogravimetric analysis (TGA). In addition, particle size distribution was studied before and after immobilization via dynamic light scattering (DLS). To assess the biocompatibility of the produced nanoconjugates, we conducted hemolysis on blood from healthy human donors and LDH cytotoxicity assays on both cancer and normal cell lines. Furthermore, platelet adhesion and partial thromboplastin time (PTT) assays were carried out to evaluate the thrombogenic properties of the nanoconjugates. Finally, the nanoconjugates were labeled with rhodamine and subsequently delivered to cells and observed under a confocal microscope. Dapi and Lysotracker stains were simultaneously delivered to label cell nuclei and endosomes, respectively.
***Results:** Results demonstrated successful immobilization of both DOX and PAC on the surface of the nanoparticles, as observed on the FTIR spectra. Moreover, immobilization efficiencies of around 8 % were achieved for both nanoconjugates and final particle size was estimated at 165 and 185 nm, for Mag-DOX and Mag-PAC, respectively. Hemolysis was found to be below 5 % in both cases, which suggests their hemocompatibility according to the ISO 10993-4, and cytotoxicity was below 20 % for concentrations up to 200 ug/mL of both nanoconjugates. In addition, platelet adhesion and PTT assays demonstrated the potential of the nanoconjugates to activate the coagulation cascade.
***Conclusion/Significance:** Finally, confocal microscopy experiments showed the ability of the nanoconjugates to translocate cells and subsequently escape endosomes, which is a crucial attribute to increase the medication's therapeutic efficiency since their effect is thought to be exerted on the nucleus.
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230 - The Sustained Release Of Basic Fibroblast Growth Factor Accelerates Angiogenesis And The Engraftment Of The Inactivated Dermis By High Hydrostatic Pressure

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***Purpose/Objectives:** We developed a novel skin regeneration therapy combining nevus tissue inactivated by high hydrostatic pressure (HHP) in the reconstruction of the dermis with a cultured epidermal autograft (CEA). The issue with this treatment is the unstable survival of CEA on the inactivated dermis. In this study, we applied collagen/gelatin sponge (CGS), which can sustain the release of basic fibroblast growth factor (bFGF), to the inactivated skin in order to accelerate angiogenesis.
***Methodology:** Murine skin grafts from C57BL6J/Jcl mice (8 mm in diameter) were prepared, inactivated by HHP and cryopreserved. One month later, the grafts were transplanted subcutaneously onto the back of other mice and covered by CGS impregnated with saline or bFGF. Grafts were

harvested after one, two and eight weeks, at which point the engraftment was evaluated through the histology and angiogenesis-related gene expressions were determined by real-time polymerase chain reaction.
***Results:** Histological sections showed that the dermal cellular density and newly formed capillaries in the bFGF group were significantly higher than in the control group. The relative expression of FGF-2, PDGF-A and VEGF-A genes in the bFGF group was significantly higher than in the control group at Week 1.
***Conclusion/Significance:** This study suggested that the angiogenesis into grafts was accelerated, which might improve the engraftment of inactivated dermis in combination with the sustained release of bFGF by CGSs.

231 - Effects Of Non-nutritive Sweeteners (nns) On Human Foreskin Fibroblast Behavior On Collagen

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***Purpose/Objectives:** Non-nutritive sweeteners (NNS) have seen increasingly common usage amongst foodstuffs in the westernized diet in the past few decades, including processed foods and flavored beverages. While various studies have been performed evaluating the epidemiologic, chronic, and acute toxicity of these compounds, a consensus has not been reached as to their safety. Organizations such as the American Heart Association and the American Diabetes Association have made recommendations that research continue as to the effects of these compounds on human health (Gardner, et al., 2012). Toxicological studies have yielded mixed results, and consensus varies from sweetener to sweetener. Some sweeteners, such as aspartame, have been posited to be carcinogenic (Morando *et al.* 2018). Still other NNS, such as stevioside, have been implicated as being anti-hypertensive, anti-carcinogenic, and anti-inflammatory in a number of contexts (Mathur *et al. 2017*; Rotimi *et al.* 2018; Armorel, 2015). In order to examine the cytological effects of NNS on human tissues, cellular behavior will be observed in the presence of sucrose, sucralose, and stevioside on human foreskin fibroblast cells and fibroblast cancer cells on collagen.
***Methodology:** Human foreskin fibroblasts and fibroblast melanoma cells COLO 829 (ATCC) were treated with increasing concentrations of the aforementioned sweeteners and allowed to proliferate for 7 days. After 7 days, cell proliferation, migration, and integrin expression were measured.
***Results:** Initial results indicate that stevioside has an inhibitory effect on initial cell adhesion, which decreases adhesion by up to 75% at concentrations of 20 mg/mL when compared to cells grown in media without stevioside ($p<0.05$). Despite this, stevioside has a pro-proliferative effect on HFF cells up to concentrations of 10 mg/mL, but is deleterious to cell survival at concentrations of 20 mg/mL ($p<0.05$). In contrast, sucralose has no effect on cell adhesion but is cytotoxic at all concentrations tested (5 mg/mL, 10 mg/mL, and 20 mg/mL). A natural sugar, sucrose, has no significant effect on cell adhesion or proliferation at any of the concentrations tested.
***Conclusion/Significance:** These data indicate that stevioside's cytotoxicity is dependent on concentration, and that stevioside may have an adverse effect on cell behavior at higher concentrations. Sucralose also is cytotoxic, albeit to a much greater degree. In contrast, sucrose (table sugar) seems to have no effect on cell proliferation at concentrations up to 20 mg/mL.

232 - Fabrication Of An Antiadhesive Material Using Human Hair Keratin

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***Purpose/Objectives:** Keratin is a major structural protein found in animal hair and nail. Compared to collagen, which is applied conventionally as a biomaterial, keratin is easily available from nonvascular origins and is less expensive. In addition, the presence of cell adhesion sequences such as RGD and LDV in the keratin molecule enhances cellular affinity and biocompatibility. Therefore, keratin has recently attracted attention as a potential biomaterial. The objective of the present work was to evaluate the potential application of keratin as an antiadhesive material.
***Methodology:** Keratin was extracted from human hair using both the oxidative and reductive methods. To eliminate hydrophobic impurities from the molecule, a process of rinsing aqueous keratin solution using a specific organic solvent was used in addition to the conventional method. Water-soluble keratin extracted by the oxidative method was processed into a film sheet, while water insoluble keratin extracted using the reductive method was processed into a nanofiber sheet. Where necessary, the film and the nanofiber were laminated into one sheet. Physical properties of the keratin sheets were evaluated based on mechanical strength, surface hydrophilicity, and surface morphology. Cellular affinity of the keratin sheets was evaluated by culturing fibroblasts (L929) on them, while their antiadhesive properties were evaluated by using them to cover a wounded site in the small intestine of a rat for 10 days. Commercially available antiadhesive sheets were also used to facilitate this comparison.
***Results:** Purification of keratin was achieved by rinsing aqueous keratin solution with chloroform. The obtained pure keratin could be processed into the film and nanofiber without using a thickener. The film and nanofiber sheets obtained from purified keratin had high cellular affinity, although the rate of cell growth on the film sheet was higher than that in the nanofiber sheet. The keratin film covering the wounded small intestine of the rat was well adsorbed on the tissue and did not fall off easily. When the wounded small intestine was left without any intervention or covered with the keratin nanofiber sheet only, adhesion of tissues was observed. In contrast, adhesion was not observed at all, when a keratin nanofiber/film laminated sheet was used.
***Conclusion/Significance:** Excellent antiadhesive properties were observed when a keratin nanofiber/film laminated sheet was used. This could be due to the differences in degradability rates and surface morphology between film and nanofiber sheets. The film sheet adsorbed on the wounded site would prevent slipping of the sheet and would be dissolved more rapidly than the nanofiber sheet. After dissolution of the film sheet, the remaining nanofiber sheet would gradually be biodegraded while maintaining the isolation of the tissues. Parallel to the biodegradation of the nanofiber sheet, serosal regeneration of the wounded site could have been achieved on the uneven nanofiber surface.

233 - Enhanced Bone Regeneration With Plasma Synthesis Of Carbon-based Nanocarriers As Bioactive Cargo

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This study proposed an innovative strategy of developing the carbon nanocarriers as drug delivery systems. Differentiation of stem cells is an essential strategy for regeneration of defective tissue. Bone morphogenetic protein-2 (BMP-2) is a well-known osteogenic differentiation factor. Nevertheless, BMPs have a very short half-life and may rapidly lose their bioactivity. Previously, BMP delivery has been designed and evaluated for osteogenic differentiation, focusing on carriers and sustained release system for delivery of BMPs. Otherwise, insulin may stimulate osteoblast differentiation to produce more osteocalcin, which would then encourage more insulin production by the pancreas and higher insulin sensitivity of skeletal muscle. Here we show that carbon-activated plasma-polymerized nanoparticles (nanoP3) can be synthesized in dusty plasmas with tailored properties, in a process that is compatible with scale up to high throughput, low-cost commercial production. We propose for the first time that dusty plasmas can be tailored to produce multifunctional nanoP3 with unique functionalization capabilities. NanoP3 retain many of the favorable surface characteristics enabling a simple one-step surface immobilization of bioactive cargo with BMP-2 and Insulin. Also, ALP activity, the secretion of type I collagen, OC gene expression, and mineralized nodule formation were increased in the BMP-2 and insulin-treated group compared with control group. This study shows the unanticipated potential of carbonaceous plasma dust for bone regeneration, facilitating simultaneous imaging and cargo delivery on cost-effective, and scalable nanoparticle platform. We demonstrate the effect of the delivery mode on osteogenic differentiation potential to be used for bone regeneration applications.

234 - Effects Of Pig Types And Processing Methods On The Characteristics Of Decellularized Porcine Scaffolds

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Purpose/Objectives: With close similarity to human tissue scaffolds, porcine scaffolds have been extensively used as medical devices and in regenerative medicine research. The characteristics of decellularized porcine scaffolds such as biochemical components and mechanical properties are important for their applications. Two major populations of pigs in the pork industry are the main sources of decellularized porcine scaffolds: market pig and sow. The two types of pigs differ in many ways, and a sow is usually twice the size of a market pig. The tissues from market pig and sow are also different in terms biochemical composition and mechanical properties. The goal of this study is to characterize and compare several decellularized scaffolds prepared from market pig and sow using different decellularization processes. **Methodology:** Porcine bladder, skin and small intestinal submucosa are harvested from both market pigs and sows. They are carried through several decellularization processes. The decellularized scaffolds are tested for DNA content, glycosaminoglycan content, collagen, histological tests and mechanical tests etc. Test data was compared for different pig types and different decellularization methods. **Results:** The decellularized tissues from market pig and sow show different mechanical properties and biochemical composition. The different decellularization methods also result in decellularized scaffolds with different characteristics. **Conclusion/Significance:** This study shows how the tissue sources of different pig types and decellularization methods can affect the characteristics of the decellularized porcine scaffolds. The results provide useful information to guide researchers in selecting the right types of pig tissues and decellularization methods in their intended applications.

235 - Designing A Biological Interface Of Cochlear Implant: Combining Human Embryonic Stem-cell Replacement Therapies With Thermoresponsive Hydrogel

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Sensorineural hearing loss is the most common sensory deficit in the nation and currently Coclear implants (CI) are the only clinical intervention to restore hearing. However, patient's ears typically have widespread loss of the spiral ganglion neurons (SGNs) creating "electrode-neuron gap" that reduce spatial selectivity. Therefore, by introducing stem cell-derived otic neuronal progenitors (ONP), precursors to spiral ganglion neurons (SGN), into scala tympani, we expect to connect electrode-neuron gaps to improve CI's performance. The influence of neurotrophins for stem cells, such as brain-derived neurotrophic factor (BDNF) has been shown to play pivotal role in synaptic plasticity. But, successful delivery of BDNF in the inner ear is still questionable, as one-time injection of BDNF to the scala tympani is not effective due to its short half-life. Alternatively, encapsulating BDNF in a thermoresponsive polymer-poly (polyethylene glycol citrate-co-N-isopropylacrylamide) (PPCN), which is suitable for delivery of therapeutics, could be our solution. It possesses a lower critical solution temperature (LCST) of 26℃, liquid status efficiently entraps chemokine when temperature is below 26℃ and gelation appears to slowly release chemokine as temperature reach above 26℃. The result demonstrates a steady long-term release when BDNF entrapped in PPCN and also increases ONPs' neurite length in vitro. Furthermore, we have been transplanting PPCN that entraps ONP cells and spheroids with BDNF into our mice's inner ears, which will be further combined with silicon films and electrodes.

236 - Effects Of Long-term Brain-derived Neurotrophic Factors Delivery On Human Embryonic Stem-cell Derived Spiral Ganglion Neurons In A Microfluidic Device

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Sensorineural hearing loss is the most common sensory deficit in the nation and the death of the hair cells of the cochlea, a major source of neurotrophic factors (NTFs), causes retrograde trans-synaptic degradation and the resultant death of afferent auditory neurons. There is ample evidence that shows that application of exogenous NTFs like brain-derived neurotrophic factor (BDNF) can prevent retrograde trans-synaptic degradation. However, others have also shown that their eventual depletion triggers an accelerated decline in neuronal survival. Herein, we apply a long-term alternative-Polyhedrin Delivery System (PODS) which BDNF is linked to polyhedrin protein and released when exposes to protease. Additionally, most of these studies have been conducted in conventional laboratory plates (i.e. 6 well plates, etc.) and do not accurately represent the microenvironments in which the cells live. In this study, we set out to investigate the effects of a sustainable long-term BDNF source on stem cell-derived auditory neurons within a microfluidic culture system-Xona, which creates a chamber for us to estimate neurite extension and migration of the cells towards the NTFs source. In addition, we also develop a three-dimensional modeling via Matlab to predict the concentration gradient of BDNF with respect to time based on the number of PODS introduced into the system. The immunocytochemistry result indicates significant amount of neurite extension and cell migration towards the BDNF source using the

number of PODS from our model, which could be further utilized in inner ears modeling and reference for in vivo experiments.

239 - Small Molecule Release From PEG-based Microparticles For Sustained Inhibition Of Cathepsin Proteases

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Cathepsins proteases have been observed to be upregulated in a variety of diseases including muscle atrophy, osteoarthritis, and cancer metastasis[1,2]. Several cathepsin inhibitors have been tested in clinical trials, but all have been abandoned due to off-target effects[3]. Local, sustained delivery of cathepsin inhibitors may reduce disease progression without deleterious side effects. Thus, we developed a microfluidic device to synthesize PEG-diacrylate microparticles for sustained release of E-64, a small molecule cathepsin inhibitor. Our goal was to explore the effects of various parameters on E-64 release kinetics.
Microparticles were fabricated using a 30-gauge needle bent at 45° and inserted into 0.7mm ID low-density polyethylene tubing. The continuous (mineral oil and 20% span-80 (500µL/min)) and discontinuous (PEG-diacrylate, Dithiothreitol (DTT), L0290 (photoinitiator), and E-64 (3µL/min)) phases were injected into the microfluidic device with syringe pumps. DTT was incorporated for hydrolytic degradation. A DQ-Gelatin assay was used to assess E-64 release and inhibitory activity (N=3).
We synthesized microparticles between 40-800µm. We found an inverse relationship between microparticle size and span-80% or continuous phase speed, whereas polymer weight% and discontinuous phase speed were proportional to size. The amount of E-64 loaded affected relative levels of inhibition. Over 14 days, loading 83µg resulted in 75% inhibition, while loading 167µg resulted in 100% inhibition.
In summary, we have developed carriers showing sustained release of an active small molecule cathepsin inhibitor that could be used to locally target imbalanced cathepsin activity in a variety of diseases.
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240 - Cross-linked Cartilage Acellular Matrix Film For Prevention Of Post-surgical Peritendinous Adhesion In A Rabbit Achilles Tendon Injury Model

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Cartilage extracellular matrix contains antiadhesive and antiangiogenic molecules such as chondromodulin-1, thrombospondin-1, and endostatin. We have aimed to develop a cross-linked cartilage acellular matrix barrier for peritendinous adhesion prevention. Cartilage acellular matrix film was fabricated using decellularized porcine cartilage tissue powder and chemical crosslinking. Biochemical analysis of the film showed retention of collagen and glycosaminoglycans after the fabrication process. Physical characterization of the film showed denser collagen microstructure, increased water contact angle, and higher tensile strength after crosslinking. The degradation time in

vivo was 14 days after crosslinking. The film extract and film surface showed similar cell proliferation, while inhibiting cell migration and cell adhesion compared to standard media and culture plate, respectively. Application of the film after repair resulted in similar tendon healing and significantly less peritendinous adhesions in rabbit Achilles tendon injury model compared to repair only group, demonstrated by histology, ultrasonography, and biomechanical testing. In conclusion, the current study developed a cartilage acellular matrix film having biological properties of antiadhesion, together with biomechanical properties and degradation profile suitable for prevention of peritendinous adhesions. **References:** A. Legrand, Molecular Biology of Flexor Tendon Healing in Relation to Reduction of Tendon Adhesions. J. Hand Surg. Am. 42 (2017) 722-726 **Acknowledgments**: Korea Health Technology R&D Project (HI14C0744) / Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2018M3A9E8023852, NRF-2018R1C1B6008883)

241 - Comparison Of Character And Cellular Interactions Of Decellularized Meniscus Extracellular Matrix Powder Originating From Three Different Zones (red, Red-white, And White) According To Vascular Supply

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Meniscus is a fibrocartilage composite tissue with three different zones (red, red-white, and white) according to vascular supply. We hypothesized that decellularized meniscus extracellular matrix (DMECM) would have different characteristics and cellular interactions according to vascular supply zones. We aimed to compare DMECM powder derived from three distinct zones in terms of biochemical character and cellular interactions regarding regeneration. DMECM powder was fabricated from porcine meniscus. Characterization of each powder was done with sGAG, collagen content assay. Proteomic analysis was done with ESI-Q-TOF. White zone powders showed highest sGAG content, while red-white zone powders showed highest collagen content among groups. Proteomic analysis showed significant differences among powders regarding composition. Adhesion and migration assays with meniscal cells were significantly better in the white zone powders. While *ex-vivo* differentiation analysis showed more sGAG expression in the white zone group, with higher percentage of S-100 (+) chondrocyte-like cells, while red zone group showed higher collagen and ligament tissue features among groups. In conclusion, DMECM showed different characteristics according to vascular supply regions and such material may be utilized specific to the region of interest for tissue engineering purposes. **References:** Jetze Visser, Crosslinkable Hydrogels Derived from Cartilage, Meniscus, and Tendon Tissue. Tissue Engineering: Part A. (21) 7,1195-1206, 2015 **Acknowledgments:** This work was supported by the Ministry of Education of the Republic of Korea and the National Research Foundation of Korea (NRF-2018M3A9E8023852 / NRF-2018R1C1B6008883)

242 - Urinary Bladder Matrix Protects Host from Respiratory Infection

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***Purpose/Objectives:** Among infectious diseases, respiratory infection (RI) is arguably the most common and significant problem, representing a major cause of mortality and morbidity, especially in high-risk patients with a compromised immune system. This problem is exacerbated by the alarming emergence of increasingly antibiotic-resistant (AR) microorganisms worldwide and the lack of effective antimicrobials to overcome the AR bacterial infection. Methicillin-sensitive and resistant *Staphylococcus aureus* (MSSA/MRSA), *Pseudomonas aeruginosa* (P. aeruginosa), and *Escherichia coli* (E. coli) have become community-acquired illnesses and are frequently associated with severe RI. An alternative approach to treat infection from these gram-positive and gram-negative bacteria is urgently needed to inhibit antibiotic-resistant bacterial infection. Urinary Bladder Matrix (UBM) is a biologically derived scaffold that has shown evidence of antibacterial and antibiofilm properties (1), and the ability to promote site-appropriate tissue remodeling in a variety of body systems (2). Herein, we describe in-vitro and in-vivo effects of UBM-based treatments for MRSA/MSSA, P. aeruginosa, and E. coli infection. In addition, we describe the delivery of these materials using a clinically relevant delivery and assessment system in a simulated human lung.
***Methodology:** In vitro bactericidal kinetics and anti-biofilm activity of UBM were first examined. In vivo treatment of mouse respiratory infection was then investigated by lung inoculation with MRSA/MSSA, P. aeruginosa, and E. coli, and subsequent treatment with UBM after infection. Bacterial burden in mouse lung and spleen, differential cell counts, inflammatory response, and tissue injury after infection were evaluated. Material characterization tests included particle size analysis and simulated human lung distribution. Characterization testing was performed using a nebulizer to aerosolize UBM and distribution analysis was conducted with a Next Generation Impactor (NGI).
***Results:** Our results indicated that UBM treatments prevented bacterial biofilm formation against MRSA/MSSA, P. aeruginosa, and E. coli. Furthermore, exogenously administered UBM exhibited host protection from induced respiratory infection in a murine pneumonia model (for all bacteria types), as shown by decreased bacterial burden in lungs and spleens, fewer recruited neutrophils, decreased inflammatory cytokines and chemokines, and attenuated lung injury. Distribution analysis of nebulized UBM within a simulated human lung also demonstrated the ability to deliver UBM-based products to treat infection within the most distal portions of the airway.
***Conclusion/Significance:** Our results demonstrate protection of exogenously administered UBM against MSSA/MRSA, P. aeruginosa, and E. coli -induced respiratory infection. Importantly, we were also able to demonstrate translation of the material used in this study using a clinically relevant delivery system, with adequate delivery of the material to the distal portions of simulated human lungs. The significantly decreased bacterial colony forming units after treatment with UBM and a suitable delivery system support further studies of UBM-based products to treat bacterial respiratory infection. These results warrant continued investigation in a larger animal model to further provide evidence in the clinical translation of UBM based treatments for pulmonary infection.
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243 - Role Of Cell-biomaterial Interactions On The Immunosuppressive Potency Of Mesenchymal Stromal Cells

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***Purpose/Objectives:** Mesenchymal stromal cells (MSCs) are widely studied for their ability to suppress immune responses *in vitro* and in pre-clinical animal models. In particular, the immunosuppressive capacity of these cells have shown promising results in pre-clinical models of wound healing, where MSCs have been shown to dampen chronic inflammatory responses that contribute to tissue degeneration. In clinical models of wound healing, however, these cells have shown mixed results, likely due both to the donor-to-donor variability of MSCs and the poor survival of the implanted cells. Biomedical engineers and clinicians have recently sought to enhance the survival of these cells for these applications by seeding these cells onto biomaterial scaffolds. However, there are currently no studies that have evaluated the functional immunosuppressive capacity of MSCs on these biomaterials.
***Methodology:** We have evaluated the immunosuppressive potency of multiple human MSC cell lines on fibrin and collagen hydrogels. MSCs were seeded onto or embedded in collagen and fibrin gels at 8 mg/mL and 3 mg/mL concentrations. After pre-stimulation with 50 U/mL IFN-γ, MSCs were co-cultured with PBMCs activated with dynabeads. After three days of co-culture, the proliferation and activation of $CD4^+$ and $CD8^+$ T-cells was determined by FACS analysis of CFSE staining and CD25 expression.
***Results:** We have shown that IFN-γ stimulation consistently enhances the ability of MSCs to reduce $CD4^+$ and $CD8^+$ T-cell proliferation and activation when seeded on 2D on fibrin gels; IFN-γ stimulation, however, did not consistently enhance these properties when MSCs were seeded on 2D collagen gels. These findings were consistent on fibrin and collagen matrices of varying concentrations, which are known to exhibit varying bulk physical properties. Preliminarily results, however, show that IFN-γ stimulation does not consistently enhance these immunosuppressive properties when the MSCs are encapsulated in 3D fibrin or collagen hydrogels.
***Conclusion/Significance:** The findings of this work may help clinicians and biomedical engineers design biomaterials that will enhance the immunosuppressive capacity of various manufactured MSCs for pre-clinical and clinical studies.

244 - The Effects Of M1 Macrophage Activation On M1-to-M2 Phenotypic Switching

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***Purpose/Objectives:** Macrophages are highly plastic immune cells that precisely control the timing and dosage of angiogenic growth factors and direct the innate immune response to biomaterials. Biomaterial-mediated macrophage modulation is therefore an attractive strategy to promote angiogenesis. A growing body of evidence suggests that early M1 macrophages initiate blood vessel sprouting, and subsequent M2 activity stabilizes these structures. Furthermore, this M2 population may originate from M1 macrophages that have undergone phenotypic switching, but the M1-to-M2 transition has not been thoroughly studied. In pathologies where angiogenesis is inhibited, such as diabetic wounds, M1 macrophages may be insufficiently activated and also fail to switch to the M2 phenotype. Thus, it is necessary to understand how M1 activation affects M1-to-M2 phenotypic switching.
To test the hypothesis that M1 macrophages are primed to undergo switching to M2, we evaluated M1 sensitivity to IL-4, the primary cytokine used to induce M2 polarization, in comparison to unactivated

M0 macrophages. We first measured IL-4 receptor-alpha (IL-4Ra) expression via flow cytometry, then measured expression of M2 and IL-4 signaling pathway genes 6 hours after treatment with varying doses of IL-4. Next, we tracked the expression of these same genes over the course of 24 hours to determine how quickly M1 macrophages switch upon IL-4 treatment. Finally, to test if M1 activation enhances subsequent M2 behavior, we measured M0- and M1-derived M2 macrophage secretion of PDGF-BB and CCL17.
***Methodology:** Primary human monocytes were cultured in complete media containing 20ng/mL macrophage colony-stimulating factor (MCSF media) for 5 days to differentiate into M0 macrophages. For flow cytometry, macrophages were activated for 48 hours in MCSF media containing 100ng/mL IFNg and LPS (M1 media) or 40ng/mL IL-4 and 20ng/mL IL-13 (M2 media), then stained for IL-4Ra and run on the BD Accuri C6. For remaining studies, M0 macrophages were cultured in MCSF or M1 media for 24 hours, then cultured in MCSF media containing 10ng/mL IL-4 for 24 hours. Nanostring was used to measure gene counts, and protein secretion was measured using ELISA.
***Results:** M1 macrophages upregulated IL-4Ra and several other IL-4 signaling pathway genes, suggesting they are primed to switch to the M2 phenotype, but did not switch at lower IL-4 doses or more quickly than M0 macrophages. Interestingly, M0-derived M2 macrophages exhibited increased expression of the M2 markers CCL22 and CD206, while the M1-derived M2 macrophages upregulated CCL17 and PDGF-BB. These data indicate that M2 macrophages preferentially upregulate different markers when they are derived from M0 vs. M1 macrophages.
***Conclusion/Significance:** This study showed that M1 macrophages may be primed to switch to M2, but this priming does not necessarily translate to heightened expression of all M2-associated markers. However, the increased secretion of CCL17 and PDGF-BB suggests that M1 activation may enhance subsequent M2 functions, particularly in the context of angiogenesis. These findings indicate that promoting the M1 phenotype may recover dysfunctional angiogenesis in part by augmenting later M2 polarization. Future studies will explore additional M2 functions and investigate a wider panel of M2 and angiogenic genes.

245 - A Human-on-a-chip Inflammation Model To Understand Adipose-liver Crosstalk In Nonalcoholic Fatty Liver Disease

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***Purpose/Objectives:** Nonalcoholic fatty liver disease (NAFLD) is a metabolic syndrome-driven liver disease affecting approximately 1 billion individuals globally and is strongly correlated with obesity and type II diabetes (T2D). NAFLD is characterized by liver steatosis and insulin resistance in liver and adipose tissue. We have developed an in vitro, microfluidic, human-on-a-chip model composed of liver and adipose tissue modules capable of modeling NAFLD and the metabolic factors that contribute to disease development and progression.
***Methodology:** The model uses serum-free, blood mimetic recirculating medium tailored to represent different human metabolic conditions: normal physiologic, elevated blood glucose and insulin mimicking T2D, increased circulating free fatty acids and inflammation mimicking obesity, and the combination of T2D and obesity.
***Results:** This platform has been characterized to show physiologically relevant disease responses caused by different treatment conditions, including liver steatosis, insulin resistance, and adipokine

profile changes in adipose tissue, as well as recovery of disease markers using the T2D therapeutics metformin and pioglitazone. Compared to healthy conditions, T2D and obesity conditions directly induced NAFLD development over 14 days. Further, the system has been used to determine the indirect influence of adipose physiology on NAFLD progression and treatment.
***Conclusion/Significance:** As NAFLD and other metabolic diseases involve a complex interplay between a variety of factors, this model provides a unique platform to evaluate an individual factor's contribution to disease development, progression and mechanism. Finally, this model can be used to evaluate efficacy and toxicity of novel therapeutics aimed at treating or preventing NAFLD and NASH.

246 - Dehydrated Amniotic Membrane Allograft Favorably Modulates Immune Responses

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***Purpose/Objectives:** Amniotic-based products are an attractive option in a variety of wound healing applications owing to their multifaceted contribution of cytokines, growth factors, and extracellular matrix (ECM) that together perpetuate accelerated healing even in chronic wounds. These materials are also intrinsically immune-privileged, a characteristic that can theoretically extend to reduction of inflammation as well as modulation of immune cell responses to promote tissue remodeling and regeneration. Here we assessed multiple facets of the immunomodulatory capabilities of a novel dehydrated amniotic membrane allograft (DAMA) to elucidate the contribution of immune-DAMA interactions on the wound healing process.
***Methodology:** The ability of DAMA to inhibit production of relevant inflammatory cytokines was first evaluated using peripheral blood mononuclear cells (PBMCs) activated with lipopolysaccharide and exposed to soluble DAMA extracts. Immune modulation was further explored through the assessment of cytokine and growth factor expression profiles of macrophages, including that of CCL22, CCL18, PDGF, IL-13, TNF-alpha, IFN-gamma, IL-6, and RANTES, after introduction to either soluble DAMA extracts or physical contact with DAMA contracts. DAMA-stimulated macrophages were directly compared to non-stimulated macrophages as well as those placed in contact with non-immune-privileged collagen constructs. Finally, a variety of fibroblast phenotypes were evaluated in the presence of macrophage-conditioned media.
***Results:** Results indicate that DAMA extracts possess a significant ability to reduce production of pro-inflammatory molecules, including that of TNF-alpha, IL-1beta, IL-8, IL-10, MIP-1a, and IL-6 by over 90% in PBMC compared to non-treated controls ($n=4$ per group; $p<0.05$). Furthermore, macrophage-specific studies indicate that exposure to both the physical DAMA constructs as well as their soluble factors alone is sufficient to encourage macrophage polarization into an alternative M2 phenotype as opposed to the classic pro-inflammatory M1 phenotype, as evidenced by especially low levels of IFN-gamma and TNF-alpha concomitant with high levels of CCL22 and CCL18 in comparison to non-stimulated controls. Conditioned media from DAMA-influenced macrophages was further shown to influence fibroblast phenotypes relevant to wound healing.
***Conclusion/Significance:** Excessive inflammation is known to have deleterious impacts on the ECM and localized cell functionality. Furthermore, macrophages are imperative for proper wound healing and re-epithelialization, and much of this functionality has been specifically attributed to their preferential polarization towards an M2 phenotype. The results herein therefore indicate that the

immunomodulatory functionality of DAMA likely plays a significant role in the overarching ability of amniotic products to promote wound healing.

247 - Indirect Antigen Recognition And T Cell Activation To Biomaterial Encapsulated Cells: Adjuvant Properties Of Alginate

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***Purpose/Objectives:** Alginate encapsulation is a promising approach to protect transplanted cells from host immune destruction. Specifically, in Type 1 diabetes, alginate encapsulated islets have shown effective immunoprotection and delayed graft rejection in rodent models. Studies have found the purity and chemical compositions of alginate playing key roles in activating host macrophages, triggering fibrosis and subsequent adaptive response [1]. While the interactions of host innate immune cells with alginate has been elucidated, the impact of alginate material on T-cell mediated antigen recognition of the encapsulated cells remains unclear. As adaptive immune responses are pivotal in immune rejection, a complete understanding of the impacts of alginate encapsulation on host adaptive immunity is needed to improve clinical translation. Our lab previously developed a robust antigen-specific *in vitro* platform to study adaptive immune responses. We established that alginate encapsulation effectively blocked donor-host contact dependent CD8+ T cell activation; however, indirect T cell activation, directed by shed-antigen primed host antigen presenting cells, was well preserved, regardless of encapsulation. Therefore, this work focuses on characterizing the impact of alginate material properties on indirect antigen recognition.
***Methodology:** Pre-conditioned media from membrane-bound ovalbumin (mOVA) cell culture was used as the antigen source to stimulate the OVA-specific OTI CD8 T cells with T cell activation subsequently quantified via flow cytometry. To delineate the adjuvant property of alginate material in indirect T cell activation, cell-free alginate microbeads were included in the antigen co-incubation. Alginate beads with different chemical compositions (high G/ M) were fabricated using PRONOVA™ UP alginate (cGMP grade) crosslinked with Ba^{2+}.
***Results:** OVA-specific CD8+ T cell activation quantification revealed intriguing observations. The inclusion of cell-free alginate microbeads to the antigen co-culture significantly enhanced the generation of OVA-specific CD8+ T effector cells, showing an over 100-fold increase in frequency from 0.8 ± 1.2% to 90.8 ± 9.4% when compared to antigen-only controls. Besides, the observed adjuvant effect of alginate was further proved to be independent of either endotoxin or TLR pathways, as established by in situ endotoxin quantification and the addition of specific pathway inhibitors. Furthermore, high G alginate exerted a more prominent adjuvant effect than high M alginate (*p=0.0094*). These results indicate unique adjuvant properties of ultrapure alginate in promoting indirect antigen recognition.
***Conclusion/Significance:** Using our platform, adjuvant effect of alginate microbeads in promoting indirect T cell antigen recognition to encapsulated cells was observed. This work provides important evidence for the further understanding of the rejection mechanism to alginate encapsulated cells. This study establishes the importance of developing active immunomodulatory biomaterials and incorporating them in cell encapsulation to dampen the indirect host immune responses. **Reference:** [1] Paredes-Juarez GA, et al. Journal of controlled release 172(3), 983-992, 2013.

248 - The Effect Of Anti-inflammatory Agents (Glucosamine Sulphate And Aloe Vera) On Mouse Mesenchymal Stem Cells

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Treatment of degenerative diseases like osteoarthritis with stem cells has gained popularity recently. Glucosamine Sulphate is a nutritional supplement used for relieving inflammation during Osteoarthritis. Aloe vera juice is a widely consumed drink used in traditional practices to reduce inflammation. In this experiment, the effect of Glucosamine Sulphate and Aloe vera on 2D and 3D proliferation of MSC cells was studied. Adhesion and proliferation of msMSC cells on collagen and fibronectin 5ug/ml and 10ug/ml matrix showed that Fibronectin 10ug/ml is a suitable matrix for msMSC cells. The effect of Glucosamine Sulphate on 2D proliferation of MSC cells was studied by exposing the cells to 0mg/ml, 0.1mg/ml, 0.5mg/ml and 1mg/ml of Glucosamine Sulphate for seven days. The cells adhered least on 0.5mg/ml and proliferated most on 0.5mg/ml as well ($p<0.05$). The effect of Aloe vera on 2D proliferation of MSC cells was studied with concentrations of 0%, 2%, 5% and 10% Aloe vera juice for seven days. Cell proliferation decreased in all conditions after Day 4 and least proliferation was observed at 10%. 3D proliferation of MSC cells in fibrin construct was studied with 0.1 mg/ml Glucosamine Sulphate and 2% and 5% Aloe vera juice. 5% Aloe vera allowed highest proliferation of cells, followed by 0.1 mg/ml Glucosamine Sulphate and 2% Aloe vera. This indicated that further analysis with 2% and 5% Aloe vera along with 0.1mg/ml as threshold could identify an optimum dose range for uptake of Glucosamine Sulphate with Aloe vera juice to enhance relief from inflammation during osteoarthritis.

249 - Biological Activities Of Pva Hydrogel Containing Chitooligosaccharides Conjugated With Gallic Acid

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Purpose: *Propionibacterium acnes* plays a key role in the onset of inflammation leading to acnes and downregulation of the defense system against oxidative stress. Therefore, antibiotics are used to reduce microbial proliferation and resulting inflammation. But, antibiotic treatment has side effects including allergy and diarrhea. According to previous studies, many researchers have focused on the development of alternative antimicrobial materials. Therefore, we designed chitooligosaccharide (COS) conjugation with gallic acid (GA) replaceable with antibiotics. **Methodology:** In this study, The COS-GA were synthesized by the H2O2 mediated method with some modification. Thereafter, we verified conjugation status through UV-vis spectra and H^1-NMR. Anti-oxidant activities are measured through DPPH radical, ABTS radical and H_2O_2 scavenging assay. And anti-bacterial activities are measured through minimum inhibitory concentration and disk diffusion assay. After we made poly-vinyl alcohol (PVA)/COS-GA hydrogel using freezing-thawing method, we performed gel fraction analysis, rheological properties, cytotoxicity test, measurement of intracellular reactive oxygen species and anti-bacterial of hydrogel. **Results:** As a result, we determined characterization of COS-GA conjugation. These experimental results

indicated that the antioxidant activity of all COS-GA was greatly improved by the conjugation with GA as compared with all COSs. The results revealed that the conjugation of COS with GA improved the antibacterial activity as compared to COS alone. The PVA hydrogel with COS-GA inhibited intracellular formation of reactive oxygen species and exerted antimicrobial action better than controls did. **Conclusion:** We suggest that the COS-GA/PVA hydrogel can be developed into a biomedical dressing or a cosmetic product.

251 - Anti-bacterial Hydrogel Based Pva And Diphlorethohydroxycarmalol (DPHC) Isolated Brown Alga For Wound In Vitro And In Vivo

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***Purpose/Objectives:** One of the main purposes of wound dressing is to protect the wound from external factors that may facilitate infection. During the wound healing process, wounds can easily be infected with harmful bacteria. Bacteria can cause serious complications by entering a wound. Phenolic compounds have potential antimicrobial activity based their ability to denature proteins, which is characteristic of compounds classified as surface-active agents. So, this study investigates antibacterial effect of diphlorethohydroxycarmalol (DPHC) isolated from *Ishige okamurae,* which consists of four phloroglucinol units, and fabricates polyvinyl alcohol hydrogels containing DPHC for its anti-bacterial effect in wound-dressing applications.
***Methodology:** This study investigate whether DPHC has an antibacterial effect by assessing its minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against S. aureus and P. aeruginosa. In addition, a hydrogel with several beneficial properties is produced by blending DPHC and PVA, and its anti-bacterial effect and wound-healing ability is investigated via *in vitro* experiments (physical characterization, bacterial inhibition, and indirect and direct cytotoxicity) and *in vivo* experiments (wound closure and histological testing).
***Results:** MIC and MBC of DPHC against *S. aureus* and *P. aeruginosa* were found to be about 128 and 512 μg/mL. Also, The PVA/DPHC hydrogels exhibited the ability to reduce the viability of *S. aureus* and *P. aeruginosa* by about 99% in ASTM E2149 testing, while not producing any toxic effect on NHDF-Neo or HaCaT cells as shown in MTT assays and in vitro FDA fluorescence analysis. In addition, the PVA/DPHC hydrogels had a strong wound healing effect when compared to non-treated groups of ICR mice in vivo.
***Conclusion/Significance:** The PVA/DPHC hydrogels produced in the present study demonstrated clear antibacterial ability and wound healing ability following microstructural, rheological, thermogravimetric, swelling, drug release, gel fraction, cytotoxicity, bacteria-killing, histological and in-vivo wound closure tests. Based on the data in this study, it is suggested that PVA/DPHC hydrogels have great potential for use in wound dressings.

252 - A Decellularized Skeletal Muscle-derived ECM Scaffolding System For In Situ Muscle Regeneration

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***Purpose/Objectives:** The cell-based tissue engineering strategies have gained attention in restoring normal tissue function after skeletal muscle injuries; however, these approaches require a donor tissue biopsy and extensive cell expansion process prior to implantation. In order to avoid this limitation, we developed a novel cell-free muscle-specific scaffolding system that consisted of a skeletal muscle-derived decellularized extracellular matrix (dECM) and a myogenic factor, insulin growth factor-1 (IGF-1). We hypothesized that muscle-derived dECM biomaterials combined with IGF-1 could provide a muscle-specific microenvironment for i*n situ* muscle tissue regeneration. We developed a novel dECM-based scaffolding system containing IGF-1 and characterized its rheological properties and in vitro biological properties.
***Methodology:** We developed a novel dECM-based scaffolding system containing IGF-1 and characterized its rheological properties and *in vitro* biological properties. In addition, we investigated the feasibility of using this dECM-based scaffolding system for *in situ* muscle tissue regeneration in a rabbit TA muscle defect model.
***Results:** The cell viability in all scaffolds had over 90% at 1, 3, and 7 days in culture. The cell proliferation in the IGF-1/dECM was significantly increased when compared with other groups. More importantly, the IGF-1/dECM strongly supported the myogenic differentiation in the scaffold as confirmed by MHC immunofluorescence. We also investigated the feasibility in a rabbit tibialis anterior (TA) muscle defect model. The IGF-1/dECM had a significantly greater number of myofibers when compared to both collagen and dECM groups at 1 and 2 months after implantation.
***Conclusion/Significance:** We developed the muscle-specific dECM-based scaffolding system and investigated the synergistic effect of the dECM with IGF-1 for in situ muscle regeneration. The dECM was obtained by the decellularization of skeletal muscle tissue, followed by the solubilization. Combining with IGF-1, the dECM showed high in vitro cellular activities, including cell viability, proliferation, and differentiation. Moreover, in vivo implantation of IGF-1/dECM scaffold in the rabbit TA muscle defect model showed an acceleration in the new muscle formation. We demonstrated that this novel muscle-specific scaffolding system could enhance the body's regenerative capability for the in situ muscle tissue regeneration.
Acknowledgments: This study was supported by the Musculoskeletal Transplant Foundation (MTF).

253 - Effects Of Common Treatment Options For Tendon Injuries On Tendon Derived Stem Cells: A Novel Therapeutic Perspective

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***Purpose/Objectives:** Tendon injuries are a common cause of musculoskeletal consultations and are often treated with pharmacological or physical therapy or a combination of both. However, despite several medications and physical treatments have been proposed thus far, still the failure of both often

leads to tendinopathy, due to impaired repair and disruption of tendon extracellular matrix. The recent discovery of tendon derived stem cells (TDSCs) shed light on the intrinsic regeneration ability of tendons, but also raised the question of whether the failure of the currently used treatments might be related to deleterious effects on TDSCs. Moving from such considerations, we evaluated the effects of medications, dietary supplements or physical therapies commonly prescribed to treat tendon injuries on human TDSC survival and proliferation *in vitro*.
***Methodology:** TDSCs isolated from calcaneal and patellar tendons of patients undergoing lower limb amputation were cultured *in vitro* and characterized for morphology, clonogenicity, proliferation, expression of mesenchymal markers and multilineage differentiation potential. Commonly used drugs, like diclofenac, triamcinolone and tiocolchicoside, or dietary supplements, like hyaluronic acid, palmitoylethanolamide and curcumin, were added to TDSC cultures using three different concentrations for each. Alternatively, TDSCs were subjected to shock waves (SW) treatment (800 impulses at 0.1 mJ/mm2) every two days for three times, or to a single one-hour treatment with electromagnetic fields (EMF) (1.5 mT, 30 Hz). Then, the effects on TDSC viability were evaluated after 72 hours by MTT assay to select the best tolerated treatment. Additionally, to assess whether a synergistic effect might result from the combined treatment with the pharmacological and physical therapy, TDSCs receiving the least cytotoxic medication were also exposed to SWs or EMFs.
***Results:** TDSCs were easily isolated and propagated in culture, characterized by small size, polygonal shape, and a doubling time of 39.11 hours. TDSCs exhibited immunopositivity for CD90, CD105, vimentin and actin, and ability to differentiate towards adipocytes, chondrocytes and osteoblasts. Single cell cloning by serial dilution demonstrated TDSC clonogenicity with formation of clones within 7-10 days of culture. Regardless of the concentration used for each drug or dietary supplement, the proliferation rate and viability of TDSCs resulted significantly increased after treatment with curcumin, SWs and EMFs. Interestingly, the combination of curcumin and both physical treatments had synergistic effects on TDSC proliferation and viability. It is also noteworthy that the viability of TDSCs exposed to high concentrations of triamcinolone was significantly reduced, confirming the arising concerns about the usefulness and safety of corticosteroid injection.
***Conclusion/Significance:** Evidence emerging from our study paves the road for a novel treatment aimed at facilitating the tendon repair by resident TDSCs that leads to a better and faster recovery. On the other hand, our results also demonstrate the possible devastating effects of the widely and commonly used corticosteroid drugs on TDSC compartment that might impair tendon repair.

254 - Analysis Of Nucleus Pulposus Endogenous Progenitor Cells And Mesenchymal Stem Cells For Their Responses To Degenerative Disc Microenvironments

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***Purpose/Objectives:** Intervertebral discs (IVDs) degeneration is the major cause for discogenic low back pain. Recently, progenitor cells were reported to reside inside the nucleus pulposus (NP), the inner compartment of IVD, in several species [1]. They display the differentiation potency towards osteoblasts, chondrocytes and adipocytes. They also have surface marker expression similar to mesenchymal stem cells (MSCs), a cell tool investigated intensively for disc regeneration. Therefore,

they are often referred as MSCs-like cells. It is currently not clear whether NP progenitor cells and MSCs behave the same in the hostile IVD microenvironment. Here we aim to analyze the similarities and differences between the impact on NP progenitor cells of the tough factors, including acidic condition, hypoxia, stiff matrix, and accumulated inflammatory factors, and compared to those on MSCs.
***Methodology:** We first systematically reviewed the literature on endogenous progenitor cells identified in the NP with the key words of 'nucleus pulposus' and 'stem cells', 'progenitor cells' or 'progenitors' in Medline, from time of inception till Mar 2019, and extracted the information of their responses to the factors in disc microenvironment. We then looked into the effects on mesenchymal stem cells and NP progenitor cells and made comparisons.
***Results:** Acidity affected MSCs and NP progenitor cells in a similar way. It decreased cell proliferation, and inhibited cell viability and ECM production. Though the incubation in acidic condition on a short term stimulated stemness marker expression of MSCs, whether it can exert a similar effect on disc progenitor cells is yet to be elucidated. As to hypoxia, it had multiple impact. It enhanced the chondrogenic differentiation of both MSCs and NP progenitor cells. Nevertheless, hypoxia caused cell apoptosis and hampered cell proliferation of NP progenitor cells, but not MSCs. It also strengthened the anti-inflammatory effect of MSCs. In addition, it inhibited osteogenesis in NP progenitor cells. Like low PH, hypoxic condition also provoked stemness marker expression of MSCs. Rigid matrix showed the same effect on NP progenitor cells and MSCs, by reducing their chondrogenic tendency and increasing their osteogenic tendency. With regard to inflammatory factors, the response of MSCs and NP progenitor cells were quite different. MSCs attenuated inflammation, while NP progenitor cells were compromised, represented by inhibited cell proliferation and upregulated cell apoptosis.
***Conclusion/Significance:** This study reveals that NP progenitors and MSCs have similarities and differences when subjected to the key disc microenvironmental factors. They behave similarly in acidic PH and stiff matrix. They also share some common responses under hypoxia, however NP progenitors are more vulnerable. The major difference lies in their reactions to inflammation factors. Taken together, these highlight our current understanding about NP progenitor cells, disclose their potentials compared to MSCs, and will facilitate the future application of them for disc repair.
Acknowledgements: This work was supported by National Natural Science Foundation of China (81702191), the Fundamental Research Funds for the Central Universities, SCUT (2018MS70).
References: 1.Lyu FJ, et al. IVD progenitor cells: a new horizon for understanding disc homeostasis and repair. Nature reviews Rheumatology 2019, 15: 102-112.

255 - Evaluating Engraftment Of Heterotopic Islet Constructs As Cell Therapy For Type 1 Diabetes

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***Purpose/Objectives:** Type 1 Diabetes (T1D) results from the autoimmune destruction of β-cells within the pancreatic Islets of Langerhans. Without functional β-cells, patients with T1D suffer from hyperglycemia due to loss of insulin production, continuous blood glucose monitoring, infusions of exogenous insulin, and diminished quality of life and lifespan. Clinical islet transplantation from healthy donors is proposed to ameliorate diabetic indications and has been investigated as a promising cell transplantation strategy. However, post-transplant outcomes have been shown to be dependent on the survival of transplanted islets, which relies on the engraftment of the islets with the recipient's

vasculature amongst other factors. Treatment strategies to improve engraftment include combining islets with Mesenchymal Stem Cells (MSC), dynamic cells capable of robust immunomodulatory and vasculogenic effects. In this study, we developed an *in vitro* model of transplantation to investigate the mechanisms that enhance rapid engraftment of heterotopic islet constructs.
***Methodology:** Self-assembled vascular beds of fluorescently stained endothelial cells served as reproducible *in vitro* transplantation sites. Heterotopic islet constructs composed of islets, endothelial cells, and MSC were transferred to vascular beds for modeling transplantation. Time-lapsed imaging was performed for analysis of engraftment. Moreover, sampling of media following modeled transplantation showed secretory profiles that were correlated with imaging analyses.
***Results:** Analysis of heterotopic islets containing islets, MSC, and endothelial cells showed markedly faster and more robust engraftment based on multiple parameters after 24 hours of modeled-transplantation. Moreover, sampling of media revealed a moderated growth factor profile that suggested greater controlled release of chemotactic and angiogenic mediators. Three-dimensional imaging provided an in-depth comparison of each islet construct after 72 hours of modeled-transplantation to demonstrate the levels of engraftment on the vascular bed.
***Conclusion/Significance:** Together, this evidence supports a promising cell transplantation strategy for T1D and also demonstrates a valuable tool for rapidly investigating candidate cellular therapies for transplantation.

256 - Novel Human-human Model Of Peripheral Myelination Using Human Schwann Cells And Ipsc Derived Motoneurons

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***Purpose/Objectives:** Myelination and Node of Ranvier formation play an important role in saltatory conduction of axonal action potentials in the peripheral nervous system. Degeneration or damage of this myelin can lead to impairment in the conduction of signals by the axons, and cause deficits in sensory and motor function. Currently, animal-based models are the primary method used to study the development / regeneration of myelination in demyelinating diseases; however, data generated in animal models generally translate poorly to humans, especially as applied to drug discovery. Here we report the development of the first human myelination model using human primary Schwann cells (SCs) and human iPSC-derived motoneurons (iPSC-MNs).
***Methodology:** We cocultured iPSC-MNs with SCs in a serum-free myelination-promoting medium formulation that facilitated myelin segment and Node of Ranvier formation over a 30-day period.
***Results:** The myelination potential of the human SCs was confirmed by monitoring expression of the transcription factor Egr2. Myelin segments were visualized using confocal microscopy for myelin basic protein surrounding neurofilament-stained iPSC-MN axons. Myelination efficiency was quantified by determining the number of S100b-stained SCs associated with myelin segments. Additionally, we observed Node of Ranvier formation via voltage-gated sodium channel expression (Nav-Pan), and Nav1.7, as well as by utilizing the paranodal membrane protein Caspr.
***Conclusion/Significance:** This novel human-based myelination model will be a much more accurate tool when it comes to the study of myelination and demyelinating diseases in humans. This system could be used to determine efficacy of novel therapeutics for demyelinating diseases such as Charcot-Marie

Tooth, Guillian-Barre syndrome, anti-MAG peripheral neuropathy, as well as the testing of treatments for the regeneration of damaged peripheral myelination due to other causes.

257 - Study Of Human Subcutaneous Adipose Tissue Microenvironments And Its Relevance In Tissue Engineering Protocols

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***Purpose/Objectives:** Human subcutaneous adipose tissue has two layers, superficial (SAT) and deep (DAT), separated by the superficial fascia. The superficial fascia is formed by loosely interlaced collagen fibers, forming extensions to the skin called retinaculum cutis (RC). The present study proposes to evaluate the contribution of Adipose stromal/stem cells (ASC) to the physiology of human subcutaneous adipose tissue in SAT, DAT and RC microenvironments.
***Methodology:** Adipose tissue samples were collected from healthy patients submitted to abdominoplasty at the University Hospital Clementino Fraga Filho (Research Ethics Committee 145/09 and 076/10). Phenotypic profile (surface markers) of stromal-vascular fraction and adipose stem/stromal cells (ASCs) was evaluated by flow cytometry. Adipogenic induction of ASCs was performed using dulbecco's modified eagle medium low glucose supplemented with 10-6M dexamethasone; 0.5 mM 3-isobutyl-1-metylxanthine; 10µM insulin and 200µM indomethacin, 10% fetal bovine serum (FBS); penicillin and streptomycin (PS). Secretory profile was evaluated by CBA kit, analyzed by flow cytometry. Adipogenic genes were evaluated by quantitative real-time polymerase chain reaction (qPCR).
***Results:** Stromal vascular-fraction revealed a higher percentage of preadipocytes in SAT compared to RC ($p = 0.0212$). ASC isolated from SAT, RC and DAT presented 90% of positive cells for CD73 and CD90. Adipogenic induced ASC from SAT showed a greater accumulation of intra-cytoplasmatic lipid compared with ASC from RC ($p < 0.0001$) and from DAT ($p < 0.0001$). Induced-ASC from SAT also had a higher number of unilocular droplets compared with ASC from RC ($p = 0.0024$) and from DAT ($p < 0.0001$). Induced-ASC from SAT presented higher expression of FABP4 compared with induced-ASC from RC ($p < 0.0001$) and from DAT ($p < 0.0001$) and higher expression of CEBPA compared with induced-ASC from RC ($p < 0.0001$) and from DAT ($p < 0.0001$). Non-induced ASC from RC had higher VEGF secretion compared with non-induced ASC from SAT ($p = 0.0485$) and from DAT ($p = 0.0112$). Induced-ASC from RC secrete higher VEGF compared with induced-ASC from SAT ($p = 0.0175$) and from DAT ($p = 0.0328$). Non-induced ASC from RC had the highest level for CCL5 compared with non-induced ASC from SAT ($p = 0.0029$). Induced-ASC from RC secrete higher CCL5 compared with induced-ASC from SAT ($p = 0.0029$).
***Conclusion/Significance:** Our results indicate differences among SAT, DAT and RC microenvironments and to a possible stem cells niche (RC) not yet described in the literature.

258 - Human Mesenchymal Stem Cell Failure To Adapt To Glucose Shortage And Rapidly Use Intracellular Energy Reserves Through Glycolysis Explains Poor Cell Survival After Implantation

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***Purpose/Objectives:** Mesenchymal stem cells (MSCs) hold considerable promise in tissue engineering (TE). However, their poor survival when exogenously administered limits their therapeutic potential. Previous studies from our group demonstrated that lack of glucose (glc) (but not of oxygen) is fatal to human MSCs because it serves as a pro-survival and pro-angiogenic molecule for human MSCs (hMSCs) upon transplantation. However, which energy-providing pathways MSCs use to metabolize glc upon transplantation? Are there alternative energetic nutrients to replace glc? And most importantly, do hMSCs possess significant intracellular glc reserves for ensuring their survival upon transplantation? These remain open questions at the forefront of TE based-therapies.
***Methodology:** MSC loaded in fibrin hydrogels were implanted ectopically in nude mice for 3 days and characterization of both the microenvironment and MSCs hallmark of hypoxia were performed (oxygen tension, LDH-a expression and MTT activity). In vitro, MSCs were cultured under either (0.1, 1, 5 or 21 % O_2) and expression of HIF-1-α and LDH-A as well as MTT activity were monitored. Viability, glycolytic reserves and ATP content of MSCs 2D-cultures were assessed during long term exposure to near anoxia (0.1% of O_2), in the presence of either exogenous glutamine, serine, glucose or pyruvate. RT-qPCR targeting expression of 84 genes implicated in the human glucose metabolism was performed for MSCs cultured under either 21 or 0.1% of O_2. Viability, glycolytic reserves and ATP content were assed for MSCs loaded into fibrin hydrogels expose to near anoxia in vitro or implanted in vivo using a diffusion chamber model in nude mice.
***Results:** In this study, we established for the first time that the in vivo environment experienced by hMSCs is best reflected by near-anoxia (0.1% O2) rather than hypoxia (1%-5% O2) in vitro. Under these near-anoxia conditions, hMSCs rely almost exclusively on glc through anerobic glycolysis for ATP production and are unable to use either exogenous glutamine, serine, or pyruvate as energy substrates. Most importantly, hMSCs are unable to adapt their metabolism to the lack of exogenous glc, possess a very limited internal stock of glc and virtually no ATP reserves. This lack of downregulation of energy turnover as a function of exogenous glc level results in a rapid depletion of hMSC energy reserves that explains their poor survival rate.
***Conclusion/Significance:** These new insights prompt for the development of glc-releasing scaffolds to overcome this roadblock plaguing the field of TE based-therapies.

259 - Optimizing Freezing Profile And DMSO-free Solution To Ensure Successful Cryopreservation Of IPS Cell Aggregates

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***Purpose/Objectives:** Induced pluripotent stem cells (iPSC) are an important cell source for tissue engineering and regenerative medicine applications. However, it has been a challenge to bank iPSC with high efficiency and consistency using conventional methods. In this study, a defined protocol is

developed to cryopreserve iPSC aggregates with optimal freezing profile and DMSO-free formulation. ***Methodology:** Slow cooling was used to capitalize on sterility, scalability and automatability. Both a controlled cooling rate and a specified temperature at which ice is formed were used. Osmolytes were used here as alternative cryoprotective agents (CPA) to dimethyl sulfoxide (DMSO) used in conventional methods. A DMSO-free solution composed of sucrose, glycerol, isoleucine, albumin and poloxamer 188 (P188) was optimized using a differential evolution (DE) algorithm. Confocal Raman cryomicroscopy was used to analyze cells frozen in CPA solution. Statistical modeling was used to investigate significance of interaction between CPA molecules. iPSC was cryopreserved as multicellular aggregates, ROCK inhibitor was NOT used, and the cells were thawed and plated without washing. ***Results:** Both cooling rate (B) and ice nucleation temperature (T_{NUC}) are important in post-thaw survival of iPSC. Multicellular aggregates are found significantly more sensitive to supercooling (low T_{NUC}) than single cells. B of -1°C/min and T_{NUC} of -4°C are found as optimal conditions to freeze iPSC aggregates with minimal intracellular ice formation (IIF) observed using Raman cryomicroscopy(1). The amount of IIF is correlated to loss of the ability for cells to attach post-thaw. Sucrose and glycerol strengthen hydrogen bonding with water therefore, inhibiting ice formation. Statistical analysis of post-thaw recovery suggests that interactions of sucrose and isoleucine, sucrose and albumin, glycerol and albumin significantly contribute to the preservation of iPSC aggregates. Raman cryomicroscopy of CPA solutions and frozen cells shows that the presence of P188 further alters the structure and shape of the ice formed, protecting the cells from ice damage. DE algorithm rapidly located optimal composition of CPA in a 4-dimensional 1296-point parameter space under 8 experiments. Using optimized B, T_{NUC} and CPA solution, cryopreserved iPSC yielded live colonies that had a cell attachment rate comparable to fresh cell passage, were 97.1% Nanog-positive, 99.1% Oct4-positive, 99.5% TRA-1-60-positive at passage confluence and displayed normal karyotype. All of which were stable over 3 freeze-thaw stress cycles(2). ***Conclusion/Significance:** Defined, optimized B and T_{NUC} in combination control ice formation to ensure high cell survival. Groups of non-DMSO CPA molecules act in concert to protect iPSC during freezing. Thawing without washing simplifies the cell processing workflow. Preservation of iPSC with high efficiency and consistency, without DMSO or ROCK inhibitor, will accelerate both stem cell research and translation of iPSC-derived cell and tissue therapy into the clinic. **References:** 1. Li R, Yu G, Azarin SM, Hubel A. Freezing Responses in DMSO-Based Cryopreservation of Human iPS Cells: Aggregates Versus Single Cells. Tissue Eng. Part C. **24**(5), 289, 2018; 2. Li R, Hubel A. Composition and Method for Cryopreservation of Cells. U.S. Provisional Patent Application No. 62/840,617. 2019. **Acknowledgments:** This work was funded by the NIBIB of the NIH under award number R01EB023880.

260 - Long-term In Vivo Survival And Retainment Of 3d Bioprinted Human Lipoaspirate-derived Adipose Tissue Intended For Treatment Of Soft Tissue Defects

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***Purpose/Objectives:** Background: Autologous fat grafting is commonly used for correction of soft tissue deformities. However, the procedure is compromised by a high rate of graft resorption and nutritional supply challenges associated with increasing size. 3D bioprinting techniques could enable tailor-made architecture of grafts, promote diffusion of nutrients and vascularisation. Furthermore, the

composition of cells in the grafts, presence of adipose tissue-derived stromal/stem cells (ASCs) and bioactive factors with tropic effect can influence vascularisation and graft survival. Aims: This study investigates the printability and proteomic signature of mechanically processed lipoaspirate containing ASCs, as well as long-term *in vivo* survival and neovascularisation of the 3D bioprinted grafts.
***Methodology:** Material and Methods: Human lipoaspirate was collected from healthy donors by conventional water-jet assisted techniques with approval from the Regional Ethics Committee of Gothenburg (Dnr 624-16) and after signed informed consent. The lipoaspirate was mechanically processed with Lipogems kit according to the manufacture's protocol (Lipogems International SpA, Italy). The obtained lipoaspirate-derived adipose tissue was thereafter gently mixed with 3% alginate and nanocellulose in a ratio of 45:15:40 (tissue:alginate:nanocellulose). The bioink was 3D bioprinted as gridded or solid constructs (10x10x3 mm), crosslinked with $CaCl_2$for 5 minutes and thereafter immediately implanted subcutaneously in BALB/c nude mice or cultured *in vitro* under normal conditions (37°C, 5% CO_2) in DMEM/F12+10%FBS+1% PS. The grafts were harvested after 30 (n=18 (9 gridded and 9 solid constructs)) and 180 (n=18, (9 gridded and 9 solid constructs)) days, respectively. The *in vitro* cultured constructs were fixed in 4% PFA+25mM $CaCl_2$ on day 0, 3, 7, and 14 (n=12 per day, (6 gridded and 6 solid constructs)).The explanted grafts and *in vitro* cultured constructs were characterized by histology and immunohistochemistry. Proteomic analysis of the lipoaspirate-derived adipose tissue was performed with nanoLC-MS/MS and the cell composition was phenotypically characterized for antigens associated with adipose tissue-derived stem cells (ASC), pericytes and endothelial cells, by multicolor flow cytometry (CD45/CD34/CD90/CD105/CD56/CD146/CD31).
***Results:** The lipoaspirate-derived adipose tissue showed high viability and good printability when combined with alginate and nanocellulose. The 3D bioprinted grafts contained intact vascular structures and a high density of mature adipocytes before and after engraftment (day 30 and 180). Already after 30 days *in vivo*, novel blood vessels were present on the graft surface, showing signs of angiogenesis into the graft, as well as vascularisation in the centre of the tissue. Histological/immunohistochemical characterisation and flow cytometry analysis confirmed the presence of potential ASCs, pericytes and endothelial cells.Proteomic characterization identified 6067 proteins, including pericyte markers, adipokines, ASC secretome, proangiogenic proteins, as well as proteins involved in adipocyte differentiation and developmental morphogenic signaling pathways. Furthermore, we found several proteins not previously described in human subcutaneous fat. Ongoing immunohistochemical analysis of the *in vitro* cultured constructs and *in vivo* grafts intend to further characterize the vascularisation process over time and determine if the existing blood vessels are of human or mouse origin.
***Conclusion/Significance:** Human lipoaspirate-derived adipose tissue contains diverse trophic factors stimulating growth, showed high printability, long-term survival *in vivo* and displayed macroscopic and microscopic signs of vascularisation.

261 - In Vitro Paracrine Effects Of Stem Cells From Human Exfoliated Deciduous Teeth On Epithelial Cells

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***Purpose/Objectives:** Various paracrine factors are released by stem cells, including growth factors and chemokines, which are involved in the tissue regeneration process. Until now, the secretome of human

exfoliated deciduous teeth (SHED) has still not been evaluated in terms of analyzing their contribution regarding epithelial regeneration/repair. The aim of this study has been to investigate the in vitro paracrine effects of SHED on the culture of keratinocytes (HaCaT cells) and other cultivations of SHED. ***Methodology:** SHED were isolated, characterized and cultivated in a controlled atmosphere. The SHED conditioned medium (SCM) was collected and filtered at 80% cell confluence microvesicles were isolated by differential centrifugation, and characterized by transmission electron microscopy (TEM) and zetasizer to determinate morphology and diameter. Different proportions of SCM and microvesicles have been tested. A total of 1,600 HaCat cells and SHED were seeded and culture in a 96-well plate. Cell viability was evaluated by MTT and Live/Dead assay. To evaluate cell migration, the scratch test assay was realized after 1 day. Results were expressed as mean ± standard deviation and statistical analysis of data was performed by independent samples t-test.
***Results:** The cells showed a proliferative peak between the fourth and sixth day. After 6 days, there was no significant difference in the viability of the SHED cultivated in SCM and their control group ($p = 0.482$), with absorbance values of 0.68 ± 0.09 and 0.62 ± 0.14, respectively. The comparison between the concentrations of 50, 60, 70, 80, 90 and 100% of SCM demonstrated that the best concentration of SCM for the culture of HaCaT cells was 50%. HaCat cells cultivated with SCM 50% showed higher viability (Abs. 0.179 ± 0.05) than those grown with the control medium (Abs. 0.135 ± 0.03), with a significative difference ($p <0.001$). The microvesicles mean size was 166.8 nm, while TEM showed microvesicles with a round shape. HaCat treated with microvesicles showed better viability with a 150% concentration, similar to the cells treated with SCM 50% (Abs. 0.173 ± 0.03 and 0.179 ± 0.04 respectively with $p= 0.99$). The higher concentration of microvesicles required to reach the same effect as SCM 50% could be due to the loss of other extracellular vesicles during centrifugation, that have been directly related to cell proliferation. Migration test using SCM 50% showed a percentage of gap closure of 89%, compared to 67% of the control group. In addition, the live/dead assay showed a visible increase in the number of HaCaT cells after treatment with 50% SCM.
***Conclusion/Significance:** The results suggest that SHED released factors have the ability to increase the migration and viability of the keratinocytes involved in the epithelial regeneration process, which means a faster healing process. This offers a new perspective and free therapeutic cell strategy for the use of the SHED secretome in epithelial repair. Financial support: MCTIC, FINEP, CAPES, CNPq, UFRGS and Instituto de Pesquisa com Células-tronco (IPCT).

262 - Quiescencent Preconditioned Human Multipotent Stromal Cells Adopt A Metabolic Profile Favorable For Enhanced Survival Under Ischemia

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***Purpose/Objectives:** A major impediment to the development of therapies using mesenchymal stem cells/multipotent stromal cells (MSC) is the poor survival and engraftment of MSCs at the site of injury. The ischemic environment (i.e. lack of both oxygen and nutrients) encountered by cells upon implantation is the prime cause of this cell death. We hypothesized that lowering the energetic demand of MSCs by driving them into a quiescent state would enhance their survival under ischemic conditions

***Methodology:** Quiescence was induced in Human MSCs by a 48h culture in serum-deprived α-MEM medium (SD-hMSCs). Cells were then characterized by cell cycle analysis, ATP/ADP and protein content measurement, seahorse analyses, mTOR western blotting and autophagy asssay in comparison with unpreconditioned cells (UP-hMSCs). RT-qPCR targeting expression of 84 genes implicated in the human glucose metabolism was performed. Viability of both SD-hMSCs and UP-hMSCs cultured under *in vitro* ischemic environment (in serum- and glucose-free medium at 0.1% O_2) for 14 days were quantified by flow cytometry. UP-hMSCs maintained in same conditions, except in the presence of glucose (5g/L), were used as (ctrl+). Cell-containing constructs were prepared in diffusion chambers and implanted in nude mice over the course of 14 days for *in vivo* cell viability analyses. Post-ischemic hMSC functionalities (proliferation, osteogenic and adipogenic differentiation potentials) were then assessed after cell reperfusion in standard culture conditions (including 21% O_2 and 1g/L glucose).
***Results:** SD-hMSCs sustained their viability and their ATP levels upon exposure to ischemic conditions for up to 14 consecutive days *in vitro*, while maintaining their hMSC multipotential capabilities upon reperfusion. Most importantly, SD-hMSC showed enhanced survival *in vivo* after implantation in an ischemic environment in mice. Quiescence preconditioning modified the energy-metabolic profile of hMSCs: it suppressed energy-sensing mTOR signaling, stimulated autophagy, promoted a shift in bioenergetic metabolism from oxidative phosphorylation to glycolysis and up-regulated the expression of gluconeogenic enzymes, such as PEPCK.
***Conclusion/Significance:** Rather than using a single pathway to ensure their survival under ischemia, SD-hMSCs likely use a multifaceted strategy to overcome this metabolic insult. The present investigation focused on the role of autophagy, the potential metabolic fuels, and on their related energetic pathways. Since the presence of pyruvate in cell culture media was critical for SD-hMSC survival, we speculate that these cells may utilize some steps of gluconeogenesis to overcome metabolic stress. These findings support that quiescence preconditioning causes a protective metabolic adaptation that might be taken advantage of to improve hMSC survival in ischemic environments.

263 - Generation Of Pancreatic Exocrine Tissue From IPS Cells And Functional Transplantation Into The Gastrointestinal Tract

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Recent advances in stem cell technologies have facilitated the generation of various human somatic cells from human pluripotent stem cells. Although several studies reported differentiation of pancreatic endocrine cells, there were few reports about the generation of pancreatic exocrine cells.
We differentiated human pluripotent stem cells (iPSCs) into pancreatic exocrine cells by stage-specific treatment with growth factors and chemical compounds. However, delivering the digestive enzymes produced in the transplanted cells to the gastrointestinal tract remains a challenge. To generate an allogenic transplantation rat model, minced pancreas was transplanted into the gastric submucosal space with ablation of muscularis mucosa. In the allogenic transplantation, transplanted pancreatic cells were engrafted. Elevated amylase was detected in gastric juice, while transplanted cells disappeared through auto-digestion without muscularis mucosa elimination. Then, we transplanted the differentiated pancreatic cells from iPSCs into the gastric submucosal space of nude rats. The transplanted cells were also engrafted, and amylase was detected in the gastric juice in some cases. These findings suggest that transplantation of pancreatic exocrine cells into the gastric submucosal

space with muscularis mucosa elimination will contribute to a regenerative approach for pancreatic exocrine insufficiency.

264 - Porphyra-334, A Mycosporine-like Amino Acid (maa), Accelerates The Cell Reprogramming Of Somatic Cells Into Pluripotency

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Porphyra-334 (P334), a mycosporine-like amino acid (MAA), is a secondary metabolite found in diverse marine and terrestrial organisms and has several beneficial effects on fibroblast proliferation, wound healing, and antioxidant activity. Here, we report that P334 accelerates the cell reprogramming process of mouse tail tip fibroblasts and human dermal papilla cells into induced pluripotent stem cells (iPSCs). We found that P334 significantly improved cell reprogramming efficiency by activating H3K4me3, which controls mesenchymal to epithelial transition (MET) during the reprogramming process. Thus, we revealed that P334 directly regulates epigenetic changes, providing an efficient approach for biomaterial-based cell reprogramming.

265 - Formation And Characterization Of Renal Organoids Generated By Urine-derived Stem Cells With Renal Specific Extracellular Matrix

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***Purpose/Objectives:** With the rapid development of regenerative medicine, three-dimensional (3D) organoid models of several organs including kidney have been developed, providing valuable research platform for drugs screening and organ transplantation for chronic kidney disease (CKD). Currently, most renal organoids are originated from tissue stem cells and human pluripotent stem cells, which inevitably caused problems such as difficult specimen acquisition, intrinsic tumorigenicity properties and medical ethical issues. Thus, a simple, safe and non-invasive method is needed. In this study, we first developed a renal organoid culture system originated from human urine-stem cells (hUSCs).
***Methodology:** hUSCs were isolated and cultured in conventional medium, renal cell medium and renal specific extracellular matrix (ECM) respectively, to form organoids in an optimized way. Their morphology is detected and characterization including proliferation and viability were assessed. Furthermore, histology including H.E., whole mountain staining and immunofluorescence for markers of renal tubule epithelial cells were evaluated. Moreover, RT-PCR and western blot were performed. Finally, erythropoietin (EPO) secretion, function assay and drug toxicity test were implemented.
***Results:** hUSCs were isolated from urine samples of three healthy individuals and cultured. Phase contrast and H.E. staining for morphology showed a compact and tight organoid with no detectable necrosis in the center, and is close to that of normal glomeruli. 10000 cells/well was determined by ATP assay to be the optimized cell concentration of USCs to generate ideal organoids, which is set as standard initial cell concentration. Furthermore, live/dead cell staining showed they were well self-organized and no significant cell death was observed within a 7-day culture period. Moreover, multiple types of renal cells were verified to be alive in compact organoid by detecting the expression of specific renal glomerular markers (Podocin, Synaptopodin and Nephrin), proximal tubule marker (aquaporin-1)

and collecting duct marker (aquaporin-4). RT-PCR and western blot showed similar results. In addition, EPO was detectable in the primary renal cells but cannot be detected in the organoids originated from second or further passage renal cells, and EPO expression increased with prolonged hypoxic culture time, reached maximum level at 24h and decreased gradually afterwards. Finally, 3D organoids were proved to be a potential nephrotoxicity model.
***Conclusion/Significance:** hUSCs can generate renal organoids induced by renal specific ECM, which provides a simple, safe and non-invasive way to generate renal organoids for drug screening, in vitro 3D human kidney model production and potential regenerative therapy for CKD.

Poster Session 2
Tuesday, December 3, 2019, 4:30 PM - 6:00 PM

267 - Effect Of Skin Decm Powder On The Mechanical Properties Of Artificial Skin Produced By 3D Printing

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***Purpose/Objectives:** In this study, we developed a paste type bioink for improving printability and mechanical property of printed tissue. The paste type bioink was made by mixing porcine skin- derived decellularized extracellular matrix (dECM) powder with collagen. Collagen has been widely used to 3D bioprinting, however, the mechanical strength of the tissue fabricated using collagen based bioink is not strong enough compared with real tissue. Also, printability of collagen is not good enough to fabricate complex structure. dECM powder that are attracting attention as a biomaterial for tissue engineering and 3D bioprinting [1], was used to reinforce collagen based bioink. The developed bioink was applied to fabricate dermis layer for artificial skin model. The dermis tissue which fabricated using paste type bioink has higher strength and form sustainability. In addition, skin dECM powder was promote differentiation of keratinocyte.
***Methodology:** Normal human dermal fibroblasts cells were mixed with collagen (3%) and skin dECM powder. The amount of skin dECM powder was altered to 10, 30 and 50 mg/mL for investigating the effect of concentration of skin dECM powder. The dermis tissue was printed by extrusion-type 3D printer (3DXPrinter, T&R Biofab) using developed pasted type bioink. To make full thickness skin model, normal human epidermal keratinocytes cells were seeded on the printed dermis layer. After seeding keratinocytes, the keratinocytes basal medium was replaced to differentiation medium and cultured under air-liquid interface condition. The medium was changed every 3 days.
***Results:** Figure 1 shows the histology of printed the dermis tissue with different concentrations of skin dECM powder. As shown in first row of figure 1, the thickness of printed tissue was gradually decreased as culture duration increased. However, the printed tissue with higher dECM powder concentration shows better form sustainability than tissues fabricated using lower dECM powder concentration.
***Conclusion/Significance:** In conclusion, the paste type bioink shows the beneficial effect on printability as well as cell compatibility. Future investigation is required to apply developed bioink for fabricating more complex structure.

268 - Bio-dot Printing For Precise Positioning And In-situ Formation Of Cell Spheroids

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***Purpose/Objectives:** Cell spheroids improve cell-to-cell contact interaction, which is widely applied to fabricate *in-vitro* tissue models. Recently, not only for contact-dependent interaction within cell spheroids, but contact-independent cellular interaction has been considered to enhance function of artificial tissue. For spheroids to interact with other cells in contact-independent manner, microfabrication techniques for spheroids arrangement have been introduced. However, conventional

methods such as microwell system required multi-step process including induction of spheroids and their arrangement. Moreover, a few bioprinting-based approaches revealed limitations in the aspect of positioning of multi types of spheroid with high resolution. Here, we introduce *in-situ* formation and precise arrangement process of cell spheroids called 3D bio-dot printing.
***Methodology:** Three types of bio-inks were designed: Matrix bio-ink, sacrificial bio-ink and fibrin based bio-ink. Matrix bio-ink contains alginate. All materials in sacrificial bio-ink were soluble during culture. Fibrin-based bio-ink was used for patterning endothelial cells (ECs). Bio-dot printing process was conducted by serial printing of polycaprolactone (PCL), matrix bio-ink and cell-laden sacrificial bio-ink. PCL line was printed to support matrix bio-ink. Sequentially, matrix bio-ink was printed between PCL line. Then, cell-laden sacrificial bio-ink was printed as dot shape into matrix bio-ink through a needle nozzle. After printing, alginate in matrix bio-ink was selectively crosslinked. In culture media, only alginate remained while soluble materials were dissolved. In this step, a non-adherent pore was formed around cells, inducing cell spheroids during culture period. For the application of contact-independent interaction, line pattern of ECs was printed followed by positioning primary mouse hepatocyte (PMH) spheroids at a distance.
***Results:** Bio-dot printing process was characterized after printing fluorescence beads-laden sacrificial bio-ink into matrix bio-ink. When the gap of viscosity between two bio-inks was minimum, distribution of beads was dense and spherical. Printing HepG2 cell, spheroids formation was identified over 3 days of culture. Morphology of spheroids from this process was similar to microwell-derived spheroids. Size of cell spheroids was controlled from 100 μm to 400 μm by adjusting dispensing time and cell concentration of sacrificial bio-ink. Especially, size of spheroids showed great repeatability. Based on the motion program, position of cell spheroids was controlled. Minimum surface-to-surface distance between spheroids was around 20 μm. With hybrid printing process, 3D construct with organized spheroids was fabricated. Furthermore, loading two types of cells, individual and mixed spheroids were arranged in hydrogel. PMH spheroids with this process showed higher cytocompatibility and secretion of albumin urea then a conventional method. Lastly, ECs and PMH spheroids were arranged in contact-dependent and -independent manner by adjusting distance. PMH spheroids at a distance from EC line pattern showed the highest albumin and urea secretion during culture period of 22 days.
***Conclusion/Significance:** We developed bio-dot printing process with precise positioning and *in-situ* formation of cell spheroids. Multi-types of cell spheroids were successfully arranged in tens of micro-level. Moreover, through this process, contact-independent configuration of PMH spheroids was achieved, which improved long-term hepatic function *in-vitro*. In the future, this process will be used for studying communication of cell spheroids, applied for a novel tissue engineering.

269 - Optimizing Spheroid Production In 3d Cell Culture Systems For Tissue Engineering

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***Purpose/Objectives:** Spheroids are three-dimensional (3D) cell aggregates that are used for various in vitro assessments and as building blocks for tissue engineering. These multicellular environments are a closer model to in vivo conditions than two-dimensional (2D) cell culture monolayers in terms of gene expression levels (1-3). The 3D culture system also captures cell-cell and cell-extracellular matrix (ECM)

interactions and metabolic gradients (1-3). Two main techniques are used to produce spheroids without biomaterials, the hanging drop method (HD) or using ultra low attachment plates (LA) (1-3). However, the optimal technique, if any, to produce spheroids has not yet been established. We developed an approach to evaluate the spheroid quality and compared two techniques (HD and LA) generating spheroids.
***Methodology:** Three cell types that include H9C2, NIH 3T3, and human dermal fibroblast cells were analyzed at different concentrations, namely 22,000, 33,000 and 44,000 cells per spheroid. The spheroids were imaged with a microscope and the mean gray value (cell density within a spheroid) and area were quantified. A rubric was designed to grade the spheroids with grade A (Area>50,000 μm2, mean gray scale<50), grade B (20,000 μm2>Area>50,000 μm2, 81<mean gray scale<50), or grade C (Area<20000 μm2, mean gray scale>81). The number of spheroids created in each dish was 348-648 in HD method and 96 in LA method.
***Results:** The ratio of grade A spheroids created with the HD method were 1.5%-23.8% whereas those with the LA method were 83.3%-92.1%. Grade A or B spheroids created with the HD method were 8.8%-44.6% whereas those with LA method were 100.0%-103.3%.
***Conclusion/Significance:** Although the HD method has the potential to create many spheroids all at once, the quality of spheroids was not as consistent as the LA method. Taken together, the insights from this study can inform spheroid selection efforts for tissue engineering.

270 - Dynamic 3d Dose-response Chip For The Prognosis Of Osteosarcoma

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***Purpose/Objectives:** Osteosarcoma is a rare tumor that affects mainly pediatric and adolescent patients, and in which the prognosis will be dependent on the response of the tumor to chemotherapy. Studies with two dimensión (2D) cultures have not been able to demonstrate a response to chemotherapy with relevant clinical applicability. One of the most plausible causes of this happening, is the different behavior of the cells when they are in three-dimensional (3D) environments, inside a bone-like matrix and supported by continuous flow. Therefore, we set ourselves the objective of developing a chip for dynamic 3D culture on bone demineralized matrix for osteosarcoma cells.
***Methodology:** We have generated a CAD design that allow us to generate 3D printings of molds for the elaboration of this disruptive chip. This chip has multiple culture chambers, separated by membranes only permeable to culture media, fed by a peristaltic pump that maintains a flow in a closed circuit. The chip has a system that opens the circuit to test up to 8 concentrations of chemotherapy (a gradient is created by the microfluidic chanenels) in scaling doses Later, after a controlled time, chemotherapyc drugs are later washed simulating body clearing, permitting us to close the circuit again. This situation makes it easier to reproduce cycles of chemotherapy as it happens in clinical practice. We use SaOS cells for the test cultures in commercially available demineralized bone matrix. We studied cell survival in the new chip and compared the results with standard 2D cultures and with static 3D cameras (traditional methodology)
***Results:** The use of dynamic 3D culture chambers offers clearly differentiated results compared to traditional culture methods, in terms of viability and cell growth.
***Conclusion/Significance:** The dynamic 3D culture chip system is a step towards the clinical applicability

of tests for the prognosis of sarcomas, especially bone related (very dependent on the spatial configuration through the Wnt pathway). We have demonstrated its viability in vitro, and the next step is to demonstrate its prognostic utility in vivo, prior to be used to guide patient specific chemotherapies.

271 - A 3d Bioprinted In-vivo Relevant Hepatotoxicity Model

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***Purpose/Objectives:** 90% of pharmaceuticals fail during clinical trials. This failure is theorized to be caused by a lack of reliable drug discovery models which are unable to recapitulate in vivo environment and account for species to species variation. As a result, there has been a large push to develop more effective drug discovery models. Two dimensions hepatocyte cultures have proven to be good short-term models of Hepatocyte functionality but these models rapidly loose typical Hepatocyte functionality in long term cultures. Challenges with scaling up of 3D tissue models involve ensuring enough oxygen and nutrient exchange occurs throughout the bulk core of the tissue. To address this, we have levered a 3D printing and sacrificial molding technique to create vertical channels within a dense hepatocyte rich tissue. The presence of channels mimics a microvasculature within the tissue construct and provides increased opportunities for oxygen/nutrient exchange.
***Methodology:** To accomplish this six pillars of Pluronic F-127 were printed within either a 96-well plate or a 24 well transwell. Primary human or Rat hepatocytes (PH) and Non-parenchymal Cells (NPCs), 10-20million/ml and 1-2million/ml respectively, were resuspended in Type 1 collagen. The PH/NCP/Collagen slurry was pipetted into the well plate and the plate was placed into the incubator to gel the collagen. After collagen gelation media was added to dissolve away the Pluronic. After seeding, hepatocytes formed visibly cell-dense constructs with cells lining the edges of each fluid-filled channel. Constructs were grown for up to two weeks with a media exchange every 24 hours. This model was tested by exposing the cell construct to Acetaminophen, a known hepatotoxic agent. Culture supernatants were collected and analysed for the presence of Urea and Albumin.
***Results:** When 14-day constructs were treated with acetaminophen, a dose-dependent color change of the construct was observed. High doses of acetaminophen caused samples to become a dark brown color while low doses showed only a slight color change. In a separate experiment, metrics of liver function were evaluated at 2 and 7 days. Urea secretion levels were 2.8 times higher at day 7 than day 2 in static cultures, demonstrating that hepatocytes maintained their functionality during longer term culture in a thick 3D tissue format. LDH in the culture medium was evaluated as a measure of cell death. LDH values were over 100 times lower at day 7 than day 2. This indicates that despite some expected cell death following hepatocyte thawing and seeding, the construct quickly stabilized and maintained viability through day 7.
***Conclusion/Significance:** In this study, we fabricated a thick, 3D hepatic tissue model. Tissues maintained functionality more than 7 days (the longest we evaluated). We validated our model by measuring response to acetaminophen, a known toxin to hepatocytes. In ongoing work, we are further validating our model by testing additional pharmaceuticals. We are continuing to assess the role of channel incorporation on model function, to develop a fully functional, 3D liver model for a variety of applications.

272 - Hydrogel Bioink Rheological Parameters For Guiding Development Of Inkjet Bioinks

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***Purpose/Objectives:** Despite inkjet and microvalve bioprinting largely encompassing the first published efforts in bioprinting, extrusion bioprinting has generated the bulk of advances in the field over the past decade. However, the need for increased resolution printing has brought a renewed interest in inkjet printing for tissue engineering. We describe rheological studies and inkjet bioprinting tests that provide a set of parameters that aid in designing inkjet compatible bioinks.
***Methodology:** A variety of common biomaterials (gelatin, hyaluronic acid [HA]), commercially available hydrogels and bioinks (CellInk, BiogelX, gelatin methacrylate), and our previously published HA and collagen bioink were assess by rheological testing and inkjet bioprinting. These gels were analyzed with a strain sweep (1% - 100% shear strain) to determine stiffness and elasticity, a frequency sweep (0.1Hz - 10Hz) to determine how the gels respond to different shear rates, and a flow sweep ($1s^{-1}$ - $100s^{-1}$) to determine viscosity changes with flow rate. Resulting data were cross-referenced with practical inkjet drop on demand printing to determine a set of rheological parameters that could predict if biomaterial formulations would be compatible with inkjet bioprinting.
***Results:** A solid that is easily deformed, with low viscosity, would be able to be jetted with sufficient precision. With this in mind, thixotropic materials are promising and largely are able to be printed, as long as they are relatively "weak" materials, i.e. low storage modulus (G' < 5 kPa) and low elasticity (elastic plateaus last less than 10%), but require sufficient strain to induce convergence of storage and loss moduli. Moreover, the bioinks should have a tan(delta) < 0.5, are shear thinning, and have a low or transient viscosity. From the collected data, we used gelatin nanoparticles to modulate the mechanical properties of our laboratory's HA-collagen bioink, converting it from an extrusion-compatible bioink to an inkjet-compatible bioink.
***Conclusion/Significance:** Empirical and quantitative parameters to help guide development of biomaterials for bioprinting has largely been lacking. Here we provide an initial set of studies and subsequent set of parameters to begin to guide development of inkjet and microvalve bioinks for 3D bioprinting.

273 - Bioprinting Bioactive Glass And Adipose Stem Cells

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***Purpose/Objectives:** In tissue engineering, 3D scaffolds are focused on utilizing growth factors and a combination of primary cells to create clinically relevant 3D tissues. However, some growth factors have been shown to have negative effects in patients, especially in pediatric populations. An alternative approach is to utilize dissolvable bioactive glasses doped with therapeutical relevant ions. Borate bioactive glass has recently: (i) helped speed the healing of dermal wounds in > 90% of elderly patients in the clinic, (ii) provided no signs of inflammation or infection surrounding the scaffolds, (iii) resulted in little to no scarring, (iv) demonstrated hair regrowth, (v) had complete healing of the dermatological wounds, and (vi) been administered safely in multiple applications. The goal of this study was to create a bio-ink with borate bioactive glass and adipose stem cells (ASCs).
***Methodology:** We used ASCs from at least three different donors, all grown sub-confluent and used

between passages 2-6. Using extrusion bioprinting, scaffolds were printed at 2 million cells / mL in different bio-inks that contained bioactive glass. Scaffolds were incubated under static or dynamic conditions and fed every 3-4 days with standard culture media. Cell viability was measured by both CyQuant and live/dead.
***Results:** Scaffolds measuring 10x10x1 mm^3 were 3D bioprinted using our novel bio-ink. Results show that ASCs can be printed in conjunction with borate bioactive glass and will survive for more than 2 weeks.
***Conclusion/Significance:** Despite the fact that borate bioactive glass is considered toxic to cells, we have developed a novel method to 3D bioprint ASCs with borate bioactive glass and maintain cell survival for more than 2 weeks. This method will allow the therapeutic benefits of boron to be incorporated into living tissue engineered strategies.

274 - Thermo-, Photo- And Electro-sensitive Ecm-based Bioinks For 3D Bioprinting Applications

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***Purpose/Objectives:** Bioink development is one of the major bottlenecks of 3D bioprinting technologies. Besides featuring high biocompatibility, ideal bioinks must exhibit sufficient mechanical properties to be extruded as a filament and to maintain shape fidelity after the bioprinting process [1]. Natural materials often fulfill these first requirements but their mechanical properties are not appropriate to be directly bioprinted [1]. A subclass of the natural materials that have gained significant attention for 3D bioprinting applications are decellularized extracellular matrices (ECMs) [2, 3]. Among the strategies that have been recently devised to overcome the limitations of ECMs for 3D bioprinting, novel crosslinking schemes have the potential to provide structural stability to bioprinted constructs while not having a detrimental impact on cell survival. Here, we propose methacryloyl-modified ECM-based hydrogels conjugated with graphene oxide (GO) nanoplatelets as thermo-, photo- and electro-sensitive platforms with potential application in 3D bioprinting of cardiac and neural tissue.
***Methodology:** The degree of the biochemical functionalization of the ECM was quantified via trinitrobenzenesulfonic acid (TNBSA) assay. Rheological time sweep experiments were performed to assess differences on the moduli before and after either irradiation or exposure to physiological temperature and ascorbic acid. Moreover, flow sweep and shear rate recovery tests were carried out to study the behaviour of the hydrogel. Transmission electron microscopy (TEM) was used to investigate the nanomaterial distribution throughout the hydrogels and impedance analyses were performed to determine the extent of electrical conductivity after in situ reduction. Finally, human mesenchymal stem cells (hMSCs) isolated from adipose tissue were embedded on the hydrogels and the resulting bioprinted constructs were electrically stimulated over time. Cellular survival was studied at different time points (0, 3 and 7 days after bioprinting) using Live/Dead assay and confocal microscopy. Differentiation on the bioprinted constructs was evaluated with the aid of immunohistochemistry and the measurement of mRNA expression of relevant genes.
***Results:** Results from rheological experiments showed superior mechanical stiffness achieved after exposure of the GO-containing methacryloyl-modified hydrogels to blue light irradiation or while maintained at 37 ºC in the presence of ascorbic acid. These two last conditions facilitated the conversion of GO into reduced GO (rGO), thereby conferring electrical conductivity to the hydrogels, as was also confirmed with the impedance analyses. Moreover, the nanocomposite hydrogels featured shear-

thinning and recovery capacity, as shown by rheological flow sweep and shear rate recovery tests. TEM imaging showed homogeneous distribution of the GO nanoplatelets along the hydrogels. Cell viability assays on the bioprinted constructs demonstrated unaffected survival by the presence of GO or rGO on the hydrogels.
***Conclusion/Significance:** This work paves the way for the development of multisensitive ECM-based bioinks with enhanced mechanical properties tunable for different tissue engineering applications.
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275 - 3D Bioprinting Of The Tunica Media Of Small-caliber Blood Vessels Using Vascular Decellularized Extracellular Matrix-based Bioinks

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***Purpose/Objectives:** Tissue engineering has emerged as a potential alternative to traditional autografts and synthetic materials for the replacement of small-caliber blood vessels. Among different biomaterials, decellularized extracellular matrix (dECM) demonstrated to be a promising candidate for cell culture and tissue engineering because it retains biochemical and biomechanical clues from the native tissue. Seeding decellularized blood vessels with stem cells, however, is a complex task, being difficult for these cells to homogeneously populate the decellularized scaffold. The development of bioprinting techniques allowed the fabrication of customized tissues with the desired shape and completely filled with cells, solving some of the main issues related to decellularized scaffold seeding. Still, to maintain the biochemical and biomechanical clues, the use of bioinks containing dECM would be a key element to the successful development of tissue-engineered blood vessels. Herein, we propose the use of vascular dECM-based hydrogels as a biomaterial for the development of the tunica media of small-caliber blood vessels using the bioprinting technique.
***Methodology:** Porcine aorta tissue was decellularized through detergent (SDS, Triton-X 100 and deoxycholate) treatments. Presence of DNA qualified the level of decellularization. Mass spectrometry-based proteomics yielded the ECM proteins composition of the dECM. The dECM was digested with pepsin and resuspended in PBS (pH 7.4). Upon warming to 37ºC, the suspension turns into a gel. Hydrogel stiffness was determined for samples with a dECM concentration of 20mg/mL. MTT assay was used to determine the hydrogel cytotoxicity. Adipose tissue-derived stromal cells (ASC) were cultured on the hydrogels to analyze cellular plasticity. Differentiation of ASC to smooth muscle cells (SMC) was induced with 10 ng/mL of TGF-β1 and SM22α used as differentiation marker. A combination of 20mg/mL dECM hydrogels and 10 million cells/mL was used as bioink for 3D bioprinting of the tunica media layer of small-caliber blood vessels in an extrusion-based 3D bioprinter. Cell viability was evaluated in the bioprinted tissue after 24 hours, 3 days, and 7 days.
***Results:** Decellularization generated dECM devoid of cells, as demonstrated by the DNA content

reduction to less than 50 ng/mg. The most abundant proteins in the dECM were, respectively, collagens, elastin, versican, perlecan, fibulins, and biglycan. The stiffness of the hydrogel derived from aorta was 6.9±0.9kPa. Cytotoxicity assay showed that the dECM hydrogel was safe for cell culture. Vascular dECM hydrogel drove spontaneous (without TGF-β1) differentiation of ASC to SMC (SM22α expression fold change: 3.2±0.4, $p<0.0001$) and the degree of differentiation was not different from the TGF-β1-induced cells ($p=0.2523$). Under culture, the 3D bioprinted constructs became a compact, highly populated tissue, demonstrating cell viability over 7 days and resembling the tunica media of small-caliber blood vessels.
***Conclusion/Significance:** Vascular dECM-based hydrogels presented biochemical and biomechanical clues mimetic to the native vascular tissue, spontaneously differentiated ASC to SMC, and supported the 3D bioprinting of the tunica media layer of small-caliber blood vessels, proving to be a promising biomaterial for manufacturing vascular grafts.

277 - A Multi-stimulus Bioreactor For Studying Complex Chemo-mechanical Microenvironments In Vitro

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***Purpose/Objectives:** In recent years, many novel bioreactor systems have been developed to expose cells to physiological stimuli such as fluid wall shear stress, cyclic stretching, hydrostatic pressure, substrate stiffness, substrate topography, and extracellular matrix proteins. However, few approaches are material-independent and allow for the systematic variation of multiple combinations of stimuli in a single device. Being able to study the interactions of these stimuli will lead to more robust tissue engineered therapies. To enable this, we have developed the MechanoBioTester- a bioreactor system for independently and dynamically varying fluid flow, stretch, applied pressure, and cell culture substrate including the substrate's stiffness, topography, and extracellular matrix components in both 2D and 3D co-culture settings.
***Methodology:** A Sylgard 184 polydimethylsiloxane elastomer (PDMS) chamber was designed with two independent inset regions, termed the cell culture regions (CCR). The CCR was first treated with sulfo-SANPAH and then filled with Advanced Biomatrix EZ-Col, 5 mg/mL type I collagen gel precursor containing human aortic smooth muscle cells (SMCs). Subsequently, green fluorescent protein expressing human umbilical vein endothelial cells (ECs) were seeded on top of the collagen gel. The CCR was also able to be filled with polyacrylamide gel, Sylgard 527 PDMS gel, and poly(1,8-octanediol citrate). Extensive finite element (FEM) and computational fluid dynamics simulations were conducted to understand the fluid-structure interactions of the chamber. ELISA assays for EC-SMC interaction (e.g. NO, ET-1, PGI_2 production) and RT-qPCR for gene regulation were used to study the cellular response to co-culture conditions and to both physiological and pathological mechanical stimulation such as fluid shear stress and cyclic stretch.
***Results:** The chamber featured a rectangular flow channel (100x10x2 mm) and a pair of perpendicularly oriented protruding struts for stretching. The location of the CCR was determined from FEM simulations of the chamber, defined as the region in which a near-uniform strain field developed; specifically, a centrally located 20x10 mm rectangle in the flow channel wall. Based on fluid-structure simulations, the system conditions were optimized to minimize variation in wall shear stress over the

CCR during stretching. The chamber was stretched using a bidirectional linear actuator; the hydrostatic pressure was varied by changing the relative height between a media reservoir and the chamber; and the flow was varied using a peristaltic pump. Strain was transferred for all CCR fillers during stretching. The chamber was shown suitable for co-culture and demonstrated unique cellular responses specific to the mechanical stimulus combinations.
***Conclusion/Significance:** The MechanoBioTester is able to decouple and independently control mechanical stimuli. The CCR allows for material-independence from the chamber construction. Moreover, it supports the testing of new approaches for material design-mechanically-stimulated co-culture systems and advance tissue engineering's ability to direct cell behavior.
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278 - Bioprinting The Blood-brain Barrier Microenvironment For The Validation Of A Computational Model For Cancer Metastasis

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***Purpose/Objectives:** The ultimate killers in cancer are the metastases—particularly those to the brain and other vital organs. Critical to understanding and developing better therapies for this metastatic process are models to study cancer progression and improved diagnostic methods. We have developed 3D printing processes to create an *in vitro* blood-brain barrier (BBB) vasculature model with controlled and reproducible geometries for the purposes of evaluating cancer metastasis to the brain. These perfusable tissue constructs allow for the observation and analysis of the metastatic events of circulating cancer cell attachment and extravasation in the context of the vascular geometry and its influence on the resulting flow dynamics. Ongoing work is focused on using printed experimental BBB model to validate an experimental computational vascular flow model.
***Methodology:** Utilizing extrusion-based bioprinting of fugitive ink materials vascular geometries can be patterned within biological hydrogels, forming perfusable channels after encapsulation and subsequent fugitive ink removal. These channels are lined with human cerebral microvascular endothelial cells, forming a confluent endothelial layer to establish an *in vitro* BBB. The barrier function of mature printed vessels is then characterized in terms of their tight-junction protein expression and permeability to 70kDa dextran. Particle image velocimetry (PIV) measurements are performed on the printed vessels to characterize the flow velocity profiles within the device at vascular features including vessel branches, side walls, and angled bends. Mouse mammary carcinoma cells can then be introduced within the device and monitored for attachment and extravasation.
***Results:** Initial results demonstrate that well-defined printed channels can be fabricated that permit highly confluent monolayers of flow-aligned cerebral endothelial cells to form under controlled perfusion of growth media. Using the bioprinted BBB bioreactor, we have tested the permeability of the endothelium and verified barrier function which is in-line with established values found in literature. Additionally, we have shown that carcinoma cells can attach to the endothelial wall during static seeding and are able to cross the endothelium where they begin to invade the surrounding matrix. Initial rounds of PIV have been performed in devices with either endothelial layers or bare gel channels to inform the

computational model's consideration of fluid dynamics within the channels.
***Conclusion/Significance:** Flow-based bioreactors have been developed which permit the formation of *in vivo*-like, endothelialized vessels possessing a wide range of pre-determined geometries, while also possessing properties of BBB function. The printed BBB tissue will be the first *in vitro* device capable of evaluating the mechanical influences over cancer metastasis to the brain through fully endothelialized channels, surrounded by hydrogel, with controllable geometries. These devices will be used to directly challenge and refine the computational model of brain cancer metastasis as the device is both capable of providing measurable flow parameters for model refinement and validating the model's predictions with relevant experimental results. *This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344. IM Release # LLNL-ABS-775022*

279 - Design Of A Perfusion Bioreactor System For Co-culture Of Vascular Cells On A Biomimetic Elastin Containing Collagen Scaffold

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***Purpose/Objectives:** Cardiovascular disease (CVD) is a serious health problem accounting for 1 in every 4 deaths in the US.[1] Although vascular tissue engineering has made significant progress towards the development of a clinical solution for the treatment of CVD, a functional tissue-engineered vascular graft (TEVG) for the replacement of diseased small-diameter vessels (< 4 mm) is still elusive. Biomimetic design of TEVG is a promising approach to recreate the native vessel niche and thereby provide the essential physiochemical cues (i.e., collagen and elastin, aligned topography) known to modulate vascular cell response.[2] However, most *in vitro* studies to assess TEVG functionality are carried out in a static culture environment in the absence of flow and hence the native biomechanical cues necessary to truly mimic the vessel microenvironment are not present. It has been well documented that vascular cells strongly respond to the biomechanical stresses induced by blood flow via mechanotransduction.[3,4] In previous work, we employed an electrochemical fabrication methodology to generate a biomimetic elastin-containing bi-layered (ECB) collagen scaffold and showed that elastin incorporation promotes contractility in smooth muscle cells (SMCs) and the mechanical properties of the scaffold improve post-culture demonstrating its potential use for the replacement of diseased small-diameter vessels.[5] The goal of the current study is to design a perfusion flow bioreactor culture system for co-culture of SMCs and human umbilical vein endothelial cells (HUVECs) on ECB scaffolds and assess cell viability after 7 days of culture in a dynamic bioreactor culture environment.
***Methodology:** The perfusion bioreactor culture system consisted of a reservoir, reactor chamber and peristaltic pump connected in a flow loop. A 100-ml polypropylene tube served as the reactor chamber and housed a 1/8" U-shaped stainless-steel-tube sample holder. ECB scaffold pre-seeded with SMCs (0.2 million cells) on the outer surface was sutured onto the sample holder and culture media (DMEM + 10% FBS + 1% sodium pyruvate + 1% pen/strep) was pumped through the scaffold lumen at a flow rate of 100 ml/min using a peristaltic pump. After 5 days, the flow loop was stopped and 2 million HUVECs were injected into the scaffold lumen and allowed to attach for 8 h before resuming flow for an additional day.
***Results:** Results revealed that it was feasible to perform an aseptic co-culture of vascular cells in the custom-designed bioreactor culture system. Further, cell viability of both SMCs and HUVECs was

maintained under the perfusion flow condition. Future work will involve performing long-term co-culture of vascular cells to assess the ECB scaffold functionality in dynamic culture compared to static culture.
***Conclusion/Significance:** Overall, results from this preliminary work have demonstrated that the custom-designed perfusion bioreactor system can be reliably used to co-culture SMCs and ECs towards the development of a pre-seeded TEVG for replacement of diseased small-diameter vessels. **References:** [1] Benjamin, EJ *et al*. Circulation. 2019 [2] Ryan AJ et al. Biomaterials. 2015 [3] Van Haaften, EE *et al*. Cells. 2018 [4] Isenberg, BC *et al*. Ann Biomed Eng. 2006 [5] Nguyen, TU *et al*. Biofabrication. 2018

280 - Fabrication Of Gold Nanoparticle-coated 3d Scaffold For Bone Tissue Engineering Applications

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Cost-effective and stable osteoinductive scaffolds are being used as alternatives to current bone scaffolds in orthopedics and dentistry. In this study, we designed scaffolds coated with gold nanoparticles (GNPs) grown on a polydopamine (PDA) coating of a three-dimensional (3D) printed polycaprolactone (PCL) scaffold. GNPs have been extensively studied for their application in bone tissue engineering due their ability to promote osteogenic differentiation. PDA is natural, eco-friendly, and highly biocompatible, and it has been recently used as a simple surface modification tool in biomedical engineering. We immobilized GNPs on PDA-coated 3D-printed PCLs in order to produce a hybrid 3D bone tissue-engineered scaffold without any toxic chemicals. This study were to develop cost-effective and osteoinductive 3D scaffolds for bone tissue engineering. The PDA layer was evenly deposited on the bare PCL scaffold, and GNPs grew uniformly on the PDA-coated scaffold. we fabricated PCLDs with their PCL surfaces using PDA . We treated PCLDs with various concentrations of $HAuCl_4$ (0.1, 0.5, 1, and 2 mM/mL) in order to establish optimal GNP growth conditions. In in vitro testing, GNP-coated scaffolds elicited markedly enhanced osteogenic differentiation with increased alkaline phosphatase activity and RUNX2 expression using human adipose-derived mesenchymal stem cells. Furthermore, in vivo testing showed that the GNP-coated scaffolds had a remarkable influence on new bone formation in a rabbit model for 4 weeks. These results demonstrated that scaffolds developed here may represent an innovative paradigm in bone tissue engineering by inducing osteogenesis as a means of remodeling and healing bone defects in restorative procedures.

281 - Effect Of The Scaffold Design In Tracheal Reconstruction With 3d Printing Technique

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The purpose of this study was to evaluate the effect of scaffold shape in maintaining airway patency and regeneration of epithelium by making different shapes of scaffolds. Scaffolds in the shape of cylinder, incomplete and complete barrel were fabricated using 3D printing technique with polycaprolactone. After making segmental tracheal defect in thirty rabbits, scaffolds were implanted to the defect. The degree of airway obstruction and regeneration of epithelium was compared. On bronchoscopic

examinations, seven rabbits in the 'cylinder', three rabbits in the 'incomplete barrel', and two rabbits in the 'complete barrel' group showed severe airway obstruction. On histologic examinations, break of epithelial lining and overgrowth of granulation tissue at the interface between native trachea and the scaffold was observed at 1 week. At 2 weeks, granulation tissue was decreased and the scaffolds were incorporated well to native trachea. At 4 weeks, regeneration of epithelium was observed at the interface. The degree of epithelial regeneration was not significantly different according to shapes of the scaffold. Barrel-shaped scaffolds have higher possibility of avoiding airway obstruction and also can improve the survival rate of the implanted animals. Further studies are needed to evaluate the effect of scaffold shape on epithelial regeneration.

282 - Viscoelastic Properties And Structure Of Porcine Myocardium Extracellular Matrix

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***Purpose/Objectives:** Currently, the gold standard treatment for heart failure patients is heart transplantation. However, due to the global shortage of heart transplants there is an urgent need to understand the fundamentals of cardiac tissue for cardiac scaffold development. In failing myocardium, the cardiac tissue undergoes continuous remodeling and can result in changes in composition, structure, and function. The myocardial extracellular matrix (ECM) plays an important role in this process. However, little is known about mechanical properties of cardiac ECM in shear. Therefore, rheological techniques were developed to measure the shear storage modulus (G'-elastic behavior), shear loss modulus (G"-viscous behavior), and Young's modulus (E-stiffness) of the ECM. Additionally, cardiac ECM was analyzed through scanning electron microscopy (SEM) and histology to bring insight into fiber alignment and ECM structure in cellularized and decellularized samples. Analyzing ECM mechanical properties provides valuable information to development of tissue engineered scaffolds.
***Methodology:** The methodology was established on fresh porcine whole hearts (n=4). Tissue sections of 1-2 mm thickness were harvested from two locations of left ventricle (wall and apex) and sectioned with three different orientations (radial (R), vertical (V), and horizontal (H)), with 6 samples in each orientation. The tissue was decellularized through a modified 3-step approach: 10mM Tris 1mM EDTA, 0.5% SDS, and PBS. The viscoelastic properties of ECM (G' and G") were measured using an oscillating disc rheometer that employed a frequency sweep and constant shear strain. A user-controlled compressive load was applied normal to the disc-shaped tissue at a constant temperature (37°C). The Young's modulus was calculated as the slope of the linear stress-strain curve from the compression testing. Student t-tests and multi-way ANOVA were used for statistical analysis.
***Results:** Results indicated that porcine myocardium G', G", and bulk modulus increased with increasing bulk compressive strain. At 0.25% rad/s, G' values at apex were H:1890±725 Pa, R:1760±497 Pa, V:2245±315 Pa and at wall were H:1969±418 Pa, R:1652±1300 Pa, V:1960±490 Pa. Correspondingly, G'' at apex were H:429±150 Pa, R:404±118 Pa, V:488±81 Pa and at wall H:447±119 Pa, R:360±265 Pa, V:421±108 Pa. There was statistical difference ($p<0.05$) of G' and G'' between the apex and wall in the vertical orientation, also for G'' in the radial direction. This suggests that mechanical properties in cardiac ECM is dependent upon orientation of the sample. SEM illustrated that in the vertical orientation, the main cardiomyocyte directionality was in the vertical plane. In contrast, fiber directionality appeared as cross sections in radial and horizontal orientations. Histological images confirmed an intact decellularized ECM. Preliminary data on the range of Young's modulus (n=3 hearts)

for apex in the vertical direction was 1921to 11864 Pa ±2838 and for wall was 1247to13981 Pa ±3888.
***Conclusion/Significance:** In conclusion, a robust protocol was developed for cardiac rheology, measuring viscoelastic shear properties of ECM *ex vivo*. Results illustrated that viscoelastic properties can depend on sample location and orientations, due to manifestation of myocardium anisotropy. This can allow for the development of complex, biomimetic cardiac tissue engineered scaffolds for specific heart locations and with particular mechanical properties.

283 - Electrospun Pga-Gelatin Scaffold In A Bioreactor System To Create A Human Tissue Engineered Vessel

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***Purpose/Objectives:** Tissue engineering for arterial conduits is a promising technology to address the need for small diameter artery applications for patients with advanced cardiovascular disease that do not have the optimal graft, autologous vein. The clinically available alternative synthetic or cryopreserved cadaveric veins are associated with early graft failure. Although there are several methods to produce a tissue engineered vessels, electrospinning is an attractive technique to fabricate the scaffold because it forms a nanofiber scaffold that can be fine-tuned with a wide range of synthetic and natural proteins to tailor the scaffold for requirements of the tissue. The objective of this study is to use an electrospun PGA-gelatin scaffold in a bioreactor system with human fibroblasts to create a human tissue engineered vessel (TEV).
***Methodology:** The electrospun scaffold was fabricated using a custom electrospinning set-up designed to electrospray sacrificial polyethylene oxide (PEO) while electrospinning PGA-gelatin. The PEO solution consisted of 120% weight/volume and the PGA-gelatin solution was 14%/1.4% weight/volume that were each injected through a 15-gauge needle at + 15 kV onto a rotating grounded mandrel. The sacrificial PEO microparticles were removed by placing the scaffold in deionized water. The TEV was formed by placing human fibroblasts on the electrospun scaffold in a bioreactor system with mechanical stretch over a 10-week culture period in supplemented media. The mechanical stretch was induced by a perfusing saline through a silicone tube on the inside of the electrospun scaffold. The human tissue engineered vessel was decellularized using a hypertonic solution and detergents.
***Results:** The electrospun PGA-gelatin scaffold had a PGA fiber diameter of 1.29 ± .27 µm with smaller gelatin fibers, and the PEO microparticle size prior to removal was 17.8 ± 2.76 µm. The human tissue engineered vessel formed a tubular structure with a 4.5 mm inner diameter and an average wall thickness of 210 µm. The mechanical strength of the TEV had a burst pressure of 1,325 ± 246 before decellularization, and a slight decrease of 1,082 ± 220 after decellularization. The mechanical properties were less than a native artery, but sufficient for implantation. The collagen matrix remained intact after decellularization by Masson's Trichrome and quantitatively by a hydroxyproline assay. The effectiveness of the decellularization was evident by a near complete removal of DNA and a total loss of intracellular proteins (MHC1 and GAPDH). The biocompatibility of the human decellularized TEV was shown by re-seeding endothelial cells on the surface and infiltration of human fibroblasts into the matrix.
***Conclusion/Significance:** The tissue engineered vessel in this study demonstrated a structure, mechanics, and preserved collagen matrix after decellularization for use as a human allogeneic clinically relevant small diameter vascular graft. The significance of this study is a proof-of-concept for electrospinning in combination with a bioreactor to generate a tissue engineered vessel, thereby leading

to a diverse platform approach to engineered vessels with specific characteristics for each vascular graft application.

284 - Preparation Of Multifunctional Cardiac Patch Using Poly(1,8-octamethylene Citrate) Elastomer

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***Purpose/Objectives:** Myocardial infarction (MI), also known as heart attack, afflicts 790,000 Americans and leads to 114,000 deaths every year.[1] In spite of current optimal clinical therapies, post-infarction heart failure and malignant arrhythmias remain challenges because of the limited regenerative potential of the cardiomyocytes. Novel engineered systems such as cardiac patches have the potential to overcome these problems. The cardiac patch as a scaffold to support and deliver stem cells and cardiomyocytes can improve cell survival and integration with native tissue.[2] In this study, we developed a novel cardiac patch with tunable mechanical and electrical properties, as well as specific topographies for regeneration of functional cardiac tissue using poly(1,8 octamethylene citrate) (POC).
***Methodology:** In general, the conductive POC patches were prepared in two steps. The first step was to fabricate the microstructured POC by curing POC pre-polymer on a micropatterned PDMS mold at 80 °C for 5 days. Followed by that, the POC film was put into PBS buffer to remove the uncured pre-polymer. Then, a conductive polyaniline (PA) layer was coated onto the POC film via in situ polymerization. Tensile strength of the patches was measured using an Instron tensile tester (5944). The surface structures were characterized using a FEI Quanta 650 scanning electron microscope (SEM). Conductivity of the patches were tested using four probe method via Agilent 4155C. Electromyography (EMG) was recorded with 10 V stimulation. L929 fibroblasts and human mesenchymal stem cells (MSCs) were cultured on the patches for toxicity test and cell morphology staining.
***Results:** In order to mimic the mechanical properties of native myocardium (0.02-0.5 MPa), a modified POC film with less crosslinking degree was fabricated to reduce the modulus of conventional POC film. It showed lower tensile modulus down to 0.2 MPa, which is comparable to that of native tissue. Conductive PA layer was successfully coated onto the POC film according to the X-ray photoelectron spectroscopy (XPS) and Fourier-transform infrared spectroscopy (FTIR) results. The conductivity (around 10^{-3} $S \cdot cm^{-1}$) and electrical signal propagation were enhanced due to the PA layer. Microgrooves with various spacing and depth were achieved on POC surface. These microstructures affected cell alignment, which is critical for cardiac tissue regeneration.
***Conclusion/Significance:** In conclusion, a novel multifunctional cardiac patch was fabricated based on POC film. Mechanical, electrical and microstructured features of the POC patches were controlled using various methods. Integration of these features is supposed to effectively regenerate the cardiac tissue. The current study will contribute to investigate the regeneration efficacy of these cardiac patches combined with stem cell therapy.
Acknowledgements: This work was supported by the Center for Advanced Regenerative Engineering (CARE), Northwestern University and American Heart Association (AHA) (Award No: 19POST34400088), and in part by the National Science Foundation (NSF) Emerging Frontiers in Research and Innovation (EFRI) (Award No: 1830968).
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285 - Evaluation Of Functionalized Electrospun Vascular Scaffolds For In Situ Endothelialization And Smooth Muscle Cell Recruitment

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***Purpose/Objectives:** Vascular tissue engineering offers a lucrative alternative to existing prosthetic modalities for small-diameter vessels. Classically, tissue-engineered vascular grafts (TEVGs) have been fabricated in an *in vitro* setting for later *in vivo* transplantation. In order to expedite the repair of damaged or occluded small-diameter vessels, recent advancements in TEVGs with the capability to attract cells *in situ* are a compelling approach. In order to achieve cellularization in situ, surface modification of a vascular scaffold can be utilized. The objectives of this study were to evaluate the use of antibodies to capture endothelial cells (ECs), as well as assessing the use of growth factors to induce smooth muscle cell (SMC) migration.
***Methodology:** Vascular scaffolds were fabricated by electrospinning a 1:1 solution of poly(ε-caprolactone) (PCL) and type I collagen. A series of in vitro experiments were performed to biofunctionalize the scaffolds to encourage the recruitment of vascular cells to the scaffold. Antibodies against von Willebrand factor (vWF), CD31, vascular endothelial cadherin (VE-cadherin), and vascular endothelial growth factor receptor-2 (VEGFR-2) were compared and paired to examine the efficacy of endothelial cell capture onto the vascular lumen. Furthermore, platelet-derived growth factor (PDGF) and stromal cell-derived factor 1-α (SDF-1α) were compared and paired to evaluate the ability to recruit vascular smooth muscle cell migration into the outer layer of the scaffold.
***Results:** Initial *in vitro* studies examining EC capture, all antibody-conjugated scaffolds captured more cells than the control condition. Notably, scaffolds conjugated with VEGFR-2 captured more cells at a statistically significant rate when compared with unmodified controls. Furthermore, the pairing of antibodies in the surface modification of scaffolds captured more cells than VEGFR-2 alone, confirming the hypothesis that the use of more than one biological factor can promote greater cell recruitment. Currently, studies are underway to examine SMC recruitment and the behavior of antibody-conjugated scaffolds in preclinical models.
***Conclusion/Significance:** Current results demonstrate synergy in the use of more than one surface-immobilized biological factor on the scaffold surface. This suggests the potential of bioconjugated TEVGs to successfully recruit functional vascular cell types *in situ*, and preclinical trials are underway to confirm this supposition. The ability of a TEVG to recruit cells allows for the capacity to create off-the-shelf vascular conduits. These advances can hasten the process of providing vessels for bypass and reconstruction in cases where prosthetic alternatives fail to provide long-term solutions.
Acknowledgments: This work was supported by the Army, Navy, NIH, Air Force, VA, and Health Affairs to support the AFIRM II effort under Award No. W81XWH-14-2-0003. The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.

286 - Modulation Of BMP Expression In Pluripotent Stem Cell-Derived Cardiomyocytes By Direct And Indirect Co-Cultures With Macrophages

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***Purpose/Objectives:** The cardiac repair patch or hydrogel comprised of cellular or acellular scaffolding materials is a potential tissue-engineered approach to treat myocardial infarction (MI). They are currently designed to be implanted or injected at the site of myocardial ischemia in order to assist in regeneration. A cellular cardiac patch would include repair cells such as cardiomyocytes (CMs), as MI results in significant losses of CM function and number. After MI, there is a highly orchestrated macrophage-mediated inflammatory response, but the mechanisms by which macrophages would interact with the cells of a repair patch or CMs are not well understood. Bone morphogenetic proteins (BMPs) have important roles in cardiogenesis and cardiac differentiation and it has been shown that macrophages can express and be recruited by BMPs released from CMs [1]. By using indirect and direct co-cultures to model the inevitable interaction with the inflammatory environment of MI, the goal of our study was to determine how non-autologous macrophages (in direct or indirect contact) affects expression of *BMP2* and *BMP4* in hPSC-CMs.
***Methodology:** Human macrophages were obtained as previously described [2] by isolating monocytes from peripheral blood *via* density centrifugation and immunomagnetic positive selection. Selection was followed by differentiation towards polarized macrophages. Pluripotent stem cells were differentiated into hPSC-CMs as previously described [3]. hPSC-CMs were seeded on Matrigel and macrophages were seeded separately via transwell inserts, allowing for indirect contact and paracrine signaling. Direct contact studies were performed by encapsulating the cells in a porcine-derived heart ECM hydrogel or commercial collagen. Cells were co-cultured for 48 hours and the gene expression analyzed via Nanostring or quantitative real time polymerase chain reaction (RT-qPCR).
***Results:** Indirect and direct co-culture of non-autologous hPSC-CMs to polarized macrophages resulted in significant downregulation of *BMP2* and *BMP4* in hPSC-CMs. These results were consistent across both hydrogel types.
***Conclusion/Significance:** These findings are an important step in our understanding of how the inflammatory environment within infarcted myocardium will influence cellular cardiac repair patches and hPSC-CMs.
Acknowledgements: American Heart Association Predoctoral Fellowship (19PRE3430006), North Carolina Translational and Clinical Sciences (NC TraCS) Institute and UNC Pluripotent Stem Cell Core Facility.
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287 - Minimizing And Characterizing Peritoneal Adhesions With A Novel Pouch And An In-vivo Bioreactor

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***Purpose/Objectives:** Tissue engineered vascular grafts require strategies to allow graft remodeling but avoid stenosis. Our approach uses pre-implantation in the peritoneal cavity, with electrospun conduits enclosed within a porous pouch, as an "*in vivo* bioreactor" to recruit autologous cells and improve graft outcomes. We previously demonstrated that pre-implanted electrospun conduits remained patent 6 weeks after autologous grafting into the aorta, and peritoneal pre-implantation reduced intimal layer thickness and macrophage marker expression[1]. We plan to overcome the potential side effect of peritoneal adhesions by designing a new pouch composed of poly(ethylene glycol)diacrylate (PEGDA) to replace the previous PTFE pouch and determine different responses when using both pouches types. While PEGDA is used to provide resistance to protein adhesion, our strategy is unique in simultaneously creating pores to enable tissue generation in the enclosed conduits. Finally, we will determine enclosed conduit viability, including after long-grafting times.
***Methodology:** Conduits were electrospun from a blend of 90/10% poly(ε-caprolactone)(PCL)/collagen. For porous pouches, hydrogels with different PEGDA concentrations were tested in compression and bending and finite element (FE) models of pouches were created to determine vonMises stresses and optimize pouch design. PEGDA pouches with the enclosed constructs were implanted in rat peritoneal cavities for 4 weeks. The formation of peritoneal adhesions was assessed (Mazuji grading scale[2]). The enclosed conduits were sectioned and stained (H&E, Alcian Blue) and proteins were assessed (BCA assay, tandem MS/MS) to compare with PTFE pouches (control). Finally, enclosed conduits were grafted into abdominal aortae. Ultrasound analysis was performed to verify patency.
***Results:** Pouches were generated from 25% PEGDA because of the highest work to fracture (n=3, one way ANOVA with Tukey). Smaller pouch with 4 pores/side were prepared because FE modeling demonstrated that this maximized the pouch integrity after implantation (i.e., minimized VonMises stress). Peritoneal implantation with PEGDA pouches significantly reduced adhesions compared to PTFE pouches and the few adhesions were easily removed. There was an average of 29% more protein found in the conduits within the PTFE vs. PEGDA pouches (*n*=7). Most of the abundant proteins found on the conduits enclosed in both types of pouches are cellular and metabolic processes. However, blood coagulation factors are more abundant in PEGDA while cytoskeletal proteins are abundant in PTFE conditions. Despite these differences, the grafts from PEGDA pouches were patent after 6 weeks. We have also demonstrated 100% patency for grafts from PEGDA pouches at 10 months (*n*=6), unlike a control condition without pre-implantation with 80% patency. We are currently analyzing the grafts to better understand the benefits of the pre-implantation step at these long grafting times.
***Conclusion/Significance:** We demonstrated that these optimized pouches were able to withstand the mechanical forces in the peritoneal cavity, prevent the potential side effect of adhesions, and still allow the generation of grafts with long-term viability. Overall, this study suggests that PEGDA hydrogels can be used as a good replacement for PTFE pouches for the *in vivo* bioreactor strategy. **References:** Shojaee et al. *Acta Biomaterialia*, 2017 **2.** Mazuji et al. *Arch. Surg.* 1964. **Acknowledgments:** AHA (18AIREA33960390).

288 - A Cost-effective In Vitro Testing Platform Using Light-assisted Functional Characterization Of Engineered Heart Tissues

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***Purpose/Objectives:** After myocardial infarction, the inflammatory response and the type of cells recruited to the site of injury play a key role in myocardial remodelling[1]. The ability to model interactions between healthy and inflammatory environments within engineered heart tissues (EHTs) in a high throughput manner is highly desirable for the study of myocardial function in the context of physiological studies, disease modeling, drug screening, and safety pharmacology. By reprogramming donor-specific adult cells into human induced pluripotent stem cells (hIPSCs), it is possible to differentiate hIPSCs into functional cardiomyocytes and other cardiac supporting cells. Current industry standard technologies capable of doing this utilize $7.5x10^4$ to $2.4x10^7$ hIPSC-derived cells per experimental condition resulting in a very costly process[2,3]. In addition, current analytical methods require single movie acquisitions of individual microtissue units to determine tissue function.
***Methodology:** We developed a novel 96-well plate-based platform for the characterization of hIPSC-derived EHTs in association with different cell types and hydrogel matrices. The platform consists of a 96-well plate patterned with polydimethylsiloxane microwells (20µL) where microtissues ($3.0x10^4$ to $2.0x10^5$ cells) are seeded in a hydrogel. Contraction of microtissues is imaged simultaneously in multiple wells using a cellphone camera by tracking the motion of high contrast posts. A light tracking algorithm correlates the motion of the posts to contractile force and contraction frequency using automated batch image processing.
***Results:** Preliminary contractility data using activated human dermal fibroblasts in the presence of 20 ng/mL TGF-β1 in 6 mg/mL collagen has shown that $3.0x10^4$ cells can produce 30% uniaxial contraction.
***Conclusion/Significance:** These results confirm that our method is faster and more cost-effective as it allows for the simultaneous capture of data from multiple wells and utilizes 800 times fewer cells per experimental condition compared to the current industry standard methods.
Acknowledgments: UNC/NCSU Joint Department of Biomedical Engineering, The North Carolina Translational and Clinical Sciences (NC TraCS) Institute and The Comparative Medicine Institute (CMI) at NCSU. **References: 1.** Frangogiannis NG. The inflammatory response in myocardial injury, repair, and remodeling. Nature Reviews Cardiology 2014; 11(5): 255-65. **2.** Zhao Y, Rafatian N, Feric NT, Cox BJ, Aschar-Sobbi R, Wang EY, et al. A Platform for Generation of Chamber-Specific Cardiac Tissues and Disease Modeling. Cell. Elsevier; 2019;176(4):913-27. **3.** Ronaldson-Bouchard, K. et al., 2018. Advanced maturation of human cardiac tissue grown from pluripotent stem cells. Nature, 556(7700), p.239.

289 - Assessment Of Cell Survival And Stability Of A Multi-layered Additive Manufactured Aortic Construct

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***Purpose/Objectives:** The current procedures that are used in order to replace aortic sections in pediatric patients involve synthetic materials that give additional support to the aortic wall. None of the current surgical options incorporate any biological component with the capability of growing along with the patient, leading to possible complications that could be avoided if a bioengineered aortic duplicate

was used. Preliminary *in vivo* results have shown an increase in vascularization of the tissue when a printed implant containing human microvascular cells is implanted subcutaneously. Building on these studies, we are researching the construction of a bioengineered aortic construct mimicking the physiology of the human aorta using a layered approach with decellularized extracellular matrix-encapsulated human microvascular endothelial cells and human smooth muscle cells.
***Methodology:** The decellularized extracellular matrix was acquired from whole porcine hearts and powdered using liquid nitrogen as well as a mortar and pestle. Using a cell ink BIO X printer and the temperature controlled printhead a 1cmX1cmX2mm cylinder was printed into a reservoir of 4% alginate and vascular smooth muscle cell media and allowed to grow for 6 weeks. The bioengineered construct was monitored for cell adhesion, survival and proliferation through fluorescent microscopy and histological analysis every week for the duration of the experiment. Experimental tissue growth results were compared to theoretical models in order to determine growth rates at larger scales for the anatomically correct construct.
***Results:** The engineered construct will additionally be subjected to mechanical testing throughout the maturity time in order to determine structural and mechanical patterns for a precise computational analysis of fluid interaction and failure parameters. The additional layers of the aortic construct that were printed on to the vascular layer allowed the smooth muscle layer to be fed by the vascular network, a problem that we had been unable to correct previously. Positive results have been seen thus far with trichrome, histological and fluorescent analysis to be done weekly as tissues mature *in vitro*.
***Conclusion/Significance:** Vascularization due to aligned human microvascular endothelial cells allow for the diffusion of oxygen and nutrients into the thicker smooth muscle layers, providing the opportunity for cell proliferation and tissue formation. The series of mechanical tests that will be done will be done prior to implantation into an animal model to ensure proper anastomosis and function.

290 - Creation Of Consistent Vascular Intimal Hyperplasia

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***Purpose/Objectives:** Intimal hyperplasia, the migration and proliferation of smooth muscle cells in the intima, has been described in all types of vascular reconstructive procedures. Wire injury model and balloon angioplasty model are commonly used to mechanically create vascular intimal hyperplasia in small animals. However, the creation of feasible and consistent intimal hyperplasia is still formidable. We aim at investigating the feature of intimal hyperplasia created by different surgical procedures.
***Methodology:** To create vascular intimal hyperplasia, wire injury (WI), ligation-stenosis (LS) and suture (ST) procedure were performed. In WI group, the 0.014-inch vascular guide wire was inserted into the left femoral artery in a mouse and was retracted and advanced 5 times to injure the intima. In LS group, abdominal aorta in a mouse was prepared and tied together with 30 gauge injection needle with 10-0 nylon, and the needle was removed to create stenosis of the abdominal aorta. In ST group, half of the abdominal aorta in a rat was incised and placed 4 stitches of 8-0 nylon to close aortotomy. At 2-week and 4-week endpoint, affected site of the vessel was harvested and sectioned to stain with H&E and Masson-Trichrome and α-smooth muscle actin. Intima/Media (I/M) area ratio was compared between each group.
***Results:** In the control group with a normal vessel, I/M ratio was 0.153±0.038. I/M ratio at 2-week endpoint was significantly higher in any procedure groups than control, while there was no significant

difference between groups (WI 0.955±0.806, LS 0.732±0.184 and ST 0.995±0.127). At 2-week endpoint, WI group showed a large deviation of I/M ratio, which was not found in both LS and ST group. At 4-week endpoint, I/M ratio was comparable to that of 2-week with no significant difference and similar distribution between groups (WI 0.874±0.186, LS 0.830±0.168 and ST 1.016±0.144).
***Conclusion/Significance:** LS and ST procedure can generate consistent intimal hyperplasia in shorter term compared to WI procedure which is the standard method of intimal hyperplasia model.

291 - Model Of Patient-specific Immune Enhanced Tumor Organoids For Immunotherapy Screening

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***Purpose/Objectives:** We have hypothesized that engineering a combined lymph node/melanoma organoid from the same patient, will allow tumor, stroma and immune system to remain viable for personalized immunotherapy screening.
***Methodology:** Surgically obtained matched melanoma and lymph node biospecimens from the same patient, were transferred to the laboratory, washed with saline, antibiotic, and red blood cell lysis buffer. Biospecimens were dissociated, and incorporated into an ECM-based hydrogel system and biofabricated into 3D mixed melanoma/node organoids. Cells were not sorted, as to preserve tumor heterogeneity, including stroma and immune cell components, resulting in immune-enhanced patient tumor organoids (iPTOs). Organoid sets, were screened in parallel with nivolumab, pembrolizumab, ipilimumab, and dafrafenib/trametinib for 72 hours. Quantification of live/dead staining and metabolism assays, recorded relative drug efficacy. Histology and immunohistochemistry were used to compare tumor melanoma cells with organoid melanoma cells.
***Results:** Ten biospecimens sets, obtained from eight stage III and IV melanoma patients were reconstructed as symbiotic immune/tumor organoids between September 2017 and June 2018. Successful establishment of viable organoid sets was 90% (9/10), although organoid yield varied with biospecimen size. Average time from organoid development to initiation of immunotherapy testing was 7 days. In three patients where a node was not available, it was substituted with peripheral blood mononuclear cells. iPTOs response to immunotherapy was similar to specimen clinical response in 85% (6/7) patients.
***Conclusion/Significance**Development of 3D mixed immune-enhanced tumor/node organoids is a feasible platform, allowing individual patient immune system and tumor cells to remain viable for studying of personalized immunotherapy response.

292 - In Vitro Recapitulation Of The Dysfunctional Neuromuscular Junction In Charcot-Marie-Tooth Disease

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***Purpose/Objectives:** Charcot-Marie-Tooth (CMT) disease is a rare condition affecting 1 in every 2,500 people. CMT develops due to genetic mutations in any of 90 associated genes, all of which cause peripheral neuropathies. The development of neurospheres using induced pluripotent stem cells (iPSC)

from CMT patients has led to further analysis of the abnormal electrophysiological properties of neurons with CMT specific mutations. While the creation of patient-derived CMT neurospheres has led to greater understanding of the disease, an *in vitro* model of the neuromuscular junction (NMJ) would allow for further study of the axonal dysfunction. For this purpose, we seek to design a compartmentalized cell culture system using a gelatin-laminin (GEL-LN) hydrogel: (1) neurospheres, (2) engineered anisotropic skeletal muscle tissue, and (3) microchannels that direct axonal growth from the neurons to the skeletal muscle. Upon validation, this platform will enable mechanistic studies of CMT, as well as discovery of novel therapeutics.
***Methodology:** Mouse skeletal muscle cells (C2C12) were cultured in high glucose growth media during expansion. Once confluent, media was switched to a low serum differentiation media to promote formation of myotubes. C2C12 cultures were performed on a 2-phase biomaterial: gelatin was crosslinked using microbial transglutaminase and first micromolded with 20 µm X 10 µm microgrooves or a compartmentalized design. A solution of laminin (10 µg/mL) and microbial transglutaminase was then incubated on top of the gelatin hydrogels for 1 hour. C2C12 cells were cultured on GEL-LN micromolded hydrogels or compartmentalized hydrogels. Human-iPSCs were differentiated into motor neurons and cultured in agitation until neurospheres formed. The control group consisted of neurospheres directly adhered to skeletal muscle cells, while the experimental group involved culturing C2C12 cells and neurospheres on a compartmentalized hydrogel.
***Results:** We chose gelatin hydrogels as the biomaterial for this platform because it has been shown to promote long-term C2C12 adhesion and myotube formation. For neurosphere adhesion, we successfully enzymatically cross-linked a layer of laminin on top of the micromolded gelatin substrate. Neurospheres cultured on the GEL-LN hydrogel had enhanced morphological and functional outcomes compared to laminin-coated glass. C2C12 cells cultured on the GEL-LN hydrogel achieved long-term culture while expressing higher levels of genes indicated in enhanced myotube differentiation, such as MyoD and M-cadherin. After fabrication of an ideal substrate and media optimization for both cell types was completed, co-culture and compartmentalized systems were assessed. Both co-cultures and compartmentalized systems were stained for synaptotagmin 1 (synaptic vesicles), myosin heavy chain (myotubes), BTX (acetylcholine receptors), and dapi. Co-localization of synaptic vesicles and acetylcholine receptor clusters indicates the presence of a NMJ. Both co-cultures and the compartmentalized system stained positive for NMJs, however compartmentalized systems contained higher densities of axons and NMJs.
***Conclusion/Significance:** GEL-LN hydrogels are an optimal substrate for the culture of both iPSC-derived neurospheres and C2C12 cells. These hydrogels utilize biomimetic extracellular matrix components to promote myotube formation and axonal growth and can be micromolded with a compartmentalized design. In future studies, functional assays will be performed, including action potential propagation through axons and activation of muscle twitching through NMJs.

293 - Personalized Chemotherapy And Immunotherapy Drug Screening In Ex Vivo Patient-specific 3d Micro-tumor Constructs

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***Purpose/Objectives:** The overarching challenge in cancer treatment design is that there is only one reliable test bed: the patients themselves. However, patients cannot be the prime model system in which early stage, mechanistic experimental treatment approaches are developed. An ideal solution would be a method by which a patient's tumor could be tracked and probed outside of the patient, where candidate treatments could be investigated in parallel to determine effectiveness without harm to the patients. We have bioengineered 3D organoids created from patients' own tumor tissues to explore, test, and validate chemotherapies and immunotherapies in a patient-specific manner.
***Methodology:** We have developed a methodology for creating patient-specific tumor organoids (PTOs) from clinical biospecimens that allow us to characterize, model, manipulate, and quantify each of these three components. To date, we have created viable PTOs and microfluidic tumor-on-a-chip devices from a variety of malignancies, including lung, colorectal, melanoma, Merckel cell, sarcoma, appendiceal, mesothelioma, myeloma, and glioma malignancies. This platform is based on a 3D biofabrication platform utilizing a hyaluronic acid and collagen Type I bioink. PTOs were subjected to a variety of chemotherapy agents, or immune-enhanced with lymph node-derived cells from the same patients, thus enabling successful immune checkpoint inhibitor screening. Drug screens were assessed using quantification of mitochondrial metabolism, ATP activity, and LIVE/DEAD staining.
***Results:** Take rates of PTOs are over 90%, compared to 25-40% in 2D cultures and PDX models. Moreover, PTOs generally showed drug responses that echoed those of the patients they were derived from. Precision medicine genomic analysis-derived mutation identification yielded targets that were successfully targeted during drug screens. Immune-enhancement of PTOs provided functional immune system components that could be leveraged to generate T cell-based tumor cell killing under PD-1/PD-L1 or CTLA-4 inhibition by immune checkpoint inhibitors.
***Conclusion/Significance:** To date, adequate models of human tumor-drug interactions are lacking, yet such models are critical for understanding the human applicability of chemotherapy and immunotherapy strategies. The research described herein provides a patient-specific platform technology, providing tumor models complete with immune system components allowing personalized drug screening to be performed, potentially providing 1) a predictive clinical diagnostic tool and 2) an advanced drug development tool.

295 - A Scaffold-free Cartilage Graft For Mandibular Condyle Repair In Rabbit Models

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***Purpose/Objectives:** The project aimed to develop a tissue-engineered scaffold-free graft to repair the mandibular condylar cartilage based on a *living hyaline cartilaginous graft* (LhCG) platform in rabbit models.
***Methodology:** The xenogenic synthetic scaffold-free graft was constructed from swine cartilage with hyaline quality, termed LhCG. It was applied for treating mandibular condyle cartilage lesions in rabbit

model in four groups. The study compared the negative control (empty defect) and grafting with decellularized-LhCG (dLhCG), as well as recellularized-LhCG (rLhCG) with allogenic bone marrow stromal stem cells (BMSCs) or allogenic chondrocytes. The assessment was on macroscopic and microscopic inspection of the restoration of cartilage thickness, promotion of integration with surrounding native cartilage, cartilage-specific matrix molecules, and re-establishment of an intact superficial tangential zone.
***Results:** The study results portray the superior healing of cartilaginous defects in groups with rLhCG with allogenic rabbit BMSCs and allogenic chrondrocytes compared with the other two groups. The fabrication process of the LhCG and dLhCG, the 2D cell morphology of rLhCG and the histology findings at 3 months post graft implantation in rabbit model are presented.
***Conclusion/Significance:** This pioneering work suggests that the graft can be used to repair damage in mandibular condyle cartilage in a rabbit model.

296 - Modeling Collagen Type 1-Hydroxyapatite Structures For Middle Ear Prosthetics

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***Purpose/Objectives:** Conductive hearing loss, due to malfunctioning of the middle ear structure, is a relevant issue that affects more than 5% of the population worldwide and more than 15% of the elderly. Prosthetics provides partial or total (TORP) replacement devices that are generally made of titanium and hydroxyapatite (HA). Although successful, relevant rejection rates and sub-optimal acoustic behavior especially at highest frequencies still represent limitations of this approach. This work aims at developing a new generation of middle ear prostheses made of Collagen Type I (COL1) and HA, the principal bone constituents, by exploiting a synergistic approach involving multiscale modeling and advanced manufacturing.
***Methodology:** Molecular dynamics was used to study the role of the material components in transmitting pressure waves in terms of velocity and energy dissipation. A virtual setup was designed to study a compact volume of COL1 mineralized with 0%, 20% and 40% of HA. At the macro-scale, we studied a finite element-based model of the TORP topology aiming at generating an optimized COL1-HA stiff structure by taking into account the displacement loads and constraints at the umbo and oval window levels measured in human temporal bones. Furthermore, we investigated how the presence and dimensions of holes on the umbo footplate can affect the overall topology in meeting the objective function. A preliminary study was also carried out to validate the possibility to 3D print COL1-HA via 3D printing.
***Results:** Our results revealed how COL1 alone is able to efficiently deliver the mechanical energy: HA represents, thus, a ceramic reinforcement that enhances this behavior reducing the energy dissipation. From a constitutive point of view, the Young's Modulus estimated from the wave speed grows when the HA mineralization increases from 0.5 GPa to about 3 GPa. The optimization of the prosthesis topology led to a piston-shaped structure with a joint to the umbo footplate strongly dependent by the presence and dimensions of two through-holes. Interestingly, larger holes have to be preferred since, inversely, the reached topology would introduce thin flextures (i.e., thickness under 0.1 mm) easily to be broken during surgical handling. Finally, a preliminary study confirmed the possibility to deposit, through a high-

pressurized nozzle, a filament of amorphous COL1-HA on a flat surface achieving the targeted topology.
***Conclusion/Significance:** The results at the molecular scale will help the development new biomimetic/bioinspired materials for prosthetic devices or replacement of damaged tissues generally loaded with transient loads. In contrast, the macro-scale study delivers a new perspective for identifying a topology able to facilitate the positioning of the prostheses with a substantial reduction of the overall weight. This research represents a twist in the consolidated approach for middle ear prosthetics delivering digital optimized devices to face conductive hearing loss through an interdisciplinary approach. Further steps will include the acoustic characterization of the devices in human temporal bones and tests to assess the otocompatibility of the prostheses. This work has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement COLLHEAR No 794614.

297 - Osteogenic Evaluation Of Xenogenically Implanted Human Adipose-derived Mesenchymal Stem Cells In A Rat Maxillary Alveolar Tooth Extraction Defect Model

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***Purpose/Objectives:** Tissue engineering aims to repair and restore full function of the damaged tissue. Current bone tissue engineering methodology involves a combined approach of developing suitable biomaterials and establishing an optimal source of viable cells (osteoprogenitors) with the potential to differentiate into bone cells, resulting in effective biological substitutes that can repair and eventually restore bone tissue function. Bone regenerative therapies have garnered a lot of attention in recent years, and pose a significant challenge in cases of larger defects, or defects that are of complex anatomical shapes and sizes, such as the oral/maxillofacial bone defects. Therefore, current treatments, based on autologous and allogenic bone grafts are still the gold standard. Even though autografts and allografts are used routinely, they suffer from inherent challenges and hence, the search for an ideal bone replacement therapy is still on.
***Methodology:** In this study, we determined whether *in vitro* cultured multipotent human adipose-derived MSCs (hMSCs) could be delivered to an alveolar tooth defect site using a commonly implemented hemostat, Gelfoam. The primary objective of this study was based on the hypothesis that the MSCs delivered via Gelfoam will home to the defect site and have the potential to regenerate bone. *In vitro* and *in vivo* assays were carried out.
***Results:** We first demonstrated that Gelfoam is cytocompatible with hMSCs and supported their adherence, proliferation, and osteogenic differentiation *in vitro*. Next, we evaluated the osteogenic effect of hMSCs in the treatment of an alveolar bone defect in 8-10 week old, mixed gender Sprague Dawley rats. 36 rats were randomly assigned to 2 treatment groups, and osseous defects were created in the maxilla. Group 1 rats were grafted with Gelfoam alone, while those in Group 2 were treated with Gelfoam seeded with $1X10^6$ hMSCs. Rats were euthanized at 1-, 4-, and 12-week time points, and the harvested bone samples were subjected to histomorphometric analyses. Despite minor complications due to variations of healing in this challenging model, Masson Trichrome staining of the decalcified bone

samples showed more consistent and significantly higher accumulation of collagen/early bone formation in rats treated with hMSCs-seeded Gelfoams.
***Conclusion/Significance:** The combination of Gelfoam and xenogenic hMSCs enhanced regeneration of maxillary defects, as compared to Gelfoam alone. Hematoxylin and eosin staining demonstrated no adverse effect due to Gelfoam or the MSCs of xenogenic origin. Results proved that Gelfoam could serve as an inert scaffold to deliver and contain MSCs to the defect site without any adverse effect on the stem cell properties, and thus, their biological function.

298 - Noninvasive Cell Motion Monitoring Using Image Analysis Algorithms: Atool For Quality Control To Discriminate Epithelial Regenerative Capacityof Oral Keratinocytes

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***Purpose/Objectives:** The lack of effective and noninvasive method to monitor cell quality for clinical use is a critical problem in regenerative medicine and is an impediment to the development of quality-assured cellular constructs for grafting. As a tool for quality control (QC) of cells, the use of image processing to quantitatively analyze information obtained from microscopic images has been investigated. Recently, we applied two image analysis algorithms, optical flow and normalized cross-correlation, to measure cell/colony motion of oral keratinocytes (OK) noninvasively and quantitatively, and found that there was a significant positive correlation between the cell/colony motion and proliferative capacity, indicating the motion indices can be used as a tool for QC at an early stage of cell culture. To apply these algorithms to our human clinical protocol, we further determine the criteria of the motion indices to discriminate substandard cell populations before manufacturing an engineered oral mucosa tissue construct (EOMTC). This study aimed to examine whether the cell motion indices at a late stage of OK cell culture correlate with their epithelial regenerative capacity, by comparing with cells receiving the environmental insults which were manipulated by culture conditions.
***Methodology:** Primary OKs were serially subcultured [1)], and passages 1-2 cells were plated into a 35-mm tissue-culture dish at a density of 7.5×10^4, and fed with completed culture medium (EpiLife® supplemented with EDG) (Thermo Fisher Scientific, Waltham, MA, USA) in the following day. For the environmental insult, OKs were fed with completed EpiLife® diluted with DPBS and incubated in a moist air atmosphere at room temperature. In the meantime, time-lapse photography was taken at 8 min intervals for 4 h, and the video files were analyzed using our software to calculate the cell motion indices. Five days later with or without the environmental insults, OKs were seeded on the scaffold and the EOMTCs were manufactured according to our standard protocol [1)]. They were then fixed and processed for histologic examinations.
***Results:** Our results demonstrated that the cell motion indices of the OKs receiving the environmental insults were significantly lower than those of the OKs without the insults. Histologically, the EOMTCs developed by OKs without the insults showed a continuous, stratified epithelial layer on the scaffold, however, the OKs receiving the environmental insults failed to develop a continuous epithelial layer.
***Conclusion/Significance:** This study validated the cell motion indices at a late stage of OK cell culture correlate with the epithelial regenerative capacity, suggesting the quantitative measurement of cell

motion is feasible and a powerful tool for QC of OKs. In a clinical setting, we should further determine reference value of the motion indices to discriminate inappropriate cells for transplantation.
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: 1792. **Acknowledgments:** This research was supported by JSPS KAKENHI Grant Numbers 17H04398G to K.I. The authors have no conflict of interest to declare.

299 - A Perfusion Bioreactor System For Cell Seeding And Oxygen-controlled Cultivation Of Three-dimensional Cell Cultures

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***Purpose/Objectives:** Bioreactor systems facilitate three-dimensional (3D) cell culture by coping with limitations of static cultivation techniques. To allow for the investigation of proper cultivation conditions and the reproducible generation of tissue-engineered grafts, a bioreactor system, which comprises the control of crucial cultivation parameters in independent-operating parallel bioreactors, is beneficial. Furthermore, the use of a bioreactor as an automated cell seeding too enables even cell distributions on stable scaffolds.
***Methodology:** In this study, we developed a perfusion microbioreactor system, which enables the cultivation of 3D cell cultures in an oxygen-controlled environment in up to four independent operating bioreactors. Therefore, perfusion microbioreactors were designed with the help of computer-aided design, and manufactured using the 3D printing technologies stereolithography and fused deposition modeling. A uniform flow distribution in the microbioreactor was shown using a computational fluid dynamics model. For oxygen measurements, microsensors were integrated in the bioreactors to measure the oxygen concentration (OC) in the geometric center of the 3D cell cultures. To control the OC in each bioreactor independently, an automated feedback loop was developed, which adjusts the perfusion velocity according to the oxygen sensor signal. Furthermore, an automated cell seeding protocol was implemented to facilitate the even distribution of cells within a stable scaffold in a reproducible way. As proof of concept, the human mesenchymal stem cell line SCP-1 was seeded on bovine cancellous bone matrix of 1 cm^3 and cultivated in the developed microbioreactor system at different oxygen levels.
***Results:** The oxygen control was capable to maintain preset oxygen levels ± 0.5 % over a cultivation period of several days. Using the automated cell seeding procedure resulted in evenly distributed cells within a stable scaffold.
***Conclusion/Significance:** In summary, the developed microbioreactor system enables the cultivation of 3D cell cultures in an automated and thus reproducible way by providing up to four independently operating, oxygen-controlled bioreactors. In combination with the automated cell seeding procedure, the bioreactor system opens up new possibilities to conduct more reproducible experiments to investigate optimal cultivation parameters and to generate tissue-engineering grafts in an oxygen-controlled environment.

300 - Tissue-specific Contrast Agents For Longitudinal Tissue Tracking

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Two fundamental and unsolved problems facing tissue tracking are nonspecific uptake of intravenously administered contrast agents by normal tissues and organs, and incomplete elimination of unbound targeted agents from the body. These problems make long-term tissue tracking extremely difficult because background is high, and therefore the signal-to-background ratio is low. To solve these problems, we have synthesized a series of near-infrared fluorophores that varied systematically in net charge, conformational shape, hydrophilicity/lipophilicity, and charge distribution. Using 3D molecular modeling and optical fluorescence imaging, we have defined the relationship among the key independent variables that dictate biodistribution and tumor-specific targeting using nanoparticles in human prostate cancers (*Nat Nanotechnol*. 2010) and small molecules in human melanomas (*Nat Biotechnol*. 2013). Recently, we have developed new pharmacophore design strategy "structure-inherent targeting," where tissue-specific targeting is engineered directly into the nonresonant structure of fluorophore, thus creating the most compact possible optical contrast agent for bioimaging and nanomedicine (*Nat Med*. 2015). The biodistribution and targeting of these compounds vary with dependence on their unique physicochemical descriptors and cellular receptors, which permit 1) selective binding to the target tissue, 2) visualization of the target specifically, and 3) provide longitudinal tissue tracking.

301 - Quantitative Characterization Of Adhesion And Cytomechnics Of Living Cells On Biomaterials And Tissues

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INTRODUCTION: The nano-mechanical analysis of cells is increasingly gaining in importance in various fields of cell biology like cancer research and developmental biology. The topography and mechanical properties of biomaterials are crucial parameters that influence cell adhesion/motility, morphology and mechanics as well as the fate of stem and progenitor cells [1, 2, 3]. **MATERIALS and METHODS:** Atomic force microscopy (AFM) is a powerful tool which allows the comprehensive study of these properties and interactions with nanometer scale resolution. The new NanoWizard® ULTRA Speed AFM, enables high-speed studies of the time-resolved dynamics associated with cellular processes. Fast AFM imaging of several frames/sec can be seamlessly combined with methods such as epi-fluorescence, confocal, TIRF, STED microscopy. **RESULTS:** Mechanical properties like the Young's modulus of biomaterials, tissues or cells can be determined. The nanostructure of biomaterials like aligned collagen matrices and cell alignment on such structures have been resolved [3]. Using Single Cell Force Spectroscopy, cell-substrate or cell-cell/tissue interactions can be measured down to single protein unbinding. **DISCUSSION and CONCLUSIONS:** We will present how the latest advances in the ULTRA Speed AFM are being applied to study a wide-range of biological specimen, from individual biomolecules to collagen type I fibrillogenesis to mammalian cells and tissues. **REFERENCES** [1] Elter et al., Eur Biophys J 2011: 40: 317-327. [2] Engler et al., Cell 2006: 126:677-689 [3] Cisneros et al., Small 2007: 3:956-63.

302 - Development Of Photo-crosslinkable Decellularized Cartilage Extracellular Matrix Bioinks

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***Purpose/Objectives:** Due to its low vascularization, cartilage has a low capacity for self-repair. In addition, the complex biochemical makeup that allows crosstalk with bone and heterogenous architecture of cartilage creates obstacles in tissue engineering approaches. Decellularized extracellular matrix (dECM) has become an attractive material for bioprinting because it provides tissue-specific biochemical cues that more accurately recreate the cell microenvironment for cell growth, proliferation, and differentiation, than commercially available natural or synthetic polymers. However, thermoresponsive hydrogels formed from dECM bioinks often lack relevant mechanical properties and architecture, particularly within musculoskeletal applications. The use of vitamin B2 as a photo-crosslinker has been shown to significantly increase mechanical properties in dECM bioinks. The objective of our work is to develop and characterize a photo-crosslinkable decellularized cartilage extracellular matrix (cdECM) bioink which retains key extracellular matrix components in order to recapitulate the physicochemical and mechanical properties of native cartilage.
***Methodology:** Previous cartilage decellularization methods were modified to be less harsh and retain important matrix components, including sulfated glycosaminoglycans. Harvested hyaline cartilage was treated with hypotonic solution, trypsin, and triton-X 100 to remove cellular material. cdECM was then dried, milled into a fine powder, and digested with pepsin for 48 hours. Following titration to physiological pH, the resulting cdECM was kept on ice until desired thermogelation at 37 °C. Biochemical characterization via hydroxyproline assay, and dimethylmethylene blue assay demonstrated retention of collagen and sGAGs, respectively. Through histology and immunohistochemistry, decellularization of cdECM was confirmed.
***Results:** After confirmation that decellularized inks retain important biochemical cues, initial rheological characterization revealed that 3 wt% cdECM is shear thinning at 4 °C with an LCST of 15 °C. In our two-step crosslinking process, cdECM thermogelation in tandem with photo-crosslinking increases the mechanical properties of printed cdECM hydrogels.
***Conclusion/Significance:** Bioprinting allows for precise control over scaffold size, shape, porosity, and pore size. In addition, bioprinting allows for highly precise architecture, which is highly important due to the heterogenous architecture of native cartilage. The versatility of bioprinting in combination with photo-crosslinkable cdECM provides an important framework for the development of tunable natural and hybrid scaffolds for cartilage tissue engineering. Long-term stability of vitamin B2 photo-crosslinked dECM hydrogels has been qualitatively observed. Future work will focus on further rheological and mechanical characterization and increasing printability of the cdECM bioink.

303 - Development Of Electrospun Decellularized Muscle-derived Scaffolds With Tunable Physicochemical Properties For Tissue Engineering Applications

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***Purpose/Objectives:** Although skeletal muscle has a high potential for self-repair, volumetric muscle loss can result in impairment beyond the endogenous regenerative capacity. Current clinical standards

fail to fully restore the structure and function of lost muscle. Therefore, improved therapies for muscle regeneration are needed. Decellularized extracellular matrix (dECM) scaffolds are an attractive platform for many tissue engineering applications, as dECM contains many biochemical cues that aid in cell adhesion, proliferation, and differentiation. However, current dECM technologies have a limited capacity to tune physicochemical properties to improve outcomes. The objective of this study is to investigate the effects of architecture and degree of crosslinking on cell growth and differentiation in electrospun scaffolds derived from decellularized skeletal muscle.
***Methodology:** Electrospun scaffolds were fabricated from decellularized skeletal muscle with tunable physicochemical properties while retaining pro-regenerative matrix components and without the need for a carrier polymer. Skeletal muscle was harvested from the hind legs of New Zealand White rabbits and decellularized using trypsin, triton x-100, and a series of hypotonic and hypertonic solutions. Upon successful decellularization, dECM was homogenized, dried, and milled into a fine powder. Electrospinning solutions were then prepared by dispersing dECM powder into chilled hexafluoro-2-propanol. Architecture was modulated by electrospinning on a stationary metal plate to form randomly-oriented scaffolds and on a rotary drum with adjustable speeds to form scaffolds with different degrees of alignment. Post-processing crosslinking was also tuned by varying the length of time that scaffolds were exposed to glutaraldehyde vapor.
***Results:** We demonstrated a successful decellularization protocol that effectively removes DNA. Through the identification of key steps in the decellularization and fabrication processes, we have fabricated scaffolds completely derived from skeletal muscle without the need for a carrier polymer. Electrospinning allows for rapid scaffold fabrication with high control over material properties, which can be optimized to mimic native muscle. Fiber alignment and mechanical properties have been shown to influence myogenic differentiation. To this end, fiber orientation and degree of crosslinking of these dECM scaffolds were modulated and the corollary effects on mechanical properties and cell behavior were investigated. In addition, these unique materials can be used long-term without the need for crosslinking agents.
***Conclusion/Significance:** Beyond application in skeletal muscle, the versatility of this technology has the potential to serve as a foundation for dECM scaffold fabrication in a variety of tissue engineering applications.

304 - Endothelial Cells Support Osteogenesis In A Vascularized 3D Bioprinted In Vitro Bone Model

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***Purpose/Objectives:** A key obstacle in the *in vivo* translation of bone tissue engineering is the lack of vascularization within the constructs [1,2]. In this work we aim to develop a vascularized *in vitro* bone model to study blood vessel morphogenesis in engineered bone, combining bioprinting of gelatin-hydroxyapatite with an interpenetrating double-network hydrogel of fibrin and methacrylated gelatin.
***Methodology:** We mixed a 50% w/v nanohydroxyapatite (Fluidinova) in a type A gelatin (Sigma-Aldrich) solution (10% w/v in deionized water) with 0.2% w/v genipin (Challenge Bioproduct Co) as crosslinker. Wood-pile scaffolds were 3D bioprinted into a 20 % w/v Pluronic acid F127 (Sigma-Aldrich) solution in deionized water (bioplotting) [3].Scaffolds seeded with $1x10^5$ hMSCs/scaffold were cultured for 7 days in

growth medium (GM=DMEM, 10% fetal bovine serum, 2% penicillin-streptomycin-fungizone). Then, GM was switched to osteogenic medium (OM=GM + 0.1 mM ascorbic acid, 10 mM βglycerophosphate, 0.1 µM of dexamethasone and 10 nM vitamin D3) for 2 weeks of differentiation. Then, scaffolds were divided in 3 groups and cultured for 2 additional weeks. Group 1: bone constructs cultured in 1:1 OM:endothelial medium (EM, Endothelial Cell Growth Medium-2 BulletKit, Lonza) (control); group 2: vascularized bone constructs (see below) cultured in 1:1 OM:EM; group 3: vascularized bone construct cultured in 1:1 GM:EM (control). All tests were conducted in triplicate.*Vascularized bone construct fabrication:* a 4:1 ratio HUVECs:hMSCs was suspended at a concentration of 10^6 cells/ml in a 1:1 solution of fibrin glue (Tisseel Fibrin Sealant kit, Baxter) and 10% w/v methacrylate gelatin (gelMA) solution (Cellink) in PBS with a 0.15% w/v Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (Sigma-Aldrich) photoinitiator. Scaffold macropores were filled by pouring 55µl of HUVECS:hMSCs-laden gelMA-fibrin hydrogel, UV-crosslinked *in situ* for 2 minutes (405 nm).
***Results:** In 14 days, HUVECs formed tubular structures within the bone constructs, assembling a complex capillary-like network. CD31 immunostaining confirmed *lumen* formation by endothelial cells. Quantitative real-time PCR was used to quantify osteogenesis and endothelial response. Alkaline phosphatase and runt-related transcription factor 2 upregulation suggests hMSCs osteogenic commitment, but cells were not fully mature (no upregulation of osteocalcin and bone sialoprotein II). Upregulation of osteopontin, vascular endothelial growth factor, and collagen type I was observed only in group 3 (vascularized construct without osteogenic medium).
***Conclusion/Significance:** We developed a vascularized *in vitro* bone model with complex, highly branched and interconnected HUVECs networks, within the scaffold macropores, simulating *de novo* morphogenesis of capillary vessels occurring during tissue development.
BIBLIOGRAPHY: (1) A. Pirosa, *et al*., Stem Cell Res. Ther., vol. 9, no. 1, e28352, 2018. (2) C. Correia *et al*., PLoS One, vol. 6, no. 12, 2011. (3) C. S. O. Bryan, et al., Mater. Res. Soc. Bull., vol. 42, pp. 571-577, 2017.**ACKNOWLEDGMENTS:** Ri.MED Foundation, NIH/CFD Research Corp R43AR072169

306 - In Situ Printed Scaffolds Controlled-Releasing Vascular Endothelial Growth Factor Facilitate Functional Recovery Post Volumetric Muscle Loss Injury

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***Purpose/Objectives:** Volumetric muscle loss (VML) overwhelms the ability of the body to regenerate, leading to fibrosis and loss of function. The limitations of current medical interventions have recently led to several tissue engineering approaches to improve tissue remodeling. However, engineering thick scaffolds required for VML injuries extend beyond the diffusion limit of nutrients and oxygen resulting in necrosis at the core of the scaffold. To overcome this limitation, angiogenesis is required. Thus, we propose using Laponite nanoclay to electrostatically bind and control-release vascular endothelial growth factor (VEGF) to promote angiogenesis in thick gelatin methacrylate (GelMA) scaffolds. VEGF-loaded GelMA hydrogels will be printed *in situ* using a handheld printer.
***Methodology:** Protein binding efficiency was evaluated by mixing Laponite at varying concentrations with a constant concentration of bovine serum albumin (BSA) in Dulbecco's phosphate-buffered saline (DPBS) and analyzing supernatant protein concentration with a bicinchoninic acid (BCA) assay. *In vitro* protein release was measured by similarly mixing Laponite and BCA or VEGF, encapsulating the protein-

loaded Laponite in GelMA or remaining unbound in DPBS, and quantifying the released protein in the samples at varying timepoints with BCA or ELISA assays, respectively. GelMA hydrogels were created by dissolving lyophilized, medium degree of methacrylation GelMA with lithium phenyl-2, 4, 6-trimethylbenzoylphosphinate (LAP) in DPBS. Protein-loaded hydrogels were made by mixing VEGF-loaded Laponite into GelMA. Mechanical properties of the GelMA hydrogels were assessed using a mechanical tester. To assess *in vivo*, VEGF-loaded GelMA hydrogels were printed into mice with hind-limb VML injury using a handheld printing device. The mice were functionally analyzed on a treadmill, and *ex vivo* analysis was performed through histological characterization.
***Results:** The handheld printer was a suitable tool to deliver hydrogels carrying protein-loaded nanoclay to *in situ* defects. Particle concentration had an impact on protein binding efficiency, and the particles were capable of the sustainable release of proteins over a period of 45 days. Mechanical testing characterized the physical properties of the GelMA hydrogels and their impact on interfacing with skeletal muscle. VEGF-loaded GelMA hydrogels printed into murine VML defects showed significant functional improvement over other VML groups. Most notably, there was no significant difference between the maximum running speed of uninjured animals and those treated with VEGF-loaded GelMA hydrogels.
***Conclusion/Significance:** A handheld bioprinter was used to in situ print and crosslink GelMA-based scaffolds carrying nanoclay particles controlled-releasing VEGF. Nanoclay particles were shown to be a suitable carrying vehicle of proteins for a sustainable and tunable time release. Sustained delivery of VEGF significantly improved the functional recovery in mice with VML injuries. This method is a versatile tool for the delivery of a variety of growth factors that can lead to improved patient outcomes.

307 - Increases In Skeletal Muscle Collagen And Crosslinking Persist In Decellularized Muscle Matrix In An Age-dependent Manner

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***Purpose/Objectives:** Extracellular matrix (ECM) based biomaterials hold promise for promoting skeletal muscle regeneration. Decellularized muscle matrix (DMM) is produced through multiple enzymatic, detergent, and saline soaks that remove the cells while retaining the skeletal muscle ECM. DMM is non-immunogenic and supports the formation of functional muscle fibers.[1] Clinically, the relevance of DMM technology may be donor-dependent. Variables such as old age may mitigate the therapeutic potential of DMM. Studies have shown that the ECM components of skeletal muscle, specifically collagen, become more crosslinked with age.[2] However, whether these changes persist in DMM has not been assessed, and it is not clear if sourcing DMM from older individuals will reduce therapeutic potential.
***Methodology:** Gastrocnemius muscle from 5 (young), 10 (mature), and 80-week (aged) old male C57BL/6 mice were collected. DMM was produced by decellularization using 0.25% trypsin, 0.1% Triton X-100, 2% sodium deoxycholate, DNase and 1x PBS. Picrosirius red staining was used to histologically assess collagen in muscle and DMM. Hydroxyproline content in muscle and DMM was measured using the Colorimetric Hydroxyproline Assay kit (Abcam). To qualify ECM crosslinking, DMM was lyophilized and cryomilled to create a DMM powder, which was digested with pepsin and then run on an acrylamide gel to assess collagen crosslinking. One-way ANOVA with Tukey's multiple comparison post-

test was used to determine significance.
***Results:** Muscle from 5, 10, and 80-week mice had age-dependent morphological differences in picrosirius red staining, with increased collagen in the endomysium of the young and aged groups when compared to mature. There was a visible thickening of the collagen in DMM from aged muscle. The hydroxyproline concentration was higher in the young and aged muscle when compared to mature muscle. This difference persisted in DMM. The aged DMM had less soluble procollagen after the pepsin digestion, indicating more crosslinking.
***Conclusion/Significance:** Age-dependent changes in skeletal muscle ECM are retained in DMM and may be mediated by crosslinking. The increases in collagen and collagen crosslinking could impact the benefits of DMM in skeletal muscle injuries. Our results show that there is donor-dependent variability in DMM, which needs to be addressed during the translation of this technology. This work was funded by the Musculoskeletal Transplant Foundation (JR Neff Award), Department of Defense, Defense Medical Research and Development Program, Neuromusculoskeletal Injuries Rehabilitation Research Award (W81XWH1810352), and the NSF Career Award (CMMI-1351162).1. McClure MJ, Boyan BD. Decellularized Muscle Supports New Muscle Fibers and Improves Function Following Volumetric Injury. Tissue Eng Part A, Vol. 24, No. 15-16, 2018. 2. Lauren WK, Susan BV. Intrinsic stiffness of extracellular matrix increases with age in skeletal muscle of mice. Journal of Applied Physiology, Vol. 117, Issue 4, 2014.

308 - Tendon-derived Subpopulations And Their Response To Inflammatory Environment

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***Purpose/Objectives:** Tendinopathy is a common musculoskeletal disorder particularly affecting amateur and professional athletes. In the pathogenesis of tendinopathy, tissue degeneration does not represent the leading cause. In fact, subclinical inflammatory cascades inducing a persistent dysregulated tissue homeostasis have re-emerged as key disease initiators[1]. A deeper understanding of the identity of different tendon sub-populations (*i.e.,* tendon stem-progenitor cells, tenoblast and tenocytes)[2], their role during the inflammatory reaction and their potential contribution to the healing process of the tissue is mandatory to design effective novel therapeutic strategies.
***Methodology:** In this study, human tendon-derived cells were isolated from semitendinosus and gracilis tendons and cultured at high and low densities to *ex vivo* discriminate the different underlying subpopulations. A thorough characterization of cell morphology, growth kinetics at different cell seeding densities, cell phenotype, gene expression profile and response to inflammatory stimulation was performed.
***Results:** Three different cell types were obtained, exhibiting distinct morphologies, phenotypic profiles and molecular signatures. Specifically, tendon cells cultured at low density immediately after tissue digestion exhibit a cobblestone morphology, a high clonogenic ability, express classic MSC markers including CD146, Stro-1 and Endomucin, possess a low multi-differentiation potential, but high level of

specific stemness-related genes (leukemia inhibitory factor, LIF). On the contrary, high density cultured cells immediately after tissue digestion possess a spindle-shaped morphology, higher multi-differentiation potential and expression of tenogenic markers but lower expression of LIF. Moreover, they possess lower expression levels of CD146, Stro-1 and Endomucin. This evidence suggests that it is feasible to *ex vivo* reproduce the tendon identities existing *in vivo*. Furthermore, tendon-derived sub-populations differentially responded to inflammation, indicating different roles during injury and healing processes. The low density cultured cells exhibit higher level of ICAM1 and VCAM1, thus suggesting a higher immunosuppressive potential[3]. On the contrary, the high density cultured ones showed higher levels of BMP2 after inflammatory stimulation, indicating a possible predisposition of this subpopulation to undergo osteogenic differentiation and possibly causing ectopic bone-formation *in vivo*.
***Conclusion/Significance:** Overall, this study aimed to identify potential molecular targets, providing evidence of how the different tendon subsets react to inflammation, understanding their roles during tendinopathy development and potentially leading to cellular targets for regeneration.
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309 - Fk506-loaded Biomimetic Sponges Enhance Skeletal Muscle Regeneration In A Rat Model Of Volumetric Muscle Loss

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***Purpose/Objectives:** Skeletal muscle is inept in regenerating after traumatic injuries such as volumetric muscle loss (VML) due to significant loss of basal lamina and the resident satellite cells. Currently, there are no approved therapies for the treatment of muscle tissue following trauma. To improve regeneration of skeletal muscle and decrease fibrotic tissue deposition, we have developed biomimetic sponges composed of collagen, gelatin, laminin (LM)-111, and FK506 using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) as a crosslinker. Collagen and LM-111 are crucial components of the muscle extracellular matrix and were chosen to impart bioactivity, whereas gelatin and EDC were used to provide mechanical strength to the scaffold. FK506 is an immunosuppressive drug that limits the immune response to injury and helps minimize fibrosis.
***Methodology:** The sponges were fabricated by mixing 3wt% porcine skin gelatin (Sigma-Aldrich) with 20mM EDC, which was pipetted into tissue culture plates along with rat tail collagen I (Gibco, 3 mg/mL) in a gelatin: collagen ratio of 70:30. LM-111 (Trevigen) was then added at a final concentration of 50 µg/ml and FK506 (Abcam) was added at a final concentration of 25 µM. The solution was allowed to freeze slowly and was lyophilized for 12 hours. A 6-mm sponge disc (n=6) was implanted in tibialis anterior (TA) muscles of male Lewis rats. Untreated VML injured muscles (n=6) served as controls. At 7, 14, and 28 days post-injury, muscles were harvested for histological and biochemical analysis. At 28 days post-injury, peak isometric force was measured.
***Results:** In sponge treated muscles, the gene expression of MyoD was significantly higher at day 7 post-injury, while the expression of embryonic myosin heavy chain (eMHC) and dystrophin was higher at day 28 post-injury compared to untreated muscles. These results suggest that sponge treatment resulted in heightened and prolonged myogenic activity at the VML defect site. In support, the histological analysis

also showed increased cellular infiltration and presence of regenerating myofibers in sponge treated muscles. Both pro-inflammatory markers (i.e., TNF-α, CCR7) and anti-inflammatory makers (i.e., TGF-β1, CD163) were significantly upregulated in sponge-treated muscles at day 7. By 14- and 28-days post-injury, the inflammatory response subsided significantly and no differences were observed between untreated and treated groups. These results indicate that sponge treated muscles supported higher cellular activity at the VML defect site at day 7 post-injury. At 28 days, peak isometric force in the sponge-treated muscles was significantly higher than untreated muscles when normalized to TA weight. This indicates that the sponges enhance functional recovery of injured skeletal muscle.
***Conclusion/Significance:** Therefore, we posit that the increased myogenic activity and reduced inflammation promoted functional muscle regeneration in sponge treated injured muscles.
Acknowledgments: This work was supported by a grant from the National Institute of Health (NIGMS) 1R15GM129731.

310 - Patterned SiRNA Presentation For Spatially Driving HMSC Osteogenesis

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***Purpose/Objectives:** Patterning the presentation of bioactive molecules in biomaterials is a promising strategy for modulating the tissue regeneration process of complex tissues (1). MicroRNA and short interfering RNA (siRNA) can silence gene expression post-transcriptionally and can guide the differentiation of stem cells, making these molecules promising agents for spatiotemporally controlled tissue regeneration strategies (2-3). For example, controlled temporal presentation of siNoggin, a pro-osteogenic siRNA against Noggin, from hydrogels enhanced the osteogenesis of co-encapsulated hMSCs (3-4). In addition, gradient presentation of siRNA in hydrogels was also reported to silence cellular gene expression of co-encapsulated cells in the gradient fashion (4). Therefore, patterned presentation of single or multiple siRNAs could be helpful in spatially controlling cell behavior and ultimately engineering tissues with specific spatial compositions and morphologies.
***Methodology:** In this work, we studied the ability of patterned siRNA presentation in hydrogels to spatially drive the differentiation of co-encapsulated hMSCs for tissue regeneration. Three different strategies were investigated, but due to space, only one is described here. The capacity of patterned-presentation of siGFP in hydrogels to spatially regulate cellular GFP expression of encapsulated cells was first investigated. “Strings” of photocrosslinked PEG-DA hydrogels were fabricated by manually fusing four square-segment gels (3.5x3.5x0.4 mm) containing homogenously distributed deGFP HeLa cells and different increasing concentrations of siGFP (0, 0.8, 2.4 and 4.0 μM in segments 1, 2, 3 and 4, respectively), and cellular GFP expression within the hydrogel was then examined. Next, the capacity of patterned siNoggin presentation to spatially control hMSC-driven osteogenesis was tested. The “string” hydrogels encapsulated with homogenously distributed hMSCs and different increasing concentrations of siNoggin were fabricated using the same method and cultured in osteogenic media for 4 weeks.
***Results:** Fluorescence confocal microscope imaging confirmed that the patterned-presentation of siGFP in the hydrogels produced a corresponding patterned-cellular GFP expression after 2 days of culture while high cell viability was maintained. When siNoggin was encapsulated in “string” hydrogels, biochemical results indicated that the DNA content in each segment was similar at each time point, while the patterned-deposition of calcium (Ca), a major component of bone extracellular matrix, was

observed to increase corresponding to the increase in patterned-presentation of siNoggin. In addition, this finding of patterned-deposition of Ca was corroborated in optical images of the hydrogels before and after Alizarin Red S staining, which showed increasingly deposited Ca in segments with higher siNoggin concentration.
***Conclusion/Significance:** These results indicate that patterned-distribution of siRNA in the hydrogels could regulate the differentiation of co-encapsulated hMSCs in the corresponding patterned-fashion, demonstrating the potential of this strategy in engineering complex tissues. **References:** 1) Jeon O, *Small* 2018,14,1800579; 2) Huynh CT, *ACS Appl Mater Interfaces* 2018,10,25936; 3) Nguyen MK, *J Mater Chem B* 2017,5,485; 4) Hill MC, *Adv Healthcare Mater* 2015,4,714. **Acknowledgments**: Funding support from NIH (R56DE022376, R01AR069564, R01AR066193).

311 - Skeletal Muscle Differentiated From ALS Patient-derived IPSCs Exhibit Defects In Muscle Differentiation, Contraction And Mitochondrial Function

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***Purpose/Objectives:** Amyotrophic Lateral Sclerosis (ALS) is a fatal adult-onset multi-factorial disease, that averages 1-3 years life expectancy after diagnosis. It is characterized by progressive motoneuron degeneration, as well as skeletal muscle wasting and weakness. The specific susceptibility of motoneurons in ALS led early researchers to adopt a “neurocentric” approach to investigating disease pathogenesis [1]. However, recent findings reported the pathological involvement of the skeletal muscle in ALS onset and progression. Skeletal muscle-specific expression of mutant SOD1 induced ALS symptoms in transgenic mice models, while neuron-specific expression did not induce motor impairment [2]. Additionally, altered skeletal muscle metabolism and atrophy are documented to precede motoneuron degeneration in both disease models and patients [2, 3]. However, the exact role of skeletal muscle in ALS pathology remains debatable, due to limited human-based ALS studies.
***Methodology:** To address this question, we utilized ALS patient-sourced induced pluripotent stem cells (PS-iPSCs), a novel avenue of developing disease-specific *in vitro* systems that have been documented to closely recapitulate human disease conditions, to recreate an ex vivo model of ALS skeletal muscle [4, 5].
***Results:** ALS PS-iPSC derived skeletal myoblasts were proliferative and expressed myogenic markers at levels comparable to wild type (WT) myoblasts. Upon myotube differentiation however, ALS PS-iPSC derived skeletal myoblasts exhibited delayed and reduced fusibility. Also, myotubes derived from ALS iPSCs contracted at a much lower amplitude and had reduced contraction fidelity under field electrical stimulation. Furthermore, subcellular analysis revealed compromised mitochondrial membrane potential and altered cell metabolism mechanisms in ALS PS-iPSC derived myotubes.
***Conclusion/Significance:** These results concur with previously published patient biopsy-derived primary muscle studies, validating this skeletal muscle system derived from ALS patient iPSCs, as an appropriate model for etiological investigations of ALS and for relevant drug testing. Moving forward, *in vitro* NMJ models will be developed using these PS-iPSC derived myotubes to elucidate their effect on NMJ degradation and motoneuron degeneration, which are key events for ALS etiology.

312 - Bone Tissue Engineering Via Growth Factor-free Osteogenic Differentiation Of Mesenchymal Stem Cells In Bioactive Hydrogel Nanocomposites Reinforced With Silica-coated Graphene Oxide

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***Purpose/Objectives:** The combination of stem cells and synthetic biomaterials has been a much sought-after solution to the unmet clinical need for bone repair. Ideal bone graft materials are expected to deliver satisfactory mechanical and biological performances. Recently, the bone tissue engineering field has witnessed intriguing applications of the 2D nanomaterial graphene and its derivatives such as graphene oxide (GO) because of their extraordinary physical and chemical characteristics. Silicon (Si) is an essential trace element for healthy bone and connective tissues and has been reported to be able to facilitate bone remodeling. Therefore, herein we hypothesize that the novel 2D hybrid nanoplatelet silica-coated graphene oxide (SiGO) can be used to prepare 3D hydrogel-based nanocomposites that enhance osteogenic differentiation of human mesenchymal stem cells (MSCs) for accelerated bone repair and regeneration.
***Methodology:** A modified Hummers method was utilized to synthesize GO, which was then converted to SiGO nanosheets via a sol-gel method reported in our previous study. The SiGO nanosheets were homogeneously dispersed in 15% methacrylated gelatin (SiGO/GelMA) at 1 mg/mL. The composite solution was subsequently used to suspend bone marrow-derived MSCs isolated from femoral heads and trabecular bone of human patients at 20 M cells/mL and photocrosslinked to obtain 3D constructs. The cell-laden scaffolds were cultured in osteogenic media for 4 weeks, without the supplement of osteoinductive growth factors. The cytocompatibility of SiGO-reinforced GelMA was examined using the Live/Dead cell viability assay. Real-time polymerase chain reaction was performed to assess osteogenic gene expression in MSCs, and osteogenesis quality was further evaluated with histological staining, immunohistochemistry (IHC), and fluorescent confocal imaging of calcein green. Scaffolds containing unmodified GO sheets (GO/GelMA) were prepared and cultured under identical conditions for comparison purposes. One-way ANOVA was employed for data analysis.
***Results:** Live/Dead staining indicates that incorporation of GO or SiGO in GelMA did not affect the viability of encapsulated cells. MSCs in SiGO/GelMA displayed highest expression levels of osteogenic marker genes, including osteocalcin (*OCN*), osteopontin, bone morphogenetic protein 2, and bone sialoprotein. Interestingly, SiGO induced a more robust and uniform calcification of MSC than GO, as revealed by alizarin red staining and von Kossa staining. This was further confirmed by calcein green imaging. In comparison, no positive staining was seen in the GelMA group. In IHC analysis, significantly more OCN and alkaline phosphatase were identified in the SiGO/GelMA group than the others. Although the mechanism of osteo-enhancing capacity of SiGO is under investigation, it is believed that the mechanically strong core and biologically active shell of SiGO nanoplatelets synergistically promoted the osteogenic differentiation of MSCs.
***Conclusion/Significance:** Drawbacks of current bone tissue engineering approaches include insufficient or non-uniform calcification and the dependence on adequate growth factors. In this research, we report the first use of SiGO nanosheets to induce robust ossification of MSCs in 3D hydrogel-based scaffolds, which is superior to pure GelMA or those loaded with unmodified GO. Our results showed the high biocompatibility and bioactivity of these novel nanocomposites. The SiGO nanoplatelets, therefore, hold strong promise for a range of bone tissue engineering applications.

313 - Spheroidal Co-culture Of HiPSC-derived Osteoblasts And Endothelial Cells As Novel Building Block For 3D Printed Bone Tissue Graft

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***Purpose/Objectives:** Vascularization is the key to create functional bone grafts by tissue engineering approaches. Human induced pluripotent stem cell (iPSCs) is a promising patient-specific cell source for pre-vascularized bone tissue graft: (1) Human iPSCs are capable of differentiating into mesenchymal stem cells (MSC), which can give rise to functional osteoblast for bone regeneration; (2) Human iPSCs are capable of endothelial commitment to vascularize the bone graft. Since numerous studies have indicated the pro-angiogenic effect of MSC 3D spheroids when co-cultured with vascular endothelial cells (EC), we aim to create 3D cell spheroids consisting of hiPSC-derived MSCs and ECs as a novel, pre-vascularized cellular component for 3D-printed bone graft.
***Methodology:** Human iPSCs (P30-40) were differentiated into ECs and MSCs using established protocols and characterized for surface markers as well as functional features. Spheroidal co-culture of MSC/EC was performed at various ratio and cell number, and vascularization visualized by CD31 immuno-fluorescent staining via confocal microscopy at pre-determined time points. The acquisition of osteoblast phenotype was evaluated at mRNA-level by PCR assay and compared to EC-free group. Spheroids were impregnated in fibrin/collagen bio-ink and printed by a 3D bio-plotter (envisionTEC) to create bone scaffold with great fidelity to the CAD model. The post-printing cell vitality was evaluated by Live & Dead staining and MTS assay.
***Results:** The iPS-ECs and iPS-MSCs prepared by our methods exhibit consistent phenotype and differentiation capacity, respectively. When the mixture of iPS-ECs and iPS-MSCs were seeded in 96 well round bottom ultra-low attachment plate, multicellular spheroidal aggregate started with the initial loose cell aggregate formation, followed by the spheroid compaction within three days. The immunofluorescent staining of CD31 revealed the outgrowth of multicellular, tube-like structures from spheroids in Matrigel, indicating the potential of spheroids to vascularize the surrounding biomaterials. Using 3D bioprinting technologies, including 3D-Bioplotter (EnvisionTEC) and BioAssemblyBot (Advanced Solutions), we have demonstrated that the cell-laden 3D constructs prepared in this manner faithfully reflected the complex geometry and porosity of the digital model. Live and Dead staining revealed high cell vitality in the scaffold 7 days after fabrication.
***Conclusion/Significance:** This study has the potential to address three major challenges that face bone tissue engineering. First of all, the employment of patient-specific iPSCs circumvents invasive isolation of primary MSCs and ECs, and offers an inexhaustible cell supply for bone tissue engineering. Secondly, the inherent pro-angiogenic potential of spheroid co-culture enables the pre-vascularization of the graft, which is identified as an indispensable driver of bone healing. Lastly, 3D bio-printing renders an alternative source of bone scaffold that can faithfully produce the desired architecture of bone graft.

314 - Effect Of Hyperglycemia On Myoblast Proliferation

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***Purpose/Objectives:** Skeletal muscle is the largest organ in the body under non-obesity conditions and composes approximately 40% of human body weight. It is not only important for voluntary and involuntary movement of the musculoskeletal system but also is a major site for glucose consumption,

and plays a significant role in the regulation of glucose and lipid homeostasis. Diabetic myopathy is well known in both type 1 and type 2 diabetic mellitus, although they differ drastically in the mechanisms of insulin insufficiency and in clinical history. However, our understanding about the mechanism for diabetic myopathy is still limited due to the complications in diabetes. It is even undefined what the direct effect of hyperglycemia, the major pathology shared by all diabetic mellitus, on muscle growth. Previous studies are mainly from animals which only partially reproduce the human system, and it is hard to isolate each individual effector. This study aimed to investigate the effect of hyperglycemia on myoblast proliferation, one of the key mechanisms for muscle growth, regeneration and repair.
***Methodology:** Human myoblasts derived from induced pluripotent stem cells (iPSCs) in a defined in vitro system were utilized. Human iPSC - derived myoblasts were seeded on collagen I-coated surfaces in 5.5, 17.5, and 25 mM D-glucose concentrations. Myoblasts in each respective serum-free medium condition were allowed to proliferate for three days before being harvested and quantified. The rate of proliferation in each D-glucose condition was then calculated.
***Results:** It was found that myoblasts in the higher D-glucose conditions proliferated at a much slower rate compared to the physiologic 5.5mM condition. Similar results were obtained from human adult myoblasts derived from human skeletal muscle biopsies.
***Conclusion/Significance:** This finding provides direct evident for the inhibition of muscle growth by hyperglycemia in a human in vitro system. It also suggests that this inhibition is true for muscle growth not only in early age from pluripotent stem cells but also in adult from satellite cells, and underscores the importance of glucose control, from gestation to the adult phenotype. This human-based iPSC-derived skeletal muscle system provides a valuable platform for investigating muscular disease etiology and therapeutic development.

315 - Muscle And Tendon Derived Extracellular Matrix Promotes Expression Of MTJ Specific Integrins In Myoblast Cell Culture

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***Purpose/Objectives:** Musculoskeletal disorders are prevalent and often debilitating. Furthermore, the combined effect of these disorders on both tendon and muscle should be considered when developing testing platforms and therapies. During development, tendon cells provide attracting signals to muscle cells that help anchor muscle to tendon, indicating how this cross-talk is extremely important to the organization.[1] Specifically, at the myotendinous junction (MTJ), muscle cells express integrin proteins like Paxillin that integrate with tendon matrix.[2]Currently *in vitro* models are often composed of one tissue which does not re-capitulate the complexity of tissue interfaces. As a first step toward developing an *in vitro* MTJ model, this research aims to determine the effects of muscle or tendon extracellular matrix on mouse myoblast cells cultured in a hydrogel and develop multi-phasic engineered tissues to create a tunable overlap region between extracellular matrix hydrogels seeded with tendon and muscle cells.
***Methodology:** Tissue specific extracellular matrix hydrogels were derived from porcine muscle and tendon tissues (mECM or tECM). To determine effects of ECM, mouse myoblast cells were suspended in Collagen, mECM or tECM hydrogels and cultured for 5 days in either regular growth media (RGM) or in muscle differentiation media (mDiff). . Relative gene expression of Paxillin was determined through q-PCR. Mouse myoblasts and mouse tendon fibroblasts were suspended in various hydrogels and seeded

on either side of a custom insert and allowed to partially cross-link before removal of the divider. This resulted in biphasic gels with an overlapping region containing both myoblasts and tendon fibroblasts of varying widths depending on time and concentration.
***Results:** Myoblasts encapsulated in 3D hydrogel environments had increased levels of relative paxillin expression in ECM environments compared to the collagen environment. Fold changes of 2-4 occurred in cells within ECM hydrogels cultured in RGM. Lower fold changes occurred in tissues cultured in mDiff, but the rank order of groups was similar. After determining the effect of tissue specific microenvironments on myoblasts, we developed a culture system that examines the interface zone with different microenvironments and different cell types. Gels were allowed to self-assemble separately before divider removal resulted in biphasic gels with varying regions of mixed hydrogels. In all 4 concentrations of hydrogels, time had a significant effect on the width of the overlap region, while the effect of gel concentration was not as pronounced.
***Conclusion/Significance:** Tissue specific derived matrix had an effect on paxillin expression in muscle cells, suggesting that mECM or tECM contained signals that helped promote MTJ expression in myoblast cells. Interestingly, exposure to tECM increased expression of the integrin, suggesting a gradient of microenvironment may be important in MTJ signaling *in vivo.*
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317 - Agent-based Computational Model Predicts Tissue Regeneration Following Volumetric Muscle Loss Injury

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***Purpose/Objectives:** After injury, skeletal muscle possesses the capability to repair and regenerate through the temporally regulated process guided by resident satellite stem cells (SSCs) that activate, differentiate, and repair myofibers [1]. However, after volumetric muscle loss (VML) injuries, the regenerative process fails and is dominated by an inflammatory and fibrotic response [1]. A number of therapeutic approaches have been developed to address the loss of muscle tissue and function from VML injury without a thorough understanding of the cellular mechanisms that drive the injury response [2-4]. Current therapeutics have failed to completely regenerate muscle tissue. To improve treatment, there is a need to better understand the cellular mechanisms associated with VML injury and repair. We present a novel agent-based computational model (ABM) of muscle regeneration following VML injury to address critical knowledge gaps and identify opportunities for improved therapeutics.
***Methodology:** ABMs offer a unique solution for probing mechanisms of VML injury and repair, as they simulate cellular behaviors and illustrate the effects of these behaviors on the entire system. The ABM platform provides an opportunity to compile and synthesize work in the field, as cellular behaviors are prescribed by literature-derived rules for cells (agents). The ABM described herein simulates events during 4-weeks of regeneration after VML injury. At each time step, all agents individually follow a probability-based decision tree to determine the outcome. The ABM includes fibroblast and SSC behaviors, inflammatory cell dynamics, and extracellular matrix properties.
***Results:** The ABM was tuned to replicate the cellular response of fibroblast and SSCs in unrepaired VML injuries. Consistent with the literature, our ABM of unrepaired muscle predicted no muscle fiber regeneration and abundant collagen deposition in the injury defect [1]. We validated the ABM by

simulating the administration of an anti-fibrotic drug (Losartan) following VML injury, and the model predictions were consistent with experimental results [5]. The model was then expanded to simulate the injury response of defects treated with acellular matrix or minced muscle graft. Our model accurately predicted the response of previous reported acellular and cellular therapies [1, 3-4]. This observation provides confidence in the capability of our initial ABM to predict tissue regeneration following VML injury and warrants its further expansion to include other treatments, such as the addition of growth factors with constructs.
***Conclusion/Significance:** Regenerative medicine therapeutics will benefit from improved understanding of the cellular and molecular mechanisms governing failed regeneration in VML injuries. We have created an ABM model of VML injury to more thoroughly investigate the intertwined mechanisms of regeneration in VML injuries. Additionally, the model can be used as a predictive tool to optimize the design of new therapies and to predict the regenerative responses to novel therapeutics. In this scenario, implementation of validated ABMs in the engineering design process can provide more rapid and efficient clinical translation of regenerative therapeutics for VML injuries. 1. Aguilar,CA. *Cell Death Discovery* 2018. 2. Passipieri,JA. *Tissue Eng Part A* 2019. 3. Garg,K. *Cell Tissue Res* 2014. 4. Aurora,A. *Biomaterials* 2015. 5. Garg,K. *J Appl Physiol* 2014.

318 - Local Injections Of Nerve Growth Factor Accelerates Cartilage To Bone Conversion During Fracture Repair

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***Purpose/Objectives:** There are no clinically relevant biological approaches to accelerate fracture healing. Biological approaches to accelerate fracture healing may be an appropriate alternative to bone graft by stimulating endochondral repair. Angiogenic and osteogenic factors to promote fracture repair have been heavily studied with variable results, however, there is limited work exploring neurogenic factors. Therefore, our objective is to establish nerve growth factor's (NGF) therapeutic efficacy in accelerating bone fracture repair by promoting cartilage to bone conversion during endochondral ossification.
***Methodology:** NGF effects on gene expression: NGF (0.5ug in 20uL DMEM) injections into calluses began either 4 or 7 days post-fracture for 3 consecutive days. Controls were injected with DMEM. Fracture calluses were then harvested 24 hours following the final NGF injection and prepared for RT-qPCR analysis of osteogenic and chondrogenic markers. Cartilage explant cultures were obtained by excising cartilage tissue from fracture callus. The cartilage tissue was then minced and cultured in hypertrophic chondrogenic media and stimulated with NGF. RNA was isolated 24 hours after NGF stimulation for RT-qPCR analysis. NGF effects on tissue composition: NGF and control injections were done on days 7-9 post-fracture, and then tibias were harvested 14 days post-fracture. Samples were fixed in paraformaldehyde then decalcified for 14 days at 4^{o}C then processed for paraffin embedding and stereological analysis. In parallel, another set of fractured tibia were similarly fixed then prepared for micro-computed tomography (MicroCT).
***Results:** Local NGF injections to the callus during the cartilaginous phase (days 3-5) promoted collagen I expression but reduced osteogenic markers osteocalcin (oc) and osteopontin (op). Interestingly, local NGF injections during the cartilaginous phase (days 7-9) resulted in an increase of osteogenic markers.

Similarly, explant cultures of hypertrophic cartilage exhibited osteoblast-like gene expression after stimulation with NGF. Histology of fracture calluses 14 days post-fracture showed an increase in newly formed trabecular bone and less cartilage. Quantitative stereology revealed no difference in callus size between treated and controls, however, bone volume was significantly higher after local NGF injections. MicroCT data showed an increase in Bone Volume Fraction and a significant increase in trabecular number and connective density.
***Conclusion/Significance:** Gene expression data of NGF-treated fracture calluses showed a more robust promotion of osteogenic markers in the cohort treated during the cartilaginous phase of endochondral repair (days 7-9 post-fracture). This resulted in increased amount of bone and decreased cartilage 14 days post-fracture compared to the control group. Finally, cartilage ex-plant cultures stimulated with NGF displayed osteogenic-like gene expression. Our data demonstrate that local NGF administration activates osteogenesis in the cartilage callus and that NGF stimulation of hypertrophic cartilage promotes cartilage to bone turnover. Moreover, our data establishes NGF's therapeutic efficacy in accelerating bone fracture repair.

319 - Handheld Bioprinting In Situ GelMA Scaffolds For Treating Volumetric Muscle Loss

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***Purpose/Objectives:** Inadequate reconstructive procedures plague individuals worldwide who suffer from volumetric muscle loss (VML). The geometry of skeletal muscle defects differs greatly case-to-case, therefore, the current standardized surgical procedures limited in their ability to facilitate tissue regeneration and functional recovery. Generating a wound specific scaffold requires an unfeasible amount of time and cost to the patient. Furthermore, it would likely require a second surgery as a result of these limitations. Therefore, we propose a new paradigm that can directly print intricate cell-laden scaffolds in one surgical procedure and alleviate the potential of fibrosis and scaffold rejection.
***Methodology:** GelMA hydrogels were prepared by dissolving medium methacrylated lyophilized GelMA at 5% and 7% (w/v) with 0.067% (w/v) lithium phenyl-2, 4, 6-trimethylbenzoylphosphinate (LAP). Mechanical testing was conducted on an Electroforce 3220 following a procedure adapted from ASTM-2458-05 and ASTM F2255-05. 11-week C57/Bl6 mice with hind-limb VML defects had 7% (w/v) GelMA hydrogel scaffolds *in situ* printed into the defect. Nine million immortalized mouse myoblasts (C2C12) were suspended in 150 µL of Dulbecco's Modified Eagle Medium (DMEM) media and mixed with pre-crosslinked GelMA/LAP solution for biological viability assessment.
***Results:** The adapted handheld printer is capable of printing complex structures and on non-flat surfaces. The adhesion and shear strength of 5% and 7% (w/v) GelMA hydrogels was evaluated and found to be within the mechanical range for skeletal muscle in an animal model [1]. Suitability of the printer's ability to print encapsulated cells was investigated up to 7 days of culture, as well as different

time points up to 24 days of post-differentiation culture. All of these results suggest that the developed easy-to-use handheld bioink printer can print GelMA hydrogels that have the mechanical properties and cell viability to form suitable scaffolds for skeletal muscle tissue.
***Conclusion/Significance:** A handheld bioprinter is developed that has the potential to represent a paradigm shift in trauma care and overcomes various challenges in the treatment of traumatic injuries. The in situ printed scaffolds can be used either in acellular or cellular forms. The adaptable hand-held printer can improve tissue regeneration and reduce the response time and inherently the fibrosis development at the injury site.

320 - Enhancement Of Tissue-engineered Skeletal Muscle Contractility By Isotonic Contraction Culture

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***Purpose/Objectives:** Recent achievements in tissue-engineering research make it viable to construct biological tissue in vitro from living cells and a scaffold material. This technology can be applied not only to patients for reconstructing malfunctioning tissue in vivo but also to other purposes such as three-dimensional tissue model for drug screening in vitro. The skeletal muscle has excellent characteristics of light weight, high flexibility, and remarkable efficiency for energy conversion in comparison with mechanical actuators that require electricity as a power source. Thus, tissue-engineered skeletal muscle has the potential to be flexible and highly efficient actuator. In this study, the tissue-engineered skeletal muscle was cultured under isotonic contractile condition and examined its contractility.
***Methodology:** Two artificial tendons were introduced at both ends of the engineered muscle in order to be fixed to the culture substrate and handled firmly. Elastic fibers and cells inside the porcine aortas were removed by gentle stirring in elastase and ethanol. The remaining collagen tissue was cut into circular forms of 3 mm in diameter with a pinhole of 1 mm in diameter at the center. The two tendons were hold with stainless steel pins placed 12 mm apart on the surface of a silicone sheet paved on a polycarbonate plate in culture dish. The C2C12 myoblasts were embedded in a cold type-I collagen gel at a density of 1.0×10^7 cells/mL. A 100 µL cold suspension was added between and on the surface of the two tendons. After gelation of the cell suspension in an incubator, the construct was placed in growth medium consisting of high-glucose Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and cultured for 2 days. To enhance muscle differentiation, the cell suspension was replaced with a differentiation medium consisting of high-glucose DMEM supplemented with 7% horse serum. The constructs were then cultured with movable pin that was adjustable with contractile force of the tissue-engineered muscle in one end. Three weeks after incubation, the contractile force was measured and the constructs were examined histologically. Control samples were cultured in same period under fixed length condition.
***Results:** The isometric twitch force of the tissue-engineered skeletal muscle cultured in isotonic contractile condition was remarkably (about 3 times) higher than that in fixed length condition. In addition, the number and concentration of myofibers in the constructs were higher. However, the isometric twitch force of the constructs cultured in isotonic contractile condition from 2 weeks after cell seeding was same as that in fixed length condition. The isotonic contractile condition may influence at early stage of myoblast development.
***Conclusion/Significance:** These results suggest that the culture in isotonic contractile condition may enhance early development of tissue-engineered skeletal muscle.

321 - In-situ Printing Of Osteogenic Material For Treatment Of Bone Defects

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***Purpose/Objectives:** Bone defects are caused by trauma, tumor, or infections which affect around 1 million cases per year in the US and 51,000 US soldiers in wars. Existing treatments for these defects are auto and allografts, which are proven to be insufficient. The implantation of scaffolds fabricated using 3D printing with biomimetic mechanical, geometrical, and biological properties has emerged as a promising alternative. However, 3D printing of scaffolds is time-consuming and challenging to use for the treatment of traumatic injuries at the acute stage. In this study, we developed a 3D pen printer which is portable, lightweight, easy-to-use, and can be used in an emergency room of hospitals. Also, it has a camera that applicable it in remote surgeries in the future. Furthermore, we used a composite material of polycaprolactone, hydroxyapatite [1] and zinc oxide which can give proper mechanical and biological properties to the printed bone structure.
***Methodology:** : Materials were characterized through techniques such as Fourier transform infrared spectroscopy (FTIR), energy-dispersive X-ray spectroscopy (EDX), X-ray crystallography, SEM, degradation tests, protein adsorption, and water contact angle. Also, the mechanical properties of the composites were evaluated for compressive, tensile moduli and adhesion to the bone tissue. The composite material was prepared as filaments by mixing and extrusion process. The printability of the material and thermal range of printing were also assessed through filled the bone defects on pig jaw and thermal camera. Furthermore, the biological behavior was investigated through the in vitro culturing of human mesenchymal stem cells (hMSCs) on printed bone composite constructs. Cellular viability and metabolic functionality were tested through Prestoblue and Live/Dead assays. Cell differentiation to osteocytes was confirmed through immunostaining specific physiologic markers and qPCR. Also, cell mineralization and calcium deposition by osteoblast evaluate through Alkaline phosphatase assay and xylenol orange staining assay respectively. Finally, the effectiveness of the in situ printed scaffolds in the treatment of bone defects was investigated in a mouse model of calvarial defect
***Results:** The composites were homogenous and offered mechanical properties comparable to non-load bearing bones and offered proper adhesion to the surrounding tissues. The scaffolds supported cellular proliferation and osteodifferentiation. The animal studies demonstrated that the materials can be printed in situ and attached to the host tissue enabling regeneration of the lost tissue
***Conclusion/Significance:** The use of handheld pen printers for in situ printing of composite scaffolds is a powerful tool for the treatment of bone defects. The chosen composite materials have the proper mechanical and biological properties facilitating tissue regeneration.

323 - Regeneration Of Branched Peripheral Nerve Defects Using Allografts And Localized Immunosuppression

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***Purpose/Objectives:** Segmental peripheral nerve (PN) defects created by injury require a bridging device for effective regeneration to occur. Ineffective regeneration leaves patients with permanent motor disability, sensory aberrations and pain syndromes. Autologous PN grafts are the gold standard for a bridging device and significant effort has gone into trying to develop an effective replacement for autologous PN grafts. However, replacements such as conduits, wraps or decellularized PN allografts are not as effective as PN autografts. Such devices are also not suited to regenerate through complex nerve structures such as branch points.
Allografts freshly harvested from immunological distinct donors are known to already be as or more effective than autografts. Use of allografts is limited by the risks and expense of non-specific systemic immunosuppressive therapy required to prevent graft rejection. The objectives of our study was to develop a method of localized immunosuppression that would promote full regeneration with an allograft that minimized the risks and expense of immunosuppression.
***Methodology:** Experiments were performed in a 2 cm sciatic nerve defect in rats that included the peroneal-tibial branch point. Localized immunosuppression was applied using a poly(ethylene glycol) norbornene (PEGNB) hydrogel that delivered immunosuppressive cells over a span of 14 days. This hydrogel was placed around the allograft at the time of surgery and no additional immunosuppression was administered.
***Results:** Regeneration with PN allografts and localized immunosuppression was equivalent to that of the autograft control. Locally-administered immunosuppressive cells infiltrated the graft, provided immunosuppression and did not engraft elsewhere in the body.
***Conclusion/Significance:** These results show that localized delivery of immunosuppressive cells for PN allografts is an effective new strategy for treating segmental PN defects that can also be used to regenerate complex nerve structures.

324 - Enhancing Neural Network Repair In A 3D Tissue System

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***Purpose/Objectives:** Current efforts to understand peripheral nerve development and repair are largely focused on in vivo studies that employ critically sized neural defects that override the peripheral nervous system's innate capacity for self-repair. Therefore, there is a need to generate in vitro models capable of replicating the complex signaling environment present in nerve repair to increase the understanding of neural network development and behavior in tissue regeneration. In this study, we report on the development of an in vivo relevant neural tissue laceration model and our efforts to understand different mechanisms to enhance repair
***Methodology:** Polydimethylsiloxane frames were fabricated with a central cavity (1 x 0.75 x 0.3 cm). Two 21G needles were inserted into opposite sides of the frame and a 25G needle was threaded through the needles. A collagen gel (6.5 mg/mL) was cast inside the frame, and the steel rod was removed after gelation to create hollow channels. Dorsal root ganglia (DRGs) harvested from prenatal chickens or rats were isolated and seeded within channels. Constructs were cut in half between DRG bodies to generate a laceration injury after four days in culture. Fresh channels (no nerves) were interfaced with the wounded channel and cultured for an additional 2 weeks, fixed, and stained to visualize axonal projections. To modulate the repair response, fresh channels were either coated in different extracellular matrix (ECM) molecules or loaded with a low-density collagen gel (2.5 mg/mL)

with or without brain-derived neurotrophic factor (BDNF).
***Results:** Two weeks after injury, many neurons extended axons into control channels, while axons appeared to be blocked from entering new channels filled with collagen hydrogels. In contrast, numerous defasciculated axons penetrated into BDNF loaded collagen hydrogel samples, demonstrating an enhanced repair response to this treatment condition. This laceration-based method of studying axonal repair mimics events found in neural repair after traumatic injuries where there is the removal of large sections of neural tissue including the supporting cell populations, namely Schwann cells, resulting in a lack of axonal infiltration into channels after injury. BDNF was added to restore soluble chemotactic cues, and facilitated neural growth within a low density collagen gel, demonstrating the ability of axons to penetrate nascent fibrous tissue that is commonly deposited after traumatic injury. Ongoing work is focused on the role of specific ECM molecules within the channels, as well as how inflammation may affect axonal survival and recovery within this laceration model.
***Conclusion/Significance:** We have developed a laceration model of neural repair. Repair was qualitatively reduced when axons migrated through collagen hydrogels, which was significantly enhanced through the addition of BDNF.

325 - Understanding The Role Of Axolotl Retinal Extracellular Matrix In Retinal Regeneration

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***Purpose/Objectives:** Retinal degeneration is the leading cause of blindness worldwide. While retinas of some lower vertebrate (axolotl) do regenerate, the human retina does not. Retinal cells implanted into the subretinal space integrate within host retina, improving visual function in models of retinal degeneration. Cell-based retinal regeneration is limited, however, due to high cell death and low integration following transplantation. The extracellular matrix (ECM) provides cells with specific physical and chemical cues that drive cell behavior during the retinal regeneration process. These observations motivate exploration of the role of retinal ECM components in supporting regenerative cell processes, including survival, migration, differentiation, and integration. The role of axolotl retinal ECM in regeneration has not been explored, yet likely contributes to regenerative potential and could be exploited to enable significant advances in regenerative medicine. To investigate the role of axolotl retinal ECM in providing regeneration-permissive cues, with the ultimate goal of incorporating such cues into a cell delivery vehicle for retinal progenitor cells (RPCs), to enable successful cell transplantation-based retinal regeneration.
***Methodology:** Isolated retinas from axolotl and porcine eyes were utilized for analysis (structure, glycomics and transcriptomics) and cell response experiments either as is, or after processing to obtain samples of soluble factors, insoluble factors, or decellularized matrix.Human RPCs response (attachment and proliferation) on induction with porcine and axolotl retinal factors were studied and related to cell signaling phosphoproteins. Fluorescently labeled porcine RPCs (pRPCs) were seeded on porcine and axolotl retinal explants mimicking retinal transplantation. Cell survival, morphology, migration and integration into the retina were analyzed using fluorescence microscopy imaging.
***Results:** Both the axolotl and porcine retina showed the presence of all characteristic retinal layers. Glycomics and immunohistochemical analyses revealed significant differences in the distinctive sulfation pattern of chondroitin sulphate and spatial organization of hyaluronic acid in porcine and axolotl

retina.Soluble and insoluble factors derived from porcine and axolotl retina did not differentially influence hRPCs initial cell attachment, but did impact cell numbers after 24, 48, and 72 h in culture. pRPCs seeded onto the distal side of a retinal explant in a culture insert exhibit markedly enhanced cell survival and integration - the processes central to regeneration - into the axolotl retina compared to pRPCs in porcine retina. In addition, pRPCs seeded in an axolotl retina explant culture displayed a neuronal morphology with defined, branched processes, relative to the more fibroblastic morphology observed in porcine retina explant culture.
Conclusion/Significance:** The axolotl retina presents cues that promote the attachment, survival, and integration of mammalian retinal progenitor cells, while a mammalian (porcine) retina does not. These results strongly motivate the exploration of lower vertebrate retinal matrix as a biomaterial providing a regeneration permissive microenvironment. ***Acknowledgements: NSF-CBET Grant 1606128 supported this research study.

326 - Incorporation Of Optiprep In Cell Encapsulation Achieves Neutral Buoyancy Without Impacting Cell Viability Or Hydrogel Mechanical Properties

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***Purpose/Objectives:** A promising long-term treatment for type one diabetes (T1D) is clinical islet transplantation (CIT). Cell encapsulation potentially improves CIT outcomes: a semipermeable membrane shields allogeneic or xenogeneic islets from the host immune system. Moreover, a macro-sized device (diameter≥1 mm) affords safe retrieval in cases of treatment cessation or beta-cell replenishment. We aim to engineer a clinically relevant device that addresses the challenges of direct immune recognition and poor oxygenation associated with current cell-based T1D treatments. To provide long-term immuno-protection, a nondegradable encapsulation platform should be used. Based on our previous work, we selected a material that provides *in vitro* and *in vivo* stability, the four-arm amide-linked PEG-norbornene (PEG-4aNB) hydrogel [1]. Problematically, islets settle within the pre-crosslinked macromer solution used for macro-sized hydrogel device fabrication. This indicates the density of the pre-crosslinked solution is less than that of islets. To increase the solution density, the reagent Optiprep [2], which has a higher density than single cells and islets, was incorporated into our encapsulation for neutral buoyancy. We evaluated its effects on PEG-4aNB hydrogel mechanical properties, beta-cell viability, and islet distribution.
***Methodology:** OptiPrep was incorporated into hydrogels of five conditions, ranging from one-sixth to one-half of the total solution volume as OptiPrep. Storage moduli was measured by rheometry. Live/Dead staining and confocal microscopy were used to examine MIN-6 beta-cell viability within these hydrogels, for conditions with one-sixth and one-third of the total volume as OptiPrep. Finally, islets were encapsulated in all five hydrogel conditions and later imaged by confocal microscopy to determine what fraction of OptiPrep in the total solution volume would achieve neutral buoyancy.
***Results:** For the physiologically relevant 5% (weight/volume) PEG-4aNB, storage moduli confirmed no significant difference among all conditions. Control hydrogels valued 231.8±42.5 Pa; all together, hydrogels with OptiPrep averaged 249.5±17.6 Pa. For hydrogels containing one-sixth and one-third of the total volume OptiPrep, Live/Dead staining revealed OptiPrep caused no significant difference in beta-cell viability. After 24 hours, control gels demonstrated 72.7±38.6% viability; gels with OptiPrep

demonstrated 92.3±8.3% viability. Confocal images of hydrogel cross-sections and z-axis projections qualitatively demonstrated that islets best distributed in solution containing one-fifth of the total volume as OptiPrep.
***Conclusion/Significance:** OptiPrep could enhance hydrogel fabrication for cell encapsulation without impacting cell viability or hydrogel mechanical properties. Future work includes functional studies, such as glucose stimulation insulin release, to characterize the potential impact of OptiPrep on nutrient diffusion. Nevertheless, our work suggests OptiPrep safely alters density in macro-sized hydrogels for cell encapsulation. **References:** [1] M. D. Hunckler *et al.*, *Adv. Healthcare Mat.*, 2019. [2] OptiPrep™ Density Gradient Medium - used for cell and subcellular organelle isolation, Sigma-Aldrich, Product # D1556

327 - Macroporous Polymeric Scaffold For Islet Transplantation

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***Purpose/Objectives:** Pancreatic islet transplantation as an established therapeutic modality is limited by insufficient number of donor organs and suboptimal efficiency of islet engraftment in portal vein branches. An alternative, more suitable site providing adequate environment for islets would improve the actual rate of engraftment and long term function of the graft as well. The crucial condition required for this site is a dense network of capillaries, an avoidance of the graft direct contact to the recipient blood and a temperature of body core, which is necessary for the prompt release of insulin from beta cells. The goals of presented work were to test the ability of marginal grafts to correct blood glucose levels, whether transplanted subcutaneously or into the greater omentum.
***Methodology:** Lewis rats (250g, Charles River) served as donors and recipients of pancreatic islets. The diabetes was induced by intraperitoneal injection of streptozotocin (60 mg/kg) after fasting overnight. Only animals demonstrating blood glucose levels over 25 mmol/l were included into experimental groups. The scaffolds were formed from a biocompatible monofilament and shaped to the rounded cylinder under sterile conditions. Together with teflon bar (preventing full obstruction of the scaffold) it was implanted to diabetic Lewis rats subcutaneously on the abdominal wall or into the greater omentum for 7 days. Under skin, the teflon bar was removed and tissue surrounding scaffold continued in healing for next 5 days before islet transplantation. In the omentum, the transplantation was performed in 7thday, immediately after teflon bar removal. Islets were isolated using collagenase digestion and cultivated overnight in tissue culture. Blood glucose levels and body weight were monitored for 100 days, IVGTT was performed in animals 40 days after transplantation.
***Results:** Polymeric macroporous scaffolds did not cause any side reaction of recipient body. In one week after implantation all scaffolds were engrafted into recipient tissues, the removing teflon bars caused vacuum-like sound. In scaffolds located under skin was adequate cavity full of membranes but enabling transplantation of islets.All islets grafts were successfully engrafted in both groups, but the normalisation of blood glucose levels was slower in subcutaneously transplanted islets (1 vs 2 weeks after Tx). IVGTT confirmed full function of all omental grafts 40 days after transplantation, but just partial function in subcutaneous granfts.

***Conclusion/Significance:** The used scaffolds are biocompatible.The greater omentum can be a better site enabling creation of optimal environment for islet graft whether optimal timing is used.
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328 - Breast And Ovarian Cell Behavior In The Presence Of Contraceptive Ingredients

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***Purpose/Objectives:** Breast cancer is the most frequently diagnosed cancer in women and the second leading cause of cancer death among women. [1] Breast cancer growth can be fueled by estrogen, progesterone, and growth hormones such as HER2/neu gene.[1] A further evaluation on the risk of breast cancer due to exposure to oral contraceptives and hormone replacement therapy is highly recommended (Brinton et al., 1998) based on the linkage found among women under the age of 55. The ovaries are the female reproductive glands and are the key source of the female hormones estrogen and progesterone.[2] Ovarian cancer risks increases with the use of estrogen hormone replacement therapy, specifically if used for long-term at high doses.[14] The overall purpose of our study was to evaluate the behavior of breast and ovarian cells in the presence of ethinyl estradiol which is one of the main ingredients in contraceptives.
***Methodology:** CHO and Human Breast (Hs 578 Bst) cells were cultured at 37°C with 5% CO_2. Cells were trypsinized, re suspended and counted using the Nexcelom Cellometer Auto T4, then seeded and treated with different concentrations of Ethinyl Estradiol dissolved in 100% ethanol. Calcein-AM treatment was performed, cells were quantified in the Filtermax F5 Multi Mode microplate reader and Imaged using an Olympus IX71 inverted microscope.
***Results:** It was observed that ethinyl estradiol (EE) impacts the proliferation of both breast and ovarian cells. In general, the initial adhesion of breast cells increased with the addition of EEl by 88% at 20 µg/ml EE, by 108% at 40 µg/ml EE, and 15% at 80 µg/ml EE. Proliferation of breast cells dramatically decreased with the addition of EE, especially at higher concentrations. The greatest reduction in proliferation on Day 7 when compared to the control was observed at 92% for 80 µg/ml EE, followed by 87% reduction for 40 µg/ml EE, and 50% reduction for 20 µg/ml EE. The morphology of the breast cells was slightly altered by Day 3, the cells started to lose their connectivity and appeared to have random shapes, even some round. By Day 7, it was clear that the cells treated with EE had completely lost adhesion to neighboring cells.
The initial adhesion of ovarian cells (CHO) had different effects at each concentration, it was not affected by the addition of 20 µg/ml EE, but it was reduced by 14% with the addition of 40 µg/ml EE and it increased by 48% with the addition of 80 µg/ml EE. Proliferation decreased by 78% with the addition of 80 µg/ml EE, by 47% with the addition of 40 µg/ml EE , and by 56% with the addition of 20 µg/ml EE. The morphology of the ovarian cells was visibly altered by Day 3, the cells started to lose their roundness and became a bit elongated. By Day 7 the morphological changes were even more visible and seemed extremely larger and more intense at the center.

***Conclusion/Significance:** Both cell lines were impacted by the addition of Ethinyl Estradiol, evaluation of Levonorgestrel is ongoing.

329 - Selection Of Interstitial Cystitis Induce Substance, Therapeutic Stem Cell And Cell Injection Route For Treatment Of Ic

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***Purpose/Objectives:** Interstitial cystitis (IC) causes serous urinary symptoms, and there is no fundamental therapy yet. This study was to establish the optimal IC animal model, stem cell source and injection route for effective treatment of IC.
***Methodology:** To establish an animal model, SD rats were treated 5 substances; HCl, acetic acid, cyclophosphamide, lipopolysaccharide or uroplakin (UPK) II. For selection of effective stem cell source, urothelial stem cell (USC), adipose-derived stem cell (ADSC), bone marrow derived stem cell (BMSC), and amniotic fluid-derived stem cell (AFSC) were injected into the established IC model. In order to establish the optimal cell injection route, stem cells were injected into the bladder mucosa directly, intravascularly through the tail vein or transurethrally. The functional and morphological comparisons were performed by cystometry, histological and PCR analysis.
***Results:** In compare of IC induce substances, LPS and UPKII group showed significant shorter voiding interval compared to the other groups. In histologic analysis, UPKII group showed epithelial cell detachment, increased infiltration of mast cells, tissue fibrosis, and expression of IL-1β, -6, MPO, MCP 1, and TLR 2 and 4 compared with the other groups. With these results, uroplakin II was selected as the IC induces substance. In compare of stem cell efficacy, USC showed significantly increased voiding interval, and decreased the inflammatory reaction and the fibrosis. In compare of cell injection route, direction injection into bladder mucosal was prolonged the voiding interval and inhibited morphologic regeneration and inflammation compared to injection via tail vein or urethra.
***Conclusion/Significance:** Subcutaneous injection of UPK II was the most effective substance to induce IC, USC was the effective stem cell source for treat of IC, and direct injection into the bladder mucosa showed functional recovery, inflammatory inhibition and histological regeneration on IC model.

330 - Immuno-reactive Colon Cancer Organoid Model To Examine Immune Checkpoint Blockade Efficacy

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***Purpose/Objectives:** As the number of available immunotherapies for solid tumors increase, their prevalence in the clinic continues to rise as well. While the results are promising, and immunotherapies have benefits over traditional chemotherapeutics, a sizable percentage of patients are non-responders to all types of immunotherapy. These differences in sensitivity can be either innate or acquired. Yet, there has been relatively limited number of in vitro model to assess tumor immune-reactivity that can

be used to further elucidate the mechanisms of immunotherapy resistance in solid tumors. Our goal was to create organoids containing cancer cells from a variety of origins paired with cytotoxic T-cells of various origins to model immunotherapy efficacy. We pursued a system in which we could validate extensive cancer cell killing due to immune cell action in an easily modifiable environment. Our choice of immunotherapies was a paired course of PD-1 and CTLA-4 immune checkpoint inhibitor antibodies, which are used extensively in the clinic for several cancer types. This system could be more generally applied to answer questions about immunotherapy resistance.
***Methodology:** We created extracellular matrix (ECM)-like structures using collagen/hyaluronic acid-based hydrogels into which primary murine tumor cells or tumor cell lines were mixed, along with T-cells. The cancer cells incorporated were either MC-38 OVA cells for a positive T-cell-mediated cytotoxicity control, freshly-isolated CT-26 colon adenocarcinoma cells, or cultured CT-26 cells. The T-cells were either derived from murine lymph nodes or were isolated from the murine tumor infiltrating mononuclear cell population. Organoids were treated with therapeutic equivalent doses of anti-PD-1 and anti-CTLA-4 antibodies. Additional factors such as microbial metabolites were evaluated for their effects on immunotherapy efficacy. We determined tumor cell viability, immune cell identities, and performed immunohistochemistry (IHC) staining on the organoids at 48 and 96 hours post treatment.
***Results:** We have been able to successfully show that the immune checkpoint inhibitor regimens stimulated T-cell-mediated tumor cell killing our organoid model using our viability assays. Checkpoint inhibitor treated samples resulted in proportionally greater loss of viability with increased significance when compared to positive controls ($p<0.01$). The results were corroborated by IHC, showing increased numbers of CD-4^+ T-cells and cytotoxic proteins such as granzyme B in the stimulated samples.
***Conclusion/Significance:** We have created an ex-vivo tumor immune-reactive tumor organoid model for studying immunotherapy. This will allow us to modulate facets of the tumor in the organoid system including cancer type, tumor cell mutations, biochemical signals, and physical properties of the microenvironment. We can then observe the impacts of these changes on immunotherapy efficacy to determine what factors could potentially be contributing to differences in patient sensitivity.

331 - Utilization Of Patient Derived Micro-tumor Constructs For Clinical Testing Of Chemotherapies

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***Purpose/Objectives:** Cases of colorectal cancer are among the top five cancers in both incidence and deaths, with both men and women equally affected. Traditional therapies for colorectal cancer include surgery and radiation, followed by chemotherapy. Despite efforts to improve survival, development of new therapies has been stagnated, with first line combinational therapies having remained the same for years. Over the last several decades, hyperthermic intraperitoneal chemotherapy (HIPEC) or multi-drug cocktails have been utilized for the treatment of advanced metastatic colorectal cancer; however, more analysis is needed to confirm efficacy and understand the mechanisms of effectiveness. To address these shortcomings and improve understanding of therapy efficacy, our group has utilized hydrogel based micro-tumor constructs (μTCs) to analyze the effectiveness of various therapies, both approved and experimental, in single and combinational treatments.
***Methodology:** Colorectal cancer cell lines HCT-116 and Caco-2 were seeded into 2D monolayer and 3D hydrogel culture and were later treated with combinational therapies of 5FU, Oxaliplatin, Irinotecan and Leucovorin to determine the differences of therapeutic efficacy between culture method and cocktail

component administration order and duration. An additional novel fluoropyrimidine chemotherapeutic, CF10, was also tested in substitution of 5FU. Additionally, a clinical procedure-mimicking HIPEC therapy with individual oxaliplatin and mitomycin C treatments was performed in the presence and absence of increased heat. Finally, micro-tumor constructs encapsulating patient cells were developed and characterized for use in HIPEC regimen testing.
***Results:** Combinational therapy was shown to be more effective than individual therapy for several regimens tested, including novel combinations of CF10. CF10 combinations also displayed increased efficacy against comparable 5FU-based combinations for cell lines. HIPEC therapy utilizing high temperatures displayed increased efficacy as compared to treatments performed at body temperatures. Acquisition and treatment of a patient tumor organoids utilizing HIPEC therapy displayed increased efficacy as compared to treatment conditions at body temperature.
***Conclusion/Significance:** Micro-tumor constructs are an effective model for the testing of chemotherapeutic regimens.

332 - Fabricating Colorectal Tumor Microenvironments For In Vivo Implantation

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***Purpose/Objectives:** Colorectal cancer represents a leading cause of cancer-related deaths by claiming 50,000 lives annually. In addition, 100,000 new cases present each year. Although these statistics are improving due to higher rates of early diagnosis, there is still a substantial need for novel therapeutics. The tumor microenvironment (TME) represents an under-studied component of tumor dynamics even though recent advances have shown it as a major contributor to tumor progression. Modeling the TME is a key to developing therapies targeting non-traditional vectors such as stromal cells or extracellular matrix (ECM). Current techniques for incorporating the TME into research are based around *in vitro* culture, including our own previous work. In this study, we present a platform for implanting bioengineered TME organoids into a mouse model for studying tumor progression and dynamics.
***Methodology:** Briefly, we use HCT-116 spheroids embedded into collagen-based hydrogels as the tumor and TME compartments of our model, respectively, and the TMEs are organized by including stromal cells or left unorganized without cells.
***Results:** Overall, organized TMEs - with parallel, widened, and lengthened collagen fibers - tend to reduce tumor cell growth and migration while unorganized TMEs promote cell spreading and, potentially, aggressive phenotype. Specifically, cancer cells in the unorganized TME undergo epithelial-to-mesenchymal transition (EMT). Consistent with previous results, we have found that TME organization can drive tumor growth and more aggressive phenotypes. When implanting pre-formed TMEs into a mouse model, this dynamic is conserved, indicating the physiologic relevance of the observed phenomenon.
***Conclusion/Significance:** In all, our results demonstrate a connection between ECM fiber organization which may govern EMT and, possibly, the reverse, mesenchymal-to-epithelial transition (MET), that often occurs in metastatic colorectal cancer. However, future analysis into the mechanisms by which changes in ECM architecture affect cancer cells is necessary to fully understand this dynamic. Further, these findings are relevant for therapeutic research targeting the stroma and TME as well as potentially presenting organization as a metric for diagnosis, prognosis, and chemotherapeutic response of tumors.

333 - Development Of A Novel Villi-Like Micropatterned Porous Membrane For Intestinal Magnesium And Calcium Uptake Studies

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***Purpose/Objectives:** Intestinal enterocytes are key players in the absorption of magnesium (Mg^{2+}) and calcium (Ca^{2+}). Understanding the exact molecular mechanisms by which their absorption behavior is regulated could greatly improve treatment strategies aiming to stimulate intestinal absorption in diseases with $Mg^{2+}$and/or Ca^{2+}deficiency. However, such studies are hampered by the lack of *in vitro* intestinal cell models mimicking the mechanical and physiological properties of the gut.
***Methodology:** In this study we develop an *in vitro* gut model based on porous micropatterned membranes with villi-like surface topography and mechanical properties closely mimicking intestinal tissue. The new membranes are prepared via phase separation micromolding using poly-ε-caprolactone/poly-lactic-glycolic acid (PCL/PLGA) polymer blend, a technique which allows tailoring of the membrane surface topography combined with membrane porosity and interconnectivity which are important parameters during *in vitro* transport studies.
***Results:** The culture of Caco-2 cells on these micropatterned membranes facilitate cellular differentiation similar to native enterocytes. In fact, cells form a brush border of microvilli with spatial differences in morphology and tight junction formation along the villous-base axis. Moreover, cells cultured on our micropatterned membranesshow a 2-fold increased alkaline phosphataseactivity at the end of differentiation. Finally, we demonstrate that cells cultured on our micropatterned membranes have a 4- and 1.5-fold increased uptake of ^{25}Mg and ^{45}Ca, respectively, compared to non-patterned membranes.
***Conclusion/Significance:** Our data indicate that mimicking the 3D geometry of the gut is very important for improving the physiological relevance of *in vitro* gut models. In the future, our micropatterned membranes with segment-specific geometries, in combination with isotopic measurements, would be applied to perform detailed studies of ion uptake and transport studies.

334 - Bioengineering Of Skeletal Muscle Tissue With Innervation Capability For Accelerated Restoration Of Pelvic Floor Muscle Function

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***Purpose/Objectives:** Pelvic floor muscles are the layer of muscles that support pelvic organs, including the bladder, bowel and uterus. Damages in the pelvic muscles often cause dysfunction of the entire pelvic urogenital system. Current treatments for pelvic muscle injury include physical therapy, autologous muscle flap transfer, and surgical interventions using synthetic and biological materials. However, none has entirely addressed the issues associated with long-term restoration of normal anatomy and function in the injured pelvic floor muscle system. In this regard, bioengineering of functional muscle tissue constructs has been proposed as a solution to this unmet medical need.

However, the current muscle engineering techniques are limited by the ability to build sizable constructs with timely innervation for successful grafting. More importantly, engineered muscle tissue must be integrated with the host nervous system (innervation) following implantation. Failure of innervation results in muscle tissue atrophy. It is evident that engineered muscle constructs, consisting of well-aligned muscle fibers and innervation-accelerating structures, are required to achieve functional restoration of large pelvic floor muscle injury. To this end, this study aims to fabricate and optimize 3-D bioprinted skeletal muscle constructs with innervation capability to repair pelvic floor muscle injuries.
***Methodology:** Bioprinted skeletal muscle construct which mimics native skeletal muscle organization were fabricated by using 'bioink' formulation of fibrin-based hydrogel containing human muscle progenitor cells (hMPCs). Agrin was treated in the bioprinted muscle constructs to pre-form neuromuscular junctions (NMJs) on muscle fibers *in vitro* and to accelerate host nerve integration *in vivo*. The muscle tissue formation and innervation capacity of the bioprinted muscle construct was investigated in a pelvic floor muscle injury model in rats.
***Results:** Our results demonstrated that the bioprinted cells in the engineered skeletal muscle constructs were able to maintain their viability and form muscle fibers *in vitro* and *in vivo*. We successfully achieved the pre-patterning of acetylcholine receptors (AChRs) on the bioprinted muscle constructs by treating agrin. Electromyography (EMG) analysis showed that the bioprinted muscle construct implantation results in functional improvement as compared with the defect only, gel only and bioprinted muscle construct without agrin implantation groups at 8 weeks (ANOVA and Tukey test, n = 3, $P<0.05$).
***Conclusion/Significance:** These results indicate that the bioprinted engineered muscle construct with innerThese results indicate that the bioprinted engineered muscle construct with innervation capability can restore pelvic floor muscle anatomy and function.

335 - Determining The Effect Of Extracellular Matrix From Human Healthy And Diseased On Cell Phenotype

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***Purpose/Objectives:** My aimed at understanding the interaction between cells and extracellular matrix (ECM) and to use this knowledge for improving regenerative interventions. ECMs are a diverse collection of three-dimensional structures that lie underneath and between most tissues. It is widely believed that the ECM in compromised organs is damaged and is not able anymore to provide the proper signals to the cells in its environment, which leads to phenotypic change, cell damage and eventually to a vicious circle of destruction. However, except for tumors where this relationship has been studied extensively, there is little experimental evidence about the role of ECM changes for cell phenotypes in solid organs. Since the earliest changes in tissue damage is often ECM remodeling, we believe that it is important to understand its interaction with cells in order to device strategies to promote cell and tissue repair and regeneration. It is currently not well understood whether and to what extend the ECM in damaged solid organs directly affects the phenotype of surrounding cells. The poor scientific evidence is partially due to a lack of adequate models to observe the interaction between cells and ECM, to study the factors involved in this interaction and the differences between disease-associated and healthy ECM. There are models that decipher architectural or chemical cues that influence cell identity, mainly based on strictly

controlled surfaces, polymers and their chemical functionalization. While these de-complexing models are excellently suited to study in detail singular aspects of cell-matrix interaction, and allow for the computational fusion of the data, this approach relies on information from more complex systems, such as native ECM. A second part of the model (besides ECM) is the cells used to study the interaction. Frequently used cell lines are highly standardized, but have little similarity with the native cells especially with regards to phenotype plasticity. Primary cells, on the other hand, show high donor dependent variability.
***Methodology:** 1. Decellularization of both healthy and disease Kidney slices 2: Differentiation of iPSC to renal cells (Established protocol in our lab) 3: Antibodies to determine cell phenotype by high content screening and 3D-imaging 4: RNA extraction and PCR analysis 5: RNAseq or array transcriptome analysis on selected conditions for a more detailed analysis.
***Results:** We have established protocols to derive native and disease kidney ECM and also established protocols to derive renal cell types from (iPSC). This allows the generation of ECM and relevant cell types to study the effects of ECM on cellular phenotypes.The recellularization work is in progress and hope until December, I will have a sufficient data to share with the confrence community.
***Conclusion/Significance:** The work directly targets the focus topic of cell matrix interaction. Using a novel model system, it will investigate the impact of ECM from compromised solid organs on cellular phenotypes and compare this with the effect of ECM from healthy organs. The model organ is the kidney, an organ where disease manifests strongly in ECM changes and where cells undergo epithelial-mesenchymal transitions and de-differentiation during disease progression.

336 - Thermoresponsive Nanogels For Drug Delivery Applications In Tissue Engineering

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***Purpose/Objectives:** Delivery of osteoinductive factors remains a major challenge in bone tissue engineering to promote the regeneration and repair of damaged tissues. The use of select bone morphogenetic proteins has shown great therapeutic potential however, supraphysiological concentrations are needed to obtain the desired effects. In contrast, the use of small molecules to induce bone regeneration is promising but has yet to be fully explored. For this purpose, thermoresponsive nanogels were synthesized, characterized, and analyzed for cytotoxicity for the delivery of osteoinductive small molecule analogs for tissue engineering applications.
***Methodology:** Nanogels composed of N-isopropylacrylamide (NIPAM) and methacrylic acid (MAA) were synthesized via solution polymerization using N,N'-methylenebisacrylamide as the crosslinking agent. The resulting nanogels were dialyzed, dried via lyophilization, and stored at room temperature. Nanogel size, polydispersity, and zeta potential was determined in either water or PBS using a Zetasizer Nano ZS system. The cytocompatibility of the synthesized nanogels was evaluated using L929 murine fibroblasts. Cells were seeded in Dulbecco's Modified Eagle's Medium (DMEM) without phenol red supplemented with 2% FBS, 1% L-glutamine, and 1% penicillin−streptomycin solution and were maintained under standard cell culture conditions (i.e., a humidified, 37°C, 5% CO_2/95% air environment) for 24 hours. Then, the supernatant medium was removed and replaced with nanogels at various concentrations in the aforementioned medium (0.125 - 1 mg/mL). After 24 hours of cell exposure to the nanogels, the CellTiter 96 Aqueous One Solution Cell Proliferation assay (Promega Corporation, Madison WI) was used to determine cell viability. Results were compared to that of the

respective controls. Loading of the cationic small molecule, methylene blue, into synthesized nanogels was performed via equilibrium partitioning. The small molecule was dissolved in either water or 0.1X PBS at 0.5 mg/mL and combined with nanogels (1:1 wt/vol) suspended in either ultrapure water or 0.1X PBS at 0.5 mg/mL. The loading took place for 1 hour at room temperature after which the nanogel/small molecule mixture was centrifuged. The supernatant was used to determine the concentration of unloaded small molecule using ultraviolet-visible spectroscopy.
***Results:** The solution polymerization method used resulted in the synthesis of monodisperse, anionic, P(NIPAM-co-MAA) nanogels. When L929 murine fibroblasts were exposed to various concentrations of the synthesized nanogels for 24 hours, the cells remained viable (>90%) under all conditions tested. Furthermore, loading of methylene blue in the synthesized nanogels in 0.1X PBS was significantly higher compared to that observed in water.
***Conclusion/Significance:** The present study demonstrated that the synthesized nanogels have no cytotoxic effects and that ionic strength affects the loading efficiency of methelyne blue. Further optimization of nanogel formulation and loading conditions to enhance osteoinductuve small molecule analog loading efficiency is currently under investigation.

337 - Materials And Process Parameters For 3D Printed Microsphere Based Tissue Engineering Scaffold

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***Purpose/Objectives:** Microspheres can be utilized to deliver factors and drugs in a therapy that aims at tissue regeneration in a controlled manner by encapsulating such substances during the production of the microspheres. The timely release of the substances is controlled by the material's degradation time in the body and the shell thickness of the microspheres. Gradient scaffolds consisting of multiple types of microspheres can release different factors at different sites of the scaffold. Current microsphere scaffold production methods, however, cannot address the need for internal architectures to meet specific requirements in different scaffold regions, e.g., certain mechanical properties, porosities, or even focal material selection for enhanced tissue regeneration. Producing scaffolds with defined internal architectures and tailored placing of such microspheres via 3D printing methods allows for high spatial control of the materials and properties within the scaffolds. However, microspheres don't allow for 3D printing unless a binding phase is added that keeps the strands of material in place. This binding phase, in turn, can negatively affect the further processing steps, such as sintering, necessary to create a stable construct that can be implanted into load bearing sites.
***Methodology:** The scaffold material was produced from a binding phase such as carboxymethyl cellulose highly loaded with monodispers poly(lactic-co-glycolic acid) (PLGA) microspheres of selected sizes for the different experimental lines. This material mix had to provide the necessary viscosity to be extruded from a syringe tip while still allowing the fabrication of mechanically stable wet green bodies and not hindering a subsequent sintering process with subcritical CO_2 to enhance mechanical properties. The created pastes were characterized for their flow behavior in a printing process. Then 3D constructs were printed with the materials using a syringe extruding process. Various CO_2 sintering parameters were applied to the scaffolds and their influence on the mechanical properties determined via mechanical testing.
***Results:** The concentration of the binding phase, the ratio of binding phase and scaffold material as well as microsphere size had significant influence on the printing process. Within the tested range, the

binding phase did not prevent sintering of the microspheres. Sintering parameters, however, have to be adhered to to create scaffolds with the desired stability and to prevent excessive sintering that potentially would damage encapsulated factors.
***Conclusion/Significance:** In a next step, multi-material scaffolds will be investigated regarding the applicability of the determined sintering parameters to find a balance for all materials, and their performance in tissue regeneration. The aim is to create scaffolds with gradients in stiffness and materials that can be used in bone/cartilage interface regions in tissue engineering applications. These materials and optimized process parameters enable the production of scaffolds with local definitions for mechanical properties, porosity, focal placement of phases, and controlled release of encapsulated factors.

338 - Modeling Tumor-associated Macrophages In Osteosarcoma Within Mechanically Tunable 3D Microenvironments

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***Purpose/Objectives:** Osteosarcoma is the most common primary tumor of the bone. Due to the large heterogeneity in osteosarcoma tumors and the lack of preclinical models that accurately mimic the native tumor microenvironment, progress in bringing new therapies to the clinic has stagnated in recent decades. Tumor-associated macrophages (TAMs) are the most prevalent immune cells in osteosarcoma and perform various functions that promote tumor progression. Using tissue engineering techniques, our laboratory has developed a mechanically tunable, three-dimensional model of osteosarcoma suitable for studying the interactions between osteosarcoma cells and TAMs. With this model, we can investigate the synergistic effects of substrate stiffness and TAM polarization in contributing to an aggressive osteosarcoma phenotype and the reciprocal effects of osteosarcoma conditioning plus microenvironment architecture and mechanical properties on TAM phenotype.
***Methodology:** Polymeric scaffolds composed of poly(ε-caprolactone) (PCL) and gelatin microfibers with varying PCL:gelatin mass ratios (100:0, 80:20, 50:50, 20:80, and 0:100) to modulate scaffold mechanical properties were fabricated by electrospinning. THP-1 human monocytes were seeded into well plates or scaffolds and differentiated into M0 macrophages by incubation in RPMI 1640 media containing phorbol 12-myristate 13-acetate (PMA), followed recovery in PMA-free media. Cell morphology and distribution was assessed by confocal microscopy. THP-1 phenotype was assessed by flow cytometry, and proliferation over 7 days post-differentiation was measured by DNA content.
***Results:** Upon exposure to PMA, THP-1s cultured in well plates and PCL scaffolds became adherent to culture substrates, and confocal microscopy revealed that cells were distributed throughout scaffolds. Flow cytometry showed that cells exhibited higher forward and side scatter profiles than undifferentiated THP-1 cells. Additionally, PMA treatment ceased THP-1 proliferation, with no significant difference in cell number between 1, 3, 5, and 7 days after differentiation with PMA in monolayer culture. Studies are currently ongoing to elucidate the combined roles of substrate architecture and the progression of macrophage infiltration on osteosarcoma phenotype. THP-1s differentiated into M0 macrophages will be co-cultured with MG-63 osteosarcoma cells in well plates or electrospun scaffolds to achieve osteosarcoma/TAM ratios that mimic initial macrophage recruitment, partial infiltration, or full infiltration. Following culture for up to 7 days, samples will be assessed for overall proliferation,

proliferation of each cell population, phenotype and proteomic profiling, and cytokine secretion. We anticipate that the results of these studies will shed light on the mechanisms of mechanotransduction, including YAP/TAZ activation, that mediate immune regulation in osteosarcoma and contribute to osteosarcoma cell phenotype, differentiation, and resistance to therapies.
***Conclusion/Significance:** Tissue engineered models of tumors featuring three-dimensional architecture, tunable mechanical properties, and multiple cell populations are versatile tools that have the potential to overcome the limitations of *in vitro* monolayer cultures and the costs of *in vivo* models currently used in the study of cancer biology and preclinical drug screens. With interest growing in developing therapies to target TAMs, study of these cells in controlled yet physiologically relevant microenvironments is warranted, especially in highly heterogeneous cancers like osteosarcoma that remain a challenge to treat in patients.

339 - In Vitro Evaluation Of A Polymeric Matrix Of Transdermal Application Of Polyethyleneglycol-Chitosan As A Possible Dressing For Wound Closure

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***Purpose/Objectives:** *The aim of this study was* to evaluate the *in vitro* properties of a poliethylenglycol-chitosan (PEG-CH) matrix of transdermal application for wound closure.
***Methodology:** Hydrogels were synthesized by photopolymerization of a homogeneous solution of polyethylene glycol diacrylate (10% in distilled water), chitosan (1% in acetic acid, Sigma-Aldrich, USA) and 2,2'-Azobis (2-methylpropionamidine) dihydrochloride) (2%; Sigma-Aldrich, USA) in a polytetrafluoroethylene mold (Escudero Castellanos, Ocampo-Garcia, Dominguez-Garcia, Flores Estrada and Flores-Merino, 2016).Hydrogels with different proportions of chitosan (0.05, 0.1, 1, 2 and 4 %) were evaluated. *The following in vitro test were carried out*: 1) wound healing assay (Swiss 3T3 fibroblasts). 2) Hemolysis test with 5 % human erythrocytes exposed to each hydrogel (1 h at 37°C. 3)Antibacterial activity, against *E.coli* ($1.5x10^8$ UFC/mL) and 4) swelling behavior by gravimetric measurement (Grada, Otero-Vinas, Prieto-Castrillo, Obagi and Falanga, 2018).
***Results:** PEG-CH hydrogels are not hemolytic (less than 5% of hemolysis). Despite they did not show antibacterial activity, there was no growth of bacteria in the hydrogels matrix. Finally, in the wound healing assay, hydrogels stimulated cell growth more quickly.
***Conclusion/Significance:** Polyethylene glycol-chitosan hydrogels could be used as a dressing, mainly in those patients whose wounds do not heal quickly.

342 - The Viability Of Stem Cells In A Selection Of Scaffolds For Use In Regenerative Medicine

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***Purpose/Objectives:** Polycaprolactone (PCL), alginate and gelatin are polymers widely used to produce scaffolds in regenerative medicine. In this study, the viability of human stem cells (SC) from human

exfoliated deciduous teeth were evaluated after their cultivation in a variety of scaffolds. The scaffolds of these polymers were produced in a number of ways, alone and associated. With synthetic PCL, polymers were produced by 3D printing and electrospinning. With the natural biomaterials alginate and gelatin, hydrogels were produced.
***Methodology:** The cells were seeded at a 400,000/well density in a 48-well tissue culture plates in the following groups: directly in the well (G1); 3D printed scaffolds of PCL (G2); electrospun scaffolds of PCL (G3); scaffolds composed of 0.1mL of 1% alginate (G4); G4 associated with 1.5% gelatin (G5); G2 associated with G3 (G6); association of G3 and G4 (G7); G2 associated with G4 (G8) and association of G2, G3 and G4 (G9). Cell viability was assessed based on a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 3 days of cultivation. Fluorescence microscopy confirmed the presence of cells.
***Results:** Results of cell viability showed statistical significance between the cells cultivated in the well plate (control group) and the majority of the groups tested. The average ± standard deviation values of absorbance obtained related to cell viability were 0.215 ± 0.030 for the G1 (control); 0.073 ± 0.023 ($p<0.01$) for the G2; 0.413 ± 0.046 ($p<0.01$) for the G3; 0.359 ± 0.078 ($p<0.01$) for the G4; 0.321 ± 0.027 ($p<0.01$) for the G5; 0.360 ± 0.014 ($p<0.01$) for the G6; 0.352 ± 0.029 ($p<0.01$) for the G7; 0.276 ± 0.037 (not significative) for the G8; and 0.335 ± 0.063 ($p<0.01$) for the G9.
***Conclusion/Significance:** As the 3D printed structures (G2) have large pores, it is possible that the cells escaped from these scaffolds to the well, thus less cell viability was achieved in this group. The combination of the 3D printing with G6, G8, and G9 statistically enhanced the proliferation of cells because the electrospun and hydrogel scaffolds filled the gaps. The lower cell viability in the well compared with the scaffolds can be attributed to the reduced space for cell proliferation on the 2D culture. A statistical difference was not found between the G4 (non-adherent hydrogels) and G5 (presence of adherent gelatin) tested, thus demonstrating that cell viability was not influenced by adherence to the proposed systems. Therefore, it is possible to affirm that the association of 3D printing with hydrogel and/or electrospinning was beneficial for the viability of the cultivated cells. The presence of the 3D printed PCL scaffolds fabricated by melted filaments facilitated the production of support with greater mechanical stability. Hence, the combined scaffolds obtained with the combination of biopolymers and synthetic polymers obtained by electrospinning and rapid prototyping could be used to produce 3D scaffolds for tissue regeneration with better features than those produced only by printing.
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343 - THE USE OF DECELLULARIZED HEPATIC SCAFFOLD IN A RAT HETEROTOPIC AUXILIARY LIVER TRANSPLANTATION MODEL

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***Purpose/Objectives:** Severe hepatic failure is the result of long-term liver injury. Liver transplantation is the only efficient treatment but is currently limited by organ shortage. In this context, the creation of a bio-artificial liver might solve this clinical problem. The aim of this work was to optimize a surgical heterotopic auxiliary liver transplantation (HALT) technique to transplant decellularized liver scaffolds (DLS).

***Methodology:** Donor Wistar rats were heparinized, anesthetized and then submitted to transverse abdominal incision. The portal vein (PV) was separated and cannulated, Teflon tube was attached to the inferior vena cava (IVC) and fixed. Then the superior vena cava (SVC) was clamped. After removal, the donor liver was perfused via PV with 5 ml of custodiol solution (organ storage solution) supplemented with 10 µl of heparin (50 IU/ml) and placed in a cold saline bath for 5 minutes. Then, the livers were transferred to be perfused through portal vein using an infusion pump at 3 ml/min with water for 1 hour followed by 1% Triton X-100 for 2 hours and SDS 1% for 24h. After total decellularization, livers were washed with distilled H_2O for 2 days. To improve the DLS shape and the HALT performance, the matrix was perfused with 10 ml of rat blood diluted in 40 ml of custodiol solution for 1 hour and washed with 3 ml of phosphate saline solution (PBS). Then, the matrix was analyzed by H&E staining. Adult Wistar rats were used as recipients for HALT. Briefly, animals were heparinized and anesthetized by vaporized isoflurane. Then, under anesthesia animals were submitted to transverse abdominal incision and the left arterial and renal vein were clamped. After that, the nephrectomy of the left-side kidney was performed. The PV and IVC of the DLS were anastomosed to the recipient rats' left arterial and renal vein in an end-to-end anastomosis, respectively. Finally, the recipients' abdomens were closed.
***Results:** After blood perfusion, the DLS shape resembled a native liver and H&E staining showed some cell retention on vessels of DLS. Active blood flow within the DLS was observed indicating that the PV and IVC of the scaffolds were able to sustain the arterial blood pressure when the circulation was re-established. No internal bleeding was observed prior to the rat abdomen closure. H&E staining and immunofluorescence analysis showed some cell retention on vessels and confirm CD31+ endothelial cells and Ki67+ cells 8 hours post HALT.
***Conclusion/Significance:** Here, we performed a HALT surgical technique to transplant DLS. Also, our findings suggest that DLS were repopulated by recipient rat cells allowing cell attachment and proliferation. The decellularization protocol preserved the structural characteristics of the native microvascular network allowing blood perfusion and the transplantation. In conclusion, our method is a promising approach of transplanting an engineered liver tissue for application in the hepatic regenerative medicine.

345 - Embossed Membrane With Guided-vessel Pattern For 3d Vascularized Tissue Engineering

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***Purpose/Objectives:** In order to reconstruct a laminated tissue using an electrospinning membrane, it must be vascularized for maintaining the transport of nutrients and oxygen to the cells. In this study, embossed membranes were generated with a vacuum forming method so that guided vascular structures would be generated.
***Methodology:** Two- or six- embossed membranes seeded with endothelial cells and pericytes were stacked and subcutaneously implanted into the mouse. The implanted membrane structures were harvested with the surrounding tissue 1, 2, 4, or 8 weeks after implantation.
***Results:** It was confirmed that blood vessels were formed in the laminated structure in the group in which the blood vessel pattern was embossed after 2 weeks of transplantation. It was possible to observe the formation of blood vessels along with the embossed blood vessel pattern in the structure of the embossing membrane laminated with 4 weeks and 8 weeks. VEGF and ANG-1 were more highly expressed in the vascularized structures. Therefore, it is shown that a structure capable of producing a

desired blood vessel shape through an electrospinning membrane embossed with a blood vessel pattern can be manufactured.
***Conclusion/Significance:** Thus, it has been shown that a variety of structures can be manufactured using the electrospun membrane in tissue engineering era.

346 - Mimicking Mesenchymal Stem Cell Condensation To Promote Articular Cartilage Regeneration

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***Purpose/Objectives:** Damage to the osteochondral unit, which includes both bone and cartilage tissue, is difficult to resolve because unlike bone, which possesses high regenerative capacity, cartilage tissue is avascular and possesses very low cell density. This often leads to bone regeneration without regeneration of the cartilage tissue, which can be painful and results in a lower quality of life for patients. In addition, current therapeutic interventions such as matrix-assisted chondrocyte implantation and microfracture have been shown to result in fibrous cartilage formation and thus fail to provide long term solutions for patients. To address this tissue engineering challenge, a thiolated gelatin microparticle (GMP-SH) platform has been developed that when combined with mesenchymal stem cells (MSCs), can mimic a phenomenon observed during embryonic cartilage development, MSC condensation. MSC condensation is the precursor to chondrogenic differentiation of MSCs and ultimately the deposition of the cartilage extracellular matrix. By mimicking this phenomenon, it is proposed that cartilage tissue can be regenerated even in adults.
***Methodology:** The thiolated gelatin is formed by reacting 1 g gelatin type B with 20, 60, or 80 mg Traut's reagent overnight under aqueous conditions to add thiols to the gelatin in a dose-dependent manner, followed by dialysis and lyophilization to obtain the final product. GMP-SHs are then formed through a water-in-oil emulsion-based technique according to a well-established protocol, with untreated gelatin as the control microparticle group (GMP), and crosslinked with glutaraldehyde before sieving to isolate particles within the 50-100 µm range when dry. The particles are then tested for thiol content via Ellman's Assay, degree of crosslinking via 2,4,6-Trinitrobenzenesulfonic acid Assay, and swelling in phosphate-buffered saline (PBS). The degradation profile of the microparticles is assessed in PBS and PBS containing collagenase. The GMP-SHs are then examined for their cytocompatibility via direct-contact assay with fibroblasts and MSCs.
***Results:** The thiolated particles demonstrate dose-dependent thiol content before and after crosslinking depending on the amount of Traut's reagent reacted with the gelatin precursor. The GMP-SHs also exhibit expected crosslinking and swelling behavior with greater percent lysine loss observed in the crosslinking of GMP-SHs compared to GMP controls and the swelling ratio matching what has previously been reported in the literature. The particles are then combined with a bifunctional linker before tethering of the MSCs to the GMP-SHs to create the mimic MSC condensate.
***Conclusion/Significance:** In conclusion, a modular, thiol-functionalized platform has been developed that can be used to chemically tether MSCs to the microparticle surface to mimic MSC condensation. The system shows promise as a tissue engineering solution given its ease of fabrication and the ability to be combined with existing osteochondral tissue engineering scaffolds.

347 - Development Of ECM Dermal Filler For Soft Tissue Augmentation

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***Purpose/Objectives:** Numerous efforts are being made to develop an ideal dermal filler that should be bio-compatibility, non-immunogenicity, long-lasting and biodegradable without a toxic secretion. Biomaterials of dermal fillers are hyaluronic acid filler, calcium filler, PMMA filler and collagen filler depending on the ingredient. Although hyaluronic acid (HA) is most widely used, it has shortages such as short shelf life and low mechanical strength compare to extracellular matrix (ECM). The cartilage ECM composed of collagen type II, proteoglycans, glycosaminoglycans (GAGs) and in a minor part with glycoproteins [1]. In this study, we developed a cartilage ECM injectable filler capable of improving biocompatibility and longevity compared with hyaluronic acid (HA) fillers.
***Methodology:** The ECM hydrogel was cross-linked by the reaction of N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) for mechanical enhancement. Prepared ECM filler was compared with cross-linked HA by butanediol diglycidyle ether (BDDE), which is the most widely used natural polymers for dermal filler. To evaluate the physical property and biocompatibility, we observed the morphology using low vacuum scanning electron microscopy (LV-SEM), assessed the rheological behavior using a rheometer, and evaluated the in vitro cell viability using Live/Dead assay. Cellular responds were also evaluated by the Ez-Cytox assay. Finally to evaluate the *in vivo* biocompatibility, durability and collagen synthesis we studied animal tests.
***Results:** In the results, cross-linked ECM filler showed fiber bundle structures unlike HA filler from the LV-SEM images. It showed the much higher storage modulus compared to HA filler from rheology analysis. Regarding the cellular responds, cell viability and proliferation rate in the ECM fillers showed similar results to HA filler. And animal tests showed long lasting than HA filler and increased collagen and elastin synthesis in surrounding tissues.
***Conclusion/Significance:** Collectively the articular cartilage ECM hydrogel has great potential as a dermal filler to improve the biophysical and biological performance.

348 - Chitooligosaccharides Integrated Fish Collagen/alginate Based Scaffold For Skin Tissue Regeneration

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***Purpose/Objectives:** An emerging paradigm in wound healing techniques is that a tissue-engineered skin substitute offers an alternative approach to create functional skin tissue. Thus, the intention of the present study is to elucidate the synergetic effect of marine derived fish collagen (FC), alginate (SA), and different molecular weight (MW) ranges of Chitooligosaccharides (COS) (1-3 kDa, 3-5 kDa, 5-10 kDa, >10 kDa) in scaffolds and to identify the ideal COS fraction containing fish collagen/Alginate **(**FCA) scaffolds for the application of skin tissue regeneration.
***Methodology:** FC was isolated from marine fish (*Paralichthys olivaceus*) collected from South Korean sea and characterized against porcine skin type I collagen. Scaffold consisted of FC and SA was initially fabricated by blending and freeze drying technique and subsequently COSs were cross-linked at the EDC involved two step cross-linking treatment. The effects of cross-linking were analyzed by Fourier

transform infrared spectroscopy. The structural morphology was examined by field emission scanning electron microscope. Mechanical properties and in vitro degradation and biological properties of fabricated scaffold were conducted to identify ideal scaffold.
***Results:** The results indicate that the homogeneous materials blending and cross-linking intensity were dependent on the molecular weights of COSs. The highly interconnected porous architecture with 160-260 µm pore size and over 90% porosity and COS's MW driven swelling and retention capacity, tensile property and in vitro biodegradation behavior guaranteed the FCA/COS scaffolds for skin tissue engineering application. Improvement of these properties enhanced the cytocompatibility of all the scaffolds, and scaffold containing 1-3 kDa COS (FCA/COS1) promoted significant growth of normal human dermal fibroblasts-neonatal (NHDF-neo) demonstrating (FCA/COS1) as tremendous tissue engineered substitute for skin tissue regeneration application.
***Conclusion/Significance:** The comprehensive physicochemical, mechanical, and biological properties demonstrate that the FC is an excellent alternative collagen source for tissue engineering, and composite scaffold enhances the unique properties of tissue engineered substitute. Overall, results suggest that the fabricated FCA/COS1 scaffold is a superior candidate for skin tissue engineering application.

349 - Biocompatible Hydrogels For High-precision Inkjet Technology

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***Purpose/Objectives:**
Biocompatible hydrogels have great potential for 3D tissue modeling. These materials are centrally important for inkjet bioprinting, which is one of the powerful technologies for precise 3D tissue construction. Hydrogels for inkjet bioprinting must have the two critical properties, biocompatibility and quick gelation. However, it has been difficult to find hydrogel materials with these properties.Recent studies developed two types of modified PEG molecules that function as hydrogel precursors[1]. One is Tetra-PEG that has an electrophilic functional group, and the other is Tetra-PEG with a nucleophilic functional group. The two materials are both soluble but mixing of the two types triggers quick gelation. In the present study, we evaluated bio-inks composed of live cells and the two types of modified Tetra-PEG molecules.
***Methodology:**
We used two bio-inks. One bio-ink contained one type of the modified Tetra-PEG, the other bio-ink contained the other type. In addition, the adhesive factor fibrinogen was added to one bio-ink, and another adhesive factor thrombin was added to the other ink. Both bio-inks contained the same type of cells. We constructed 3D models by ejecting the bio-inks using the piezoelectric inkjet bioprinter that can precisely eject cells with high viability[2]. Typically, we fabricated alternating layers of the two bio-inks. The models were cultured for one week.
***Results:**
First, we built 3D models with a thickness of up to 1 mm. Cells in the models exhibited adhesion and spreading and the cell viability was >90% even after one week.Second, we constructed 4-layer models, approximately 200 µm thick, composed of cells labeled with two different fluorescent dyes. Cross section images were acquired with confocal laser scanning microscopy. Each layer was approximately 50 µm thick, and the ejected cells rarely contaminated adjacent layers.

The high viability observed in our models suggests that the hydrogel materials are highly biocompatible supporting cell adhesion and providing biomolecule permeability. The stability of the layered structure suggests that the gelation is sufficiently fast to immobilize cells at the target position and thereby enabling 3D structure formation.

***Conclusion/Significance:**

Our results demonstrate that the modified Tetra-PEG hydrogels have properties suitable for 3D bioprinting. Biofabrication techniques based on inkjet bioprinting and the hydrogel materials may enable the construction of complex biological systems.

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350 - Isolation And Identification Of Osteogenic Peptide From Marine Teleost Bone And Application Of The Bone Regenerative Scaffold By 3d Print System

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***Purpose/Objectives:** Fish bone is considered as a valuable source of an essential element for the health promoting components. In the fish bone, osteogenesis regulating peptides were found and these peptides play functionally similar roles as mammalian. Although these peptides promote osteoblastic differentiation, such bioactivity has been rarely reported for peptides isolated from fish bone. The objective of this study was to investigate the effects of a bioactive peptide isolated from the fish bone, Johnius belengerii, on the osteoblastic differentiation of MC3T3-E1 and to fabricate bioactive scaffolds for bone tissue regeneration.

***Methodology:** After mincing *J. belengerii* bone, the fish bone powder was digested with pepsin in 5% acetic acid solution for 48 h. After enzyme inactivation, the hydrolysate was removed insoluble matters by filtration and centrifugation. The soluble hydrolysate was lyophilized to obtain a fish bone extract (FBE). Post consecutive purification by liquid chromatography, a potent osteogenic peptide, composed of 3 amino acids was identified and determined osteogenic activities and signaling pathways on the MC3T3-E1 pre-osteoblasts. To fabricate osteogenic composites with FBE and PCL, we used a computer controlled tree-axis robot system supplemented with a dispenser. The fabricated scaffolds were investigated characterization by FT-IR, universal testing machine (UTM), and scanning electron microscope. Additionally, we investigated the osteogenic activity of the fabricated scaffolds by p-NPP assay, alizarin red staining, EDS spectroscopy and RT-PCR.

***Results:** Post consecutive purification by liquid chromatography, a potent osteogenic peptide, composed of 3 amino acids, Lys-Ser-Ala (KSA, MW: 304.17 Da), was identified and determined that the isolated fractions and the peptide induced osteoblast differentiation. We then designed and fabricated 3D interconnected porous scaffolds consisting of polycaprolactone (PCL) and fish bone extract (FBE) by 3D printing and coating process for bone tissue regeneration. The FBE was provided good cell attachment and osteoblast differentiation activities. FBE/PCL scaffolds were characterized mechanical properties and determined that these scaffolds induced osteoblast differentiation activities.

***Conclusion/Significance:** We determined that cellular activities were promoted by exposing the

scaffolds to bioactive FBE. It was observed that the PCL/FBE significantly enhanced cell proliferation and calcium deposition. The results indicate that 3D scaffolds coated with FBE solution have outstanding biological properties, strongly promote calcium deposition, and increase the expression of mRNA levels compared to the PCL.

351 - 3D PCL/fish Collagen Composite Scaffolds Incorporating Osteogenic Abalone Protein Hydrolysates For Potential Bone Regeneration: In Vitro And In Vivo Studies

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***Purpose/Objectives:** An effective induction of bone formation with proper materials is an utmost research theme in bone tissue engineering field. Hence, 3-dimensional (3D) printing technology is an emerging field of study that can merge materials with architecture of tissue to produce structurally similar native bone tissue
***Methodology:** Here, we fabricated a novel method to combine PCL scaffolds with fish collagen (Col) or sodium alginate (Sa) and the osteogenic abalone intestine gastro-intestinal digests (AIGIDs) from *Haliotis discus hannai* for bone regeneration. And then, mouse mesenchymal stem cells (MSCs) were seeded onto the fabricated scaffolds. After *in vitro* culturing, the cell proliferation, ALP activity, and mineralization were investigated.
***Results:** The results indicated that the ALP activity and mineralization in PCL/AIGIDs/Col was higher than that of the other scaffolds. In addition, RT PCR analysis found that ALP and OSC mRNA levels were significantly higher in the PCL/AIGIDs/Col. In the *in vivo* experiment, the two fabricated scaffolds were inserted in a rabbit tibia defect. PCL/AIGIDs/Col group exhibited strong osteoinduction capability in the rabbit tibia defect model.
***Conclusion/Significance:** These prompted biological responses *in vitro* and *in vivo* suggest that the PCL/AIGIDs/Col scaffold provide adequate mechanical strength and are thus promising material to induce bone formation in bone tissue regeneration.

352 - Magnetic SPCL Scaffolds Exposed To Magnetic Field As Cell Signaling Instructive Platforms For Tendon Tissue Engineering

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***Purpose/Objectives:** As mechanosensitive tissues, mechano conditioning of tendons is essential and a critical parameter of the native environment for tissue homeostasis and proper functioning. Thus, to

induce the regenerative process of tendon, biophysical stimuli are imperative with the right combination of tendon mimetic scaffolds and triggered cells. In a previous study, we reported that targeted activin receptor type IIA (ActRIIA) in human adipose stem cells (hASCs), using magnetic nanoparticles (MNPs) and magnetic stimulation, promotes phosphorylation of Smad2/3 inducing tenogenic transcriptional responses, through TGF-β/Smad2/3 signaling cascade [1]. In this work, we aim to access the synergistic effect of hASCs labeled with anti-ActRIIA functionalized MNPs seeded onto magnetic scaffolds and exposed to an alternate magnetic field (AMF) provided by a custom-designed solenoid device, specifically assessing the expression of tendon related genes and proteins.
***Methodology:** Aligned magnetic fibrous scaffolds were fabricated by 3D printing made of a blend of starch and polycaprolactone (SPCL) and incorporating iron oxide MNPs (magSPCL) [2]. After a labeling period of 30min with functionalized MNPs, hASCs were seeded onto magSPCL scaffolds and AMF exposed for 10min twice a week (F=50Hz, B=1mT) for up to 21 days of culture.
***Results:** MTS assay and dsDNA quantification were performed to assess hASCs viability and proliferation, respectively, and the tenogenic commitment assessed by real time RT-PCR, immunocytochemistry and Sirius Red/Fast Green Collagen assay. The combination of magnetic field with magnetic responsive scaffolds stimulates overall expression of tendon related genes and the deposition of tenomodulin protein by hASCs, in comparison to non-stimulated conditions.
***Conclusion/Significance:** The engineered magnetically-responsive system seems to influence the mechanosensing reply of hASCs towards tenogenesis, holding therapeutic promise.
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353 - A Smoothened Agonist Non-phospholipid Liposome Immobilized Hybrid Scaffold To Facilitate Bone Regeneration

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***Purpose/Objectives:** Hybrid scaffolds possess the ability to carry bioactive agents that promote tissue regeneration and therapeutic activity. Especially, these have great potential when combined with liposomal formulations, which can be amplified their benefits for biomedical applications. In the present study, we fabricated a hybrid scaffold with smoothened agonist non-phospholipid liposome (SmAL) to improve bone repair via bio-inspired dopamine chemistry.
***Methodology:** Poly(lactic-co-glycolic acid) (PLGA) 3D scaffolds were employed to provide space and structure, biocompatibility, and mechanical properties for tissue support and regeneration. Thereafter, PLGA 3D scaffolds were immobilized with SmAL. This fabrication was performed by brief incubation of the scaffolds in a weak alkaline solution of dopamine, followed by secondary incubation with desired SmALs. The osteogenic potential was evaluated via alkaline phosphatase, alizarin red S staining, and quantitative real-time polymerase chain reaction (qRT-PCR) with bone marrow stromal cells (BMSCs).
***Results:** The SmAL-immobilized PLGA 3D scaffold (SmAL-Sc) provided highly biocompatible property and supported cellular attachment and proliferation without cytotoxicity in BMSCs. In addition, SmAL-Sc

significantly enhanced the osteogenic differentiation of stem cells corresponding to the osteoinductive activity of SmALs. Alkaline phosphatase and alizarin red S staining and colorimetric assay demonstrated synergistic smoothened activation of two potent smoothened agonists. As a consequence, the expression of osteogenesis-related genes (*ALP*, *Runx-2*, and *OCN*) and hedgehog pathway-related genes (*PTCH* and *Gli1*) from the SmAL-Sc were significantly up-regulated compared to controls.
***Conclusion/Significance:** These results indicated that a new hybrid scaffold has simple fabrication method and remarkable pro-osteogenic efficacy via the combination effect of two smoothened agonist, OHC and Pur. Thus, the scaffold will serve an intriguing way to guide bone tissue regeneration.

354 - Novel Zeolite-loaded Polymer Promotes A Pro-osteogenic, M2-like Macrophage Phenotype And Osteoblast Maturation

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***Purpose/Objectives:** The present study compared the macrophage response to PEEK versus a novel, zeolite-laden polymer, ZFuze, in vitro and in vivo. Further, the ability of PEEK and ZFuze to promote osteoblast maturation was interrogated directly, as well as indirectly through modulation of macrophage phenotype.
***Methodology:** Primary murine bone marrow derived macrophages or human osteoblast-like cells were exposed to either PEEK or ZFuze scaffolds for 24 hours. Phenotypes were determined for exposed cells using quantitative polymerase chain reaction, protein expression using immunofluorescence, and secreted products were characterized using ELISAs. In addition, macrophages that had been exposed to either PEEK or ZFuze were allowed to condition media for 5 hours, after which the media was collected and added to a culture of MG-63 cells for 24h. MG-63 cell phenotype was evaluated as described above.
***Results:** Results suggest that PEEK promotes a pro-inflammatory macrophage phenotype characterized by robust expression of iNOS, IL1β, and IL-6, whereas ZFuze promoted a more pro-osteogenic macrophage phenotype with expression of Fizz1, an M2-like marker. Importantly, ZFuze-stimulated macrophages, but not PEEK-stimulated macrophages, supported osteoblast maturation, as determined through increased expression of osteoblast maturation markers including BMPs and osteocalcin.
***Conclusion/Significance:** Results of the present study suggest that ZFuze, but not PEEK, promotes osteointegration indirectly through modulation of macrophage phenotype and directly promotes osteoblast maturation. The immunomodulatory properties of ZFuze could be attributed to the presence of surface-bound metal cations.

355 - Micropatterned Fibrin Scaffolds For Cardiomyocyte Alignment

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***Purpose/Objectives:** A major cause of heart failure is a myocardial infarction (MI), which leads to cardiomyocyte death, and, ultimately, scar tissue formation.[1]The heart has limited capacity to restore cardiac function after an MI because cardiomyocytes have little ability to regenerate. In an effort to restore functional tissue properties, cardiac patches can be used replace diseased tissue; however, they

only provide passive structural support. We developed a composite fibrin hydrogel and microthread patch with a custom tension culture system to create an anisotopically aligned cardiac patch; however, the nuclear alignment of the cardiomyocytes, and the contractility of the scaffold was limited.[2]Because cellular alignment is critical for restoring contractile function, we investigated the use of topographical cues to align cells and create the organized hierarchy necessary to produce a mature cardiomyocyte phenotype and to maximize the cellular conduction velocity. To increase cardiomyocyte alignment, we propose to micropattern fibrin scaffolds and create topographical cues that will produce highly aligned, contractile cardiac tissue analogs.
***Methodology:** A photomask of aligned channels was used to create a master silicon wafer with channels of 10, 15, 25, 50, or 100 µm in width and 20 µm in depth. Polydimethylsiloxane (PDMS) negative replicates were created from the wafer, and 1×1 cm fibrin scaffolds, composed of 1.6 U/mL thrombin and 10 mg/mL fibrinogen in DMEM, were polymerized on the surfaces of the micropatterned replicates. C2C12 mouse myoblasts were seeded on the scaffold at a density of 25,000 cells per surface and allowed to attach for 2 hours at 37°C, 5% CO_2. Hydrogels were then rinsed and incubated overnight. Samples were fixed with 4% paraformaldehyde and stained with phalloidin and Hoechst. Nuclear alignment of the was measured in ImageJ with respect to the direction of the channel. Values were binned in 15° increments and plotted as a histogram to present the distribution of nuclear orientations for each channel morphology.
***Results:** Cells in micropatterned channels with 10, 15, 25, or 50 µm widths exhibited nuclear alignment values that were comparable to native cardiac tissue (46% of cells within 15° of the long axis).[2]In contrast,100 µm channels displayed 35±11 % of cells within 15° of the long axis. These were compared to the unpatterned control, where 16±2% of the cells were aligned with the channels, suggesting random orientation.
***Conclusion/Significance:** In this study, we demonstrated the ability to create topographical cues on fibrin scaffolds to direct myoblast orientation. By measuring the nuclear alignment of initial cell attachment, the influence of topographical cues as opposed to cell-cell signaling can be used to determine the role of micropatterning. Future studies will focus on assessing the attachment of cardiomyocytes to achieve an aligned, hierarchal cellular organization with physiologically relevant contractile strains.
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356 - Utilizing Air-gap Electrospinning To Wrap Polymer Nanofibers Around Bioactive Glass Microfibers For Regenerating Critical Size Cortical Bone Defects

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***Purpose/Objectives:** Critical size bone defects are classified by sufficient bone loss preventing natural healing within a patient's lifetime, clinically occurring when the length of missing bone is twice the diameter. Most research focuses on the inner trabecular bone, which makes up more bulk volume than cortical bone, but the physiology of the outer cortical bone is necessary for proper healing. Osteons are the repeating unit throughout cortical bone, consisting of canals filled with blood and nerve vessels surrounded by concentric lamella of hydroxyapatite-containing collagen fibers, which provides mechanical strength. The osteogenic cells residing in this bone matrix are responsible for the constant

remodeling of the bone, by either adding more bone matrix to strengthen the bone or by removing bone matrix to release the mineral ions into the blood for further use. Creating a biodegradable scaffold that mimics the osteon structure is crucial for optimizing cellular infiltration and ultimately the replacement of the scaffold with native cortical bone. Recent studies have shown that highly aligned nanofibers increased directional cell migration, fabricated by electrospinning across two positively charged plates called air-gap electrospinning.
***Methodology:** In this study, a modified air-gap electrospinning setup was exploited to continuously wrap highly aligned polycaprolactone polymer nanofibers around individual 1393 bioactive glass microfibers, resulting in a synthetic structure similar to osteons. By varying the disc diameter, charge, rotation speed, and the location on the glass fiber, polymer fibers that were wrapped at angles between 10-30^{o} to the glass fiber were chosen, although fibers wrapped as large as 45-90^{o} were possible. Mechanical testing included flexure strength, porosity, and glass conversion, among other methods to determine the effects of wrapping the polymer fibers around the glass. Different *in vitro* tests confirmed interactions between the cells and the scaffold.
***Results:** There was no change in the fiber diameter, although the porosity decreased from 90% to 80% due to consolidation of the aligned fibers during wrapping, but is still sufficient for ion exchange. Encapsulating the glass with polymer nanofibers caused viscoelastic deformation during 3-point bending, as opposed to typical brittle glass fracture. Scaffold degradation was not cytotoxic. The aligned polymer fibers demonstrated unidirectional cell migration, increasing the cell movement speed. The ion release and thus the rate of bioactive glass conversion was similar between the bare and wrapped glass, resulting in hollow hydroxyapatite fibers.
***Conclusion/Significance:** This composite aims to lengthen the possible distance for growing cortical bone in critical size bone defects through two processes. First, the converted, hollow hydroxyapatite fiber is expected to allow blood vessel ingrowth to supply nutrients to the cells. Simultaneously, the aligned polymer fibers will stimulate cell migration along the scaffold while allowing infiltration radially into the scaffold. The scaffold should eventually be replaced by native tissue. When used in conjunction with a trabecular scaffold, the combination should allow for total regrowth of the critical size bone defects.

357 - Transcatheter Tissue-Engineered Vein Valve

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***Purpose/Objectives:** About 2.5 million people in the U.S. have regurgitant vein valves[1] with approximately 20% severe cases that could benefit from a vein valve replacement. The strongest evidence that a prosthetic vein valve can be beneficial was shown by Pavcnik *et al.* in a clinical trial of 15 patients that showed at 3 months after implantation, 80% of the patients had clinical improvement in symptoms[2]. However, in the venous system all attempts with bioprosthetic valve have failed in long-term performance.
***Methodology:** We have developed a novel engineered tissue vein valve with embedded nitinol stent for transcatheter delivery[3]. The tissue is developed from fibroblast-seeded fibrin gel remodeled by the cells and then decellularized to give an acellular collagenous matrix. The matrix covers the nitinol stent in developed vein valve. Vein valves were tested in vitro for hydrodynamic properties, stent-tissue integration and eventually were implanted into the ovine iliac vein to assess valve function and

regeneration.
***Results:** The hydrodynamic testing of the valve demonstrated it to have forward pressure drops in the range of 2-4 mmHg, low closing volume, and nil regurgitation. Further hydrodynamic tests were performed after crimping and then again after 1 million cycle durability testing, showing no degradation of valve performance or any visual damage to the matrix. The valve held over 600 mmHg back-pressure after the durability testing, ensuring the valve would withstand pressure spikes well outside of the normal *in vivo* range.
At 2 weeks after implantation, angiography showed functional leaflets; however, function was lost by 8 weeks. The explanted valve showed leaflets adhered to the valve root with histology showing thin recellularized leaflets, including endothelialization, without thrombosis.
***Conclusion/Significance:** The tissue engineered vein valve developed demonstrate acceptable hydrodynamic properties and promising outcome in preliminary implant with regenerative capacity.
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359 - Poly (n-isopropylacrylamide)-collagen Hydrogels For Tunable Drug Delivery

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Efficacious drug delivery systems necessitate strict regulation of the distribution of therapeutic molecules in a biocompatible manner. Multi-component hydrogels have been used increasingly for this purpose. Poly(*N*-isopropylacrylamide) (PNIPAM), a thermo-responsive polymer, has gained attention because of its physiologically relevant hydrophilic-to-hydrophobic switch above 32°C, which leads to a change in the retention of water and a macroscopic volume change. The properties (i.e. swellability, solute release, and biocompatibility) of PNIPAM hydrogels can be tailored by altering the hydrophilic-hydrophobic balance of the polymer network via integration of copolymers with different physical and chemical properties. In this study, PNIPAM-collagen hydrogels were synthesized at varying mass ratios to investigate the effects of hydrogel compoosition on hydrogel swellability, model drug delivery, and biocompatibility. Analysis of hydrogel microstructure using scanning electron microscopy revealed that increasing collagen content disrupted the organization of PNIPAM networks and resulted in markedly diverse matrix porosities and morphologies. At 37°C, all hydrogels exhibited a collapse in volume; however, increased collagen content decreased both the rate of syneresis and equilibrium swelling ratios. Increased collagen content also attenuated the release rate of bovine serum albumin (BSA) several hours after temperature-dependent changes in polymer conformation, as well as over the duration of 30 days. Biocompatibility studies showed that the presence collagen increases fibroblast viability on the hydrogel network. Collectively, these results suggest that hybrid PNIPAM-collagen hydrogels can be a useful tool in developing tunable drug delivery systems and may be broadly applied to other synthetic/natural polymer blends.

360 - Enhanced Hemocompatibility And Antibacterial Activity On Titania Nanotubes With Tanfloc/Heparin Polyelectrolyte Multilayers

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Introduction: Thrombus formation is still a major concern for cardiovascular implants. Another barrier for successful implementation of medical devices is bacterial infection. Therefore, it is vital to develop multifunctional surfaces, that can prevent both clotting formation and bloodstream infections. A promising technique investigated to enhance compatibility of biomaterials is modifying surfaces with polyelectrolyte multilayers (PEMs), using naturally derived polymers. Heparin is highly negative charged polymer and a natural glycosaminoglycan, which in the body prevents blood coagulation. Tanfloc is a natural polymer found in plants and is a hydrophilic amino-functionalized tannin. Recently, it has attracted considerable interest due to its antifouling and antithrombogenic properties.
Methods: In this work, tanfloc/heparin PEMs were developed on titanium. First, the surface topography was modified by making titania nanotubes (NT) via anodization process. Then the surface was coated using tanfloc and chitosan as polycations, and heparin and hyaluronan acid as polyanions. These surfaces were incubated in S. aureus and P. aeruginosa, and bacteria adhesion and morphology on different surfaces were studied. These surfaces were also incubated in blood plasma, and the platelet adhesion and activation were investigated. Clotting time studies were also performed.
Results: After 24 hrs, tanfloc/heparin PEMs on NT decreased the adhesion and proliferation of S. aureus and P. aeruginosa bacteria. These surfaces also reduced platelet adhesion and activation, as well as delayed the clotting time on the surfaces.
Discussion and Conclusion: The novel surfaces developed showed enhanced antithrombogenic and anti-microbial activities, thus be a promising approach to improve tissue compatibility on cardiovascular implants.

361 - Polyethylene Glycol Based Vocal Fold Augmentation Material

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Objective : long- term vocal fold augmentation materials must be biostable, remain at the site of injection. In this study, we explore polyethylene glycol(PEG) as vocal augmentation material. Method : Sixteen rabbit were injected in the lateral aspect of their right thyroarytenoid muscle with o.3cc of PEG or calcium hydroxyapatite(CaHA) 1 week after Right recurrent laryngeal nerve section.Endoscopic examinations were conducted at 4,8 and 16weeks after injection. Then larynges were harvested, High speed video camera examination was done for comparing degree of medialization of paralyzed vocal fold between PEG and CaHA. we also conduct histological and immunohistochemical examination Result: Endoscopic examinations showed that. Injected PEG remained well in the paralyzed vocal fold

without significant decrease after 16 weeks than CaHA group. there were no lnflammatory response both group, glottic gaps were decreased in PEG group. Histology performed 16 weeks after injection. Injected PEG remained at the injected site, whereas CaHA migrated surround area.There were no inflammatory response in the surround tissue nearby injected site .The residual volume was larger in the PEG group than CaHA group Conclusion : PEG could be new candidate for vocal fold injection material

363 - Gelatin/hydroxyapatite Microsphere Embedded Stromal Cell-derived Factor-1 Promoting Alveolar Bone Regeneration

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Periodontitis is a severe inflammatory condition of the periodontium that progressively damages the soft tissue and destroys the alveolar bone, which supports the teeth. This type of bone loss is naturally irreversible due to the limited reparability of teeth. Advances in tissue engineering have provided the means for the effective regeneration of osseous defects with suitable dental implants or tissue-engineered constructs. Bone regeneration can be accomplished through three different mechanisms: osteogenesis, osteoinduction, and osteoconduction. However, alloplastic grafts are typically only osteoconductive. Therefore, the development of a novel alloplastic graft with osteoinduction for alveolar bone regeneration is necessary. In this study, gelatin/hydroxyapatite microsphere (GHM-S) was used to embed stromal cell-derived factor-1, a well-characterized chemokine for attracting stem cells and thus a strong candidate for promoting regeneration. Synthesized hydroxyapatite was found to be similar to hydroxyapatite of natural bone tissue. The organic and inorganic components of the GHM-S was measured by TGA analysis, which confirmed that part of the GHM-S was similar to the natural bone tissue. SDF-1 protein could be released in a controlled manner from the GHM-S to form a concentration gradient in a culture environment to attract the stem cell migration. Gene expression and protein expression indicated that stem cells could differentiate or develop into preosteoblasts. The *in vivo*bone formation was assessed in rats with alveolar bone defects and bone augmentation by GHM-S was confirmed by micro-CT imaging and histological examination. Our findings demonstrated that the GHM-S is a feasible approach for alveolar bone regeneration.

364 - Surface-functionalized Electrospun Polycaprolactone/Fibrinogen Scaffold Enhances Stability Of Platelet-derived Growth Factor

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Traditional wound dressings act as a physical barrier to prevent debris from entering the wound and help control hemostasis. However, most dressings do not enhance the healing process. Platelet-derived growth factor (PDGF) plays an integral role in all aspects of the wound healing process. Therefore, to improve maxillofacial soft tissue regeneration after craniofacial trauma, we have modified polycaprolactone (PCL) fibrinogen electrospun scaffolds to allow for controlled release of PDGF by means of covalent attachment with heparin groups. To stabilize PDGF, PCL was functionalized to express heparin through the use of 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide hydrochloride and N-

hydroxysulfosuccinimide. Scaffolds were characterized using vibrational spectroscopy, electron microscopy, and pycnometry to study internal composition, topography, and porosity. Successful heparin incorporation was confirmed via toluidine blue heparin binding assays. Additionally, PDGF release was evaluated over 3 days at 37 °C. Spectral data and heparin binding assay results demonstrated a clear increase in heparin activity on the surface of the scaffolds as a function of introduced reactive groups indicating control over the extent of the functionalization process. Further, the extent of PCL functionalization was shown to influence the PDGF release profile. This study demonstrates the viability of heparinization as a means of improving PDGF stability and tailoring release kinetics. Future studies will investigate cell viability and migration as well bioactivity over 90 days.

365 - Effect Of Basement Membrane Structure On Cell Behavior And Function On Decellularized Aorta

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Decellularized tissues which is the extracellular matrix (ECM) have attracted a great deal of attention because of its high biocompatibility and functionality. The mechanism of biocompatibility and functionality of decellularized tissue are poorly understood. Previously, we reported that decellularized aorta prepared by high-hydrostatic pressurization (HHP) showed early endothelialization and anti-thrombogenicity[1)]. We proposed the hypothesis that these phenomena occur on the HHP treated aorta since its basement membrane structure and function is maintained [2)]. In this study, using human umbilical vein endothelial cells (HUVECs) [1, 3)], the cell proliferation and gene expressions were evaluated to clarify the cell behavior and physiological function on aorta with various basement membrane structures.
H-E staining showed that the basement membrane structure was well maintained, and the nuclei were removed in HHP treated aorta. The surface structure of heated HHP aorta was relatively maintained and that of SDS treated aorta was destroyed. The calcein-AM staining revealed that the HUVEC were well-aligned and proliferated on HHP aorta, whereas the HUVEC hardly attached on SDS aorta. The expression of MMP2 involved in breakdown of ECM was low and that of TIMP1, inhibitor of MMP2, is upregulated on HUVEC in HHP aorta. From these results, it is suggested that the maintenance of basement membrane is one of the key factors for functional expression of recellularized HUVEC.
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366 - Modulating Neutrophil NETosis By Template-Released Manuka Honey

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***Purpose/Objectives:** Neutrophils can extrude their DNA into large, fibrous neutrophil extracellular traps (NETs) through an antimicrobial form of programmed cell death, called NETosis. When dysregulated, this process is implicated in thrombosis, fibrosis, and chronic inflammation. Additionally, electrospun polydioxanone (PDO) tissue engineering templates with small fiber diameters (SD, 0.25-0.45 µm diameters) significantly up-regulates the release of NETs compared to large fiber diameters (LD, 1.0-2.0 µm diameters), which precludes cell infiltration and initiates fibrosis, thus inhibiting tissue regeneration. In this study, we investigated the ability of Manuka honey to attenuate NETosis when used as a template additive. Manuka honey, a natural wound additive with anti-inflammatory and pro-healing properties, was recently shown to reduce neutrophil pro-inflammatory responses. Therefore, we hypothesized that incorporating Manuka honey into the electrospun PDO templates would reduce biomaterial-induced NETosis.
***Methodology:** PDO and Manuka honey were dissolved in 1,1,1,3,3,3 hexafluoro-2-propanol and electrospun with optimized parameters to generate SD templates and LD templates with 0.1, 1, or 10% v/v Manuka honey. To characterize honey release, 8 mm diameter punches of the templates (n = 4) were incubated in phosphate-buffered saline (PBS) for 21 days, and the absorbance of the supernatant was measured at 340 nm and normalized to the mass of the template. After characterizing honey release, fresh peripheral blood human neutrophils were seeded on the templates (n = 4) for 3 and 6 hours, fixed with formaldehyde, and stained with SYTOX orange and DAPI to visualize extracellular DNA and intact nuclei, respectively. Images of the templates were then analyzed with a custom MATLAB program to measure the percent surface area covered by NETs.
***Results:** The honey release study indicates that 60-80% of the incorporated honey is released as a burst within 24 hours, with consistent low-level release over the following 20-day period. Additionally, the honey release was proportional to the amount of incorporated honey with the SD templates releasing more honey than the LD templates. These data suggest most of the honey is released within the first 24 hours, which is the ideal timeframe to combat NETosis. Furthermore, the preliminary results from the NETosis experiment revealed that honey incorporation in the SD templates decreased NET release with 10% honey decreasing the area covered by NETs from 11 ± 8% to 5 ± 5%. Contrasting on the LD templates, honey incorporation increased NETosis slightly from 1 ± 2% area on the 0% honey template to 3 ± 2% area on the 10% honey template. Together, these results suggest that Manuka honey may be a useful additive for decreasing neutrophil NETosis on electrospun tissue engineering templates.
***Conclusion/Significance:** Biomaterial-induced NETosis may significantly impair tissue regeneration by increasing thrombosis, fibrosis, and inflammation. In this study, we demonstrated that it is possible to electrospin PDO templates with Manuka honey to attenuate NET release *in vitro* on SD templates. By decreasing NETosis, Manuka honey has the potential to improve tissue-template integration and regeneration. Future work includes identifying the intracellular mechanism by which Manuka honey reduces NETosis and verifying the work *in vivo* with a rat model.

367 - MICRONIZED LIPOASPIRATE SHOWS IN VITRO POTENTIAL FOR WOUND HEALING

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***Purpose/Objectives:** In our recent study we demonstrated that micronized lipoaspirate act as a natural scaffold for stem cells and give rise to spontaneous cell outgrowth, together with a paracrine effect on resident cells that overcome the limitations of the extensive time and manipulation necessary for the

use of MSCs therapy in tissue regeneration. On the basis of the results obtained on cartilage regeneration, in this work tried to demonstrate *in vitro* if micro-fragmented lipoaspirate can promote and accelerate wound healing.
***Methodology:** Human micronized lipoaspirate as residual material destined to be disposed of after liposuction was obtained from healthy female patients at Image Istitute. The outgrowth study was done in a 3D collagen matrix culture; the cytokines released by lipoaspirate in culture media to understand its paracrine effect were assessed using Human Cytokine Antibody Array-Membrane. Proliferation migration and contraction tests on fibroblasts and keratinocites have been done using MRC5 and HaCat cell lines using respectively ATP quantification kit, scratch Wound healing and collagen contraction assay. Antibacterial effect of lipoaspirate was tested using agar test and E-coli count. The data were analyzed statistically using the SPSS for Windows software.
***Results:** We showed the ability of resident cells in lipoaspirate to grow out from microfragmented adipose tissue and their capability of repopulating an organ culture of human skin. At the same time it was shown its paracrine effect on the proliferation rate, migration and contraction of fibroblasts and keratinocytes and the release of trophic/reparative cytokine from lipoaspirate together with its antibacterial activity.
***Conclusion/Significance:**

368 - Antiseptic Wound Dressings Based On Bacterial Nanocellulose

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***Purpose/Objectives:** Bacterial nanocellulose (BNC) is matrix has made its way into the clinic as a novel wound dressing. In addition to properties such as high tear resistance and flexibility, it also consists of nearly 95% water. This hydrophilicity allows the material to absorb and release water-soluble substances. We tested how well BNC can absorb antiseptic substances and whether it can be used to create antimicrobial wound dressings.
***Methodology:** BNC-based wound dressings were placed in four different antiseptic substances and punch biopsies were taken at different time points. The concentration of the antiseptic agent contained therein was analysed. Two PHMB-containing solutions, one octenidine-containing and one povidone-iodine-containing solution were tested. In addition, the release of the substances from the punch biopsies were examined. In order to test the size-dependence of uptake and release of molecules in BNC dressings, the same experiments were also carried out with FITC-labelled dextrans in different sizes. Finally, the antimicrobial activity of BNC wound dressings loaded with antiseptic solutions were tested against Staphylococcus aureus
***Results:** With the use of FITC-labeled dextranes, a size-dependent uptake and release of molecules into and out of the BNC dressing was observed. Large molecules were absorbed more slowly and released more slowly than smaller ones. All antiseptic solutions showed excellent uptake into the BNC as well as

release. Especially the PHMB- and octenidine-containing solutions already showed high values after only 30 minutes. The overall achieved concentrations were all highly effective against Staphylococcus aureus and were all higher than the minimal bactericidal concentration against MRSA. However, although the size of the active ingredient of the antiseptic solutions was known, the uptake and release kinetics did not quite match the size-dependency seen in the FITC-labelled dextran molecules, especially for the povidone-iodine-containing solution.
***Conclusion/Significance:** The uptake of molecules into BNC wound dressings is size dependent. Antiseptic, water-based solutions are excellently absorbed in a very short time and are released steadily over a period of time dependant on the size of the molecules. All tested antiseptic solutions reached effective antibacterial concentrations making them all suitable for the making of antiseptic BNC-based wound dressings. However, when using a commercially available solution and not a solution containing only the active ingredient, it must be taken into consideration that all ingredients have an effect on the uptake of the active substance and thus influence the maximum uptake and release concentration.

369 - 3d Printing Of Hemoglobin Releasing Hydrogels For Skin Regeneration And Healing Of Chronic Wounds

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***Purpose/Objectives:** Critical skin wounds result in immense suffering for patients and are a major cost for the health sector[1]. One key factor for improved wound healing is the development of advanced wound dressings. Our group has previously established a three-layered 3D-printed patient-specific wound dressing. This wound dressing moisturizes, drains out excess wound fluids and resists infections. The lowest layer, a 3D-printed hydrogel that moisturizes, can also be loaded with active compounds. Recently, it has been shown that one way to facilitate healing of chronic wounds is through topical addition of hemoglobin (Hb) the oxygen carrying protein in our own blood. We believe that our wound dressing infused with Hb wound lead to profound improvements in wound healing. [3].
***Methodology:** A hydrogel composed of TUNICELL nanofibrous cellulose (TUNICELL ETC, Ocean TuniCell) and alginate (SLG 100, Nova Matrix) was mixed with a commercial hemoglobin (Hb) spray (10% w/v) (Granulox, SastoMed, Germany). The resulting hydrogel was printed into small discs (Ø=10 mm, h=1.6 mm, w=0.1g) with a honeycomb infill using 3D Bioprinter Inkredible (CELLINK, Sweden). The diffusion rate of Hb from the hydrogels was assessed through UV-Vis spectrophotometry. Hydrogel composition was tuned for slow release and the hydrogel discs were assessed using *in vitro* scratch-assays. Human fibroblasts were seeded on top of the printed discs and cell attachment and morphologies were evaluated, post incubation.The performance of these hydrogels was compared to hydrogels without Hb
***Results:** The tuning of the hydrogel resulted in a loaded gel with a steady diffusion rate of approximately 0.726 mg Hb/g gel/hour. The cells treated with Hb infused wound dressings closed the scratch more quickly, whereas cells covered by wound dressings without Hb suffocated beneath the construct. The attachments tests showed a complete lack of attached cells when tested with human dermal fibroblasts.
***Conclusion/Significance:** The addition of Hb to hydrogels results in an easily printable ink with robust shape fidelity. This can be used to print wound dressings that fit to patient-specific needs and deals with difficult wounds located on hard to bandage areas, such as the armpit or around the tailbone. It was also

shown that the hydrogels can easily be adjusted to tune diffusion rates by varying biopolymer composition while still retaining their desired properties: shape fidelity, shear thinning and biocompatibility. The lack of cell attachments is also desirable when designing a wound dressing, as the goal is not to incorporate it permanently.
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370 - Decellularized Human Skin-derived ECM Improves Mechanical Strength And Cell Growth In Bioprinted Fibrin Skin Constructs

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***Purpose/Objectives:** Burn injuries represent a significant clinical burden in the United States, with 1.1 million injuries annually requiring medical attention. For patients with full-thickness burns, the healing process proves particularly challenging with success rates below 50%. The standard of care for burn injuries includes autologous skin grafting, which requires sufficient amounts of harvest sites and can be scarce in patients with severe wounds. Bioprinting has been proposed as a complementary method for *in vitro* fabrication of full-thickness skin with multiple cell types organized into biomimetic layers, which utilizes biomaterials to mimic the native tissue microenvironment and encourage cellular attachment and proliferation within engineered constructs. Fibrin hydrogel, the potential bioink, has limited post-printing mechanical strength and lacks signaling molecules and native extracellular matrix [ECM] proteins crucial for skin regeneration. It has been shown that the addition of collagen significantly increases fibrin hydrogel mechanical strength. While collagen is the major component of human skin ECM, other ECM proteins may foster additional cellular attachment and activity. Thus, this project aims to evaluate the efficiency of decellularized human skin-derived ECM supplement in improving the mechanical strength and biological performance of bioprinted fibrin skin constructs.
***Methodology:** Human skin was decellularized and solubilized into an ECM solution. Decellularization efficiency was evaluated through scanning electron microscopy [SEM] and histology. Total protein, collagen concentration and, glycosaminoglycan content were compared pre- and post- decellularization. Surface structures and rheological properties, and printability of fibrin hydrogel and Fibrin-ECM gel [FEG] were characterized. To access biological performance, fibroblasts isolated from human skin were cultured in hydrogels. Cell proliferation and viability and cell-laden construct mechanical strength and structure were evaluated. Immunohistochemistry was performed to compare relative levels of ECM proteins.
***Results:** Preservation of the ECM protein network was confirmed by SEM and histology. FEG displayed similar viscosity and shear thinning properties as fibrin hydrogel, but ECM addition prevented the hydrogel from transitioning to a complete liquid at high temperature, a factor known to improve printability. Increasing ECM concentrations slightly decreased fibrin hydrogel's pre-crosslinking storage modulus [G'], but post-crosslinking G' was significantly improved by 1% ECM addition (FEG G'=790Pa, Fibrin G'=400Pa). Alamar Blue assay revealed that higher ECM concentration yielded a decreased rate of cell proliferation in 2D culture, but an increased rate in 3D culture over 7 days. Cell viability was better maintained in FEG over 15 days. All cell-laden constructs exhibited a decline in mechanical strength over

15 days, but FEG displayed superior storage modulus than fibrin hydrogel at all time points. Histological assessment of the constructs illustrated improved maintenance of structural integrity in the FEG construct.
***Conclusion/Significance:** Decellularization and solubilization of human skin yielded ECM solution, a potential supplement to fibrin hydrogel for skin bioprinting. We have demonstrated that 1% ECM solution is the optimal concentration for FEG. In conclusion, human skin-derived ECM enhanced mechanical strength and structural stability of fibrin hydrogel over time and improved cell viability and cell proliferation. ECM solution protein composition, hydrogel printability, cellular activity, and ECM remodeling of engineered skin constructs require further evaluation.

371 - The Effect Of New Platelet Derived Growth Factor (PDGF) Peptide On Human Foreskin Fibroblast (HFF) Proliferation And Migration On 2D And In A Novel 3D Fibrin Bead

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***Purpose/Objectives:** Chronic wounds are an increasingly prevalent problem in the healthcare industry with the recent rise in diabetes, heart disease, obesity, and other conditions that adversely affect the rate of wound healing. Although numerous factors affect the mending of the skin, platelet derived growth factor (PDGF) is an excellent candidate for use in chronic wound therapy due to its potential to increase cellular migration and proliferation. However, as entire growth factors can be costly and limited in their effective dosages, this paper explores the use of shorter PDGF-mimetic peptide sequences to affect cell behavior in 2D and 3D wound healing models. In this study, the effects of one PDGF peptide on human foreskin fibroblasts (HFFs) were investigated *in vitro* using a cell monolayer as well as a previously developed fibrin bead model.
***Methodology:** The effects of the PDGF peptide on the growth of HFF cells were studied both through their growth and migration. Cell proliferation was evaluated using alamarBlue assays in 2D and calcein-AM in a novel 3D fibrin bead. Cell migration was measured out of a novel 3D fibrin bead. Rhomadine phalloidin staining was used to evaluate cell membrane ruffling and a quantitative ELISA was performed to evaluate cell activation.
***Results:** Of the peptides tested, one peptide of sequence AECK shows similar cell membrane ruffling and gene transcription as that caused by PDGF. This experimental peptide also showed statistically significant increases in HFF proliferation in a 2D model and showed an increase in HFF migration out of the fibrin bead by 35% more than the control condition.
***Conclusion/Significance:** These studies suggest promise for the use of short sequence peptides to replicate the important wound healing functions of PDGF and the use of a novel 3D fibrin bead to perform proliferation and migration assays for wound healing studies.

372 - Contribution Of Zona Spongiosa To The Make-Up Of Placental Membrane: Advantages In Wound Healing

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***Purpose/Objectives:** The *zona spongiosa*, or intermediate layer, is an under-characterized layer of placental membrane. The intermediate layer (IL) is the spongey, acellular barrier between the fetal-facing amnion and maternal-facing chorion layers[1]. Commercially available placental membrane allografts exclude the IL due to the challenges IL inclusion presents during processing. However, IL plays an important mechanical and chemical role in native membranes that should be considered when developing placental membrane products for wound healing applications. The IL is composed of a matrix of collagens, glycosaminoglycans and proteoglycans, including hyaluronic acid[2]. This composition allows the amnion to glide and stretch along the chorion, providing strength and elasticity to the membranes[3]. The efficacy of amnion and amnion/chorion allografts is especially well-studied in chronic wounds where an imbalanced biochemical environment promotes breakdown of the wound[4]. The extracellular matrix and cytokines found in the amnion and chorion layers provide scaffolding and signaling proteins that facilitate healing and homeostasis[1]. However, there remains minimal data demonstrating the composition of the IL and the complete placental membrane and its potential utility in wound healing.
***Methodology:** A dehydrated complete human placental membrane (dCHPM) allograft was developed, which retains the IL throughout processing. Histological examination demonstrated the conservation of IL between the amnion and chorion layers. Isolated IL composition was compared to dCHPM using quantitative protein assays including 1,000 targets involved in the processes of tissue remodeling, regulation of inflammation, angiogenesis, and host defense.
***Results:** Results demonstrated over 850 detected regulatory proteins in dCHPM, greater than 98% of which were also detected in IL alone. The IL contained over 120 cytokines identified in tissue remodeling (including PDGFs, FGFs, EGF, HGF, TGFa's, TGFb's, Matrilin-2, progranulin, epiregulin, and TIMPs), over 90 regulators of inflammation (including IL-4, IL-6, IL-11, IL-10, IL-13, and IL-1ra), over 60 protein regulators of angiogenesis (including VEGF, SDF-1b, Angiogenin, Endocan, and TGFb2), and more than 20 contributors to host defense (including S100A8, Granulysin, IL-28A, and IFNb).
***Conclusion/Significance:** The IL was found to contribute to the overall biochemical composition of regulatory proteins in dCHPM. The specific proteins detected in the IL suggest a therapeutic benefit to the inclusion of IL in placental membrane allografts intended for wound healing applications. Initial clinical use of dCHPM in the treatment of chronic wounds has been promising. Further investigation, including animal studies and clinical evaluation, is warranted to further understand the potential therapeutic and regenerative role of *zona spongiosa* in placental membrane allografts.
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373 - Jet-based Drug Delivery In Chronic Wound Healing

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***Purpose/Objectives:** The chronic non-healing wound has become a major worldwide healthcare challenge with increased mortality rate. Faster wound closer and controlling primary infection and preventing secondary sepsis plays a crucial role in these patients and has the potential to limit the morbidity, mortality, and amputation. Extreme hypoxia from the lack of angiogenesis, immune-modulated hyper inflammation, biofilm formation, and bacterial infection interrupts the physiological process to wound healing. Topical antibiotics are generally used to prevent the ulcer from getting infected, but due to the formation of necrotic tissue, it is not practical. The necrotic tissue and debris allow bacterial attachment, and wounds are susceptible to infection due to impaired host immune response making chronic wound and ideal environment for biofilm formation. Biofilms can have a significant impact on wound healing by contributing to bacterial infection, inflammation, and delayed wound healing. Traditional wound care routines are limited to topical delivery of antimicrobial properties and require high maintenance and frequent dressing change for applying drugs and withdrawing exudates from the wound bed. The liquid jet -based injection is a needle-free drug delivery method used for insulin, human hormone growth, and vaccine administration. Here, we've suggested using jet injector as a drug delivery method to deliver antibiotics and therapeutics to chronic wounds. Due to the high jet velocity, using this method will make it possible for any drug and antibiotic solution to pass through the necrotic tissue and microbial communities.
***Methodology:** Agarose (3%w/vol) was used to model the necrotic tissue in the *in-vitro* study. A comparison test was performed between jet, topical delivery, and hypodermic needle-delivery to investigate the distribution of Rhodamine-B in an agarose gel block. Computer modeling was developed to track the fluid dispersion from the jet exit. A thin agarose layer was placed on the top of the insert to mimic skin barrier and Cefazolin, and bovine serum albumin (BSA) was delivered using jet injector and compared to topical delivery of antibiotic and albumin. *In-vivo* study for investing the effect of vascular endothelial growth factor (VEGF) delivery with jet injection on chronic wounds was performed on diabetic mice model (B6.BKS(D)-Leprdb/J). The wound closure was monitored and evaluated during a 20-day healing period. Tissue histology was performed post-termination to study granulation and vascularization.
***Results:** The release study results of BSA and cefazoline delivery shows a significant difference in therapeutics delivery between jet group compared to a topical delivery. The delivery of vascular endothelial growth factor through the jet injector demonstrated much faster wound closure rate compared to topical delivery in diabetic mice wound model. With histological evaluation, the junction of the surgical wound and adjacent skin of all treatment groups had a healing site, which was mild to moderately thickened by granulation tissue.
***Conclusion/Significance:** Drug delivery in chronic wounds can lead to faster wound healing using the jet-injection method. Due to high jet velocity, therapeutics and antibiotics can pass through necrotic tissue and deliver growth factors and antimicrobial agent into deeper layers of the wound.

374 - Property Correlation Between Mechanical, Structural And Compositional Tissue Characteristics: A Comparative Study Between Human Skin And Swine Skin

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***Purpose/Objectives:** Skin is the largest multifunctional organ in the body. Understanding, comparing, and differentiating the physiological, chemical, and biophysical characteristics of the dermal compartment between humans and swine assists in building a proper approach for therapeutic management strategies of skin diseases and improving skin tissue engineering methods. Many instruments have been developed to evaluate skin ultrastructure, elasticity and firmness. It's becoming difficult to decide which equipment and mechanical property analytics are the optimal platform to attain data on the characteristics of different skin types. The aim of this study was to compare and correlate parameters assessed with different techniques across different animal/tissue models, to better understand how each method characterizes properties of various tissues.
***Methodology:** Swine dorsal skin is commonly utilized as a model for studying cutaneous wounds and defects, as well as active ingredient assessment in dermatological and cosmetic fields. Structural, mechanical and compositional comparison between abdominal human skin and porcine back skin was conducted using Raman microscopy, mechanical testing and ballistometry, atomic force microscopy (AFM), environmental scanning electron microscopy (ESEM), and multiphoton microscopy (MPM). MPM, ESEM and AFM were utilized to evaluate the ultrastructure of the skin at micrometer and nanometer resolution and qualify the fiber arrangement within various skin cross sections. Finally, mechanical properties of both skin types were assessed using microscale mechanical tests which were compared against structural and chemical measurements.
***Results:** Our findings highlighted similarities and some differences between full-thickness swine skin and human skin in terms of ultra-structure, collagen, and lipid composition. AFM imaging revealed swine dorsal thoracolumbar skin to have a high degree of organization of collagen fibrils, whereas human abdominal skin had a more random orientation. The static indentation results showed significant difference between human skin and porcine skin, as swine skin had a higher tensile modulus and lower indentation values. Collagen to elastin ratio as measured using Raman microscopy was greater in porcine skin, corresponding to higher stiffness as confirmed by AFM scans. AFM and MPM images also confirmed a more random orientation of collagen fibrils within human skin. This behavior indicated a higher proportion of matrix dispersed between the collagen fibrils in the human abdominal skin tissue, which is expected given the anatomical area. Raman microscopy of both species' skin showed similarities in the presence of collagen, keratin, amide and elastin signature peaks. Comparison performed using microscopy and mechanical characterization via ballistometry and mechanical testing revealed functional differences between human and swine skin due to varied ECM organization and fibrillar arrangement. By combining different characterization techniques, correlative similarities were revealed between properties such as AFM-based fibrillar arrangement and skin firmness, and Raman spectra-based collagen-to-elastin ratio and elastic modulus.
***Conclusion/Significance:** Accurate measurement of the properties of skin has uses for several applications such as in surgical repair, aging and cosmetic product assessment. We have performed a comparative analysis of human skin and swine skin using innovative characterization techniques and identified correlative properties for development of a unique non-destructive methodology for the complete assessment of elasticity and firmness of human skin.

375 - Hydrophobic Bacterial Cellulose Matrix For Incorporation Of Essential Oils With Antimicrobial Properties For Potential Use As Wound Dressing

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Commercial bacterial cellulose (BC) dressings haven't antimicrobial properties and are hydrophilic. Incorporating essential oils and vegetables can be a good strategy for controlling infection in the wound environment and accelerating the healing process. The incorporation of significant amounts of oils is possible through chemical modifications to change the hydrophilic/hydrophobic character of BC. The objective of this work was insert hydrophobic groups in BC nanofibrils for addition of essential oils. The fibrils were modified by direct contact with solution of methyltrimethoxysilane (MTMS), silicon derivative reagent. Aerogels was produced by dry freeze method. FTIR, SEM, contact angle and liquid (water and oils) were analyzed for absorption capacity with unmodified and silanized airgels. SEM micrograph analysis showed a significant difference between the materials. Silanized airgel presented a more porous structure with larger porous size. The FTIR analysis proved the success of chemical modification, since after chemical treatment with MTMS, the characteristic silane vibrations were identified at 780 cm -1, 905 cm -1 and 1275 cm -1. In contact angle test, the unmodified BC airgel showed a highly hydrophilic character resulting in low contact angles (<10 °). While the hydrophobized airgel has a contact angle of 127 °. In the liquid absorption capacity test, unmodified and silanized airgel absorbed about 60 and 14 times, respectively, the dry weight in water. Using essential oils, this value changes to 50 and 90, respectively. This preliminary work showing a promising system for the delivery of oils with antimicrobial properties, aiming at a future application as a dressing.

376 - Papain Immobilized In Calcium Alginate Membrane For Wound Dressing

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Natural-based dressings are of considerable interest in the pharmaceutical industry, given their similarity to extracellular matrix and other polymers in the human body. Alginate is a polysaccharide found in the cell walls of brown algae. It has excellent properties for use as biomaterial (biodegradable, biocompatible and non-toxic). In order to improve its therapeutic properties, it was proposed the incorporation of an enzyme (papain) to accelerate wound healing. Papain is found in the composition of ointments wounds treatment, as it is capable of promoting the debridement of the devitalized or necrotic tissues. The development of a dressing based on alginate and papain aggregates the healing properties of both materials. The adsorption of the enzyme on a support can stabilize its structure and it allows the papain release in a controlled manner. Papain was immobilized by physical adsorption. The best conditions for immobilization were evaluated and the yield of immobilization was measured by quantifying the protein retained. The enzyme activity stability during storage during 28 days as well as the release profile using Franz cell were also evaluated. Physical-chemical and *in vitro* biological characterization were performed. Best immobilization conditions were in neutral medium, using 20 mg/mL papain concentration and temperature at 25 °C. The enzyme remained active after immobilization and over time immobilized papain had a smaller decrease in relative activity compared to free-papain. Franz cell showed a release profile of 64.1% of the enzyme after 24 hours. The biological assays indicate a material with low cytotoxicity and not hemolytic

377 - Retinal Pigment Epithelium Wound Healing Model To Target Injury Induced Scarring In The Eye

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Introduction: The Retinal Pigment Epithelium (RPE) is a quiescent monolayer integral to the function of the retina. Retinal injury can trigger RPE proliferation and epithelial to mesenchymal transition (EMT) leading to scarring in the eye. The aim of this study is to identify key molecular players in RPE EMT for potential therapeutic targets and develop a high-throughput wound assay for effective drug screening.
Methods: EMT-like phenotype was validated in an *in vitro* wound healing model using immunohistochemistry and gene expression analysis of multiple iPSC-derived-RPE samples. An siRNA screen identified NOX4 as a candidate gene for inducing EMT in RPE cells. The functional role of NOX4 in RPE-EMT was validated in the wound model using Western Blot, immunohistochemistry, and migration analysis after shRNA and pharmacological inhibitors mediated NOX4 knockdown.
Results: Mechanical wounding induced RPE EMT as shown by the upregulation of EMT genes (ZEB1, Vimentin, SMA). NOX4 genetic knockdown or pharmacological inhibition suppressed the expression of these genes. Scanning electron and confocal microscopy images revealed NOX4 role in EMT initiation and cell migration, which was blocked by NOX4 inhibition.
Conclusions: Our findings suggest NOX4 is a novel regulator of EMT and a potential druggable target. Our wound model for RPE EMT mimics the *in vivo* process and can be used in a high throughput screen. With our high throughput wound healing assay, we will be able to test different drugs that can suppress EMT and keep the fate of RPE, avoiding both scar formation and excessive proliferation.

378 - Renal Regenerative Effect Of Induced Nephron Progenitor Cells (inpcs) In Diabetic Nephropathy Mice

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***Purpose/Objectives:** In vivo safety and efficacy of renal regeneration using induced nephron progenitor cells (iNPCs) was evaluated using diabetic nephropathy mouse.
***Methodology:** Cells were injected directly into the right kidney, and histopathology, change of renal function, inflammation/fibrosis, oxidative stress, renal regeneration and cell fate were analyzed at week 8.
***Results:** The iNPCs injected diabetic mice showed diminished glomerular hypertrophy, reduced tubulointerstitial fibrosis, low BUN, serum creatinine and albuminuria value, decreased inflammation/fibrosis, and enhanced anti-inflammation and renal regeneration compared to the untreated mice. Also, iNPCs injected kidney did not show histological malformation or migration into other distant organs.
***Conclusion/Significance:** The injected cell tracking is an important factor to prove in vivo safety, which contains cell distribution, duration and migration analysis. The injected iNPCs were evenly distributed in

the cortex area, which means that injected cells have immunoregulatory ability of the host tissue, thus they are not isolated with cystic fibrosis in the kidney. In aspect of duration, iNPCs were identified at week 1 and vanished at week 8, which means that injected cells have a short life span like a mesenchymal cells. In migration analysis, iNPCs did not show any positive signal in spleen, liver and lung at week 1 meaning that injected cells were not migrated into distant organs, because these organs were not injured and did not release chemoattractant factors by inflammation. Thus, iNPCs have a similar fate as mesenchyme cells in vivo, not embryonic cells.

379 - Developmentally Inspired Dynamic Hydrogels For Endodermal Differentiation Of Pluripotent Stem Cells.

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***Purpose/Objectives:** Synthetically defined hydrogels are increasingly used for lineage-specific differentiation of humna induced pluripotent stem cells (hiPSCs). However, existing synthetic hydrogels do not fully recapitulate the dynamic stem cell microenvironment, which may experience sequential stiffening and softening during the differentiation processes. The objective of this project is to design synthetically defined hydrogels with modularly assembled matrices and developmentally inspired dynamic properties for enhancing endodermal differentiation of hiPSCs.
***Methodology:** hiPSCs were encapsulated in photopolymerized thiol-norbornene hydrogels for in situ expansion and directed differentiation into endodermal and endocrine lineages. The hydrogels were modularly crosslinked with different weight contents of poly(ethylene glycol)-octa-norbornene (PEG8NB) and various peptide crosslinkers to identified ideal microenvironment suitable for endodermal differentiation. Specifically, four peptide linkers were employed to construct instructive stem cell niche, including (1) matrix metalloproteinase (MMP) sensitive linker (for cell-mediated cleavage), (2) sortase A (SrtA) sensitive linker (for user-controlled gel softening), (3) vitronectin-derived linker (for cell improving survival and differentiation), and (4) inert linker (for control). Appropriate combination of these linkers produced various modular microenvironment suitable for growth and differentiation of hiPSCs. In addition, these peptide linkers contained tyrosine residues that served as substrates for enzyme-induced dynamic stiffening. Cell-laden hydrogels were differentiated using STEMdiff^{TM} pancreatic progenitor kit (STEMCELL Technologies) and cell morphology and lineage specific differentiation outcome were evaluated via immunostaining and real-time PCR.
***Results:** We found that hydrogels with lower crosslinking density (i.e., soft gel with shear moduli G' ~ 1 kPa) promoted higher degree of cell survival. Lower cell encapsulation density ($2x10^6$ cells/mL) yielded larger clusters, whereas stiffer matrices (G' ~ 3kPa) decreased cell viability while the surviving cells exhibited highly irregular protrusions. The ability of cells to remodel matrix greatly affected their survival in the chemically defined hydrogels as inert matrices led to extensive cell death. On the other hand, vitronectin-derived sequence promoted cell cluster growth, confirming the critical role of appropriate matrix motifs on promoting cell morphogenesis. The addition of tyrosine residues on the peptide linkers permitted user controlled matrix stiffening, a process known to alter cell fate processes. We found that on-demand matrix stiffening via tyrosinase-indced di-tyrosine crosslinking was essential for promoting endodermal differentiation of hiPSCs. Finally, the inclusion of SrtA-cleavable sequence afforded an orthogonal method of manipulating hydrogel stiffness, which encouraged subsequent endocrine

differentiation.
***Conclusion/Significance:** We showed that synthetically defined hydrogels can be designed to exhibit a wide range of tunability to recapitulate the dynamic stem cell niche. Specifically, we demonstrated that on-demand and orthogonal hydrogel stiffening and softening can be achieved through designing simple peptide linkers composed of all natural amino acids. Using this synthetically simple hydrogel system, we found that matrix properties can be readily tuned to mimic stem cell developmental process. This developmentally inspired dynamic hydrogel system may be used for other lineage-specific differentiation.

380 - Controlled Delivery Of Stem Cell-derived Trophic Factors To Treat Acute Kidney Injury

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***Purpose/Objectives:** Renal disease is a worldwide health issue. Besides transplantation, current therapies revolve around dialysis, which is limited to delay disease progression through filtering metabolic wastes in the blood and unable to replace other renal functions, such as synthesizing erythropoietin. To address these limitations, cell-based approaches have been proposed to restore damaged kidneys as an alternative to current therapies. Particularly, recent studies have shown that stem cell-derived secretomes could enhance tissue generation. However, the therapeutic effects were only observed with the administration of a high dose of highly concentrated conditioned medium (CM) due to rapid degradation of various growth factors. Thus, we developed a gel-based delivery system for controlled delivery of trophic factors in the CM derived from human placental stem cells (hPSCs) and evaluates the effects of trophic factors on renal regeneration.
***Methodology:** This study explores a gel-based delivery system for controlled delivery of trophic factors secreted from human placental stem cells and evaluates the effect of trophic factors on renal regeneration. *In vitro* cell viability and proliferation assays were tested to observe the effects proliferation effect of the CM. Platelet-rich plasma was used as a delivery vehicle for CM *in vivo*. The feasibility of controlled delivery test was done on the PRP encapsulated CM and the release kinetics of CM from the gel was evaluated.
***Results:** *In vitro* cell viability and proliferation assays demonstrated that CM treatment significantly enhanced cell proliferation when compared with the control without CM. To test the feasibility of controlled delivery, CM was encapsulated within the Platelet-rich plasma (PRP), which was used as a delivery vehicle for CM, and the release kinetics of CM from the gel were analyzed both *in vitro* and *in vivo*. The release profiles show that CM can be released from PRP in a controlled manner by altering gel stiffness. An *In vivo* study using a rat acute kidney injury model showed that CM delivery using the gel system into the injured kidney tissue facilitated less renal tissue damage, leading to rapid functional recovery than that of saline, CM or vehicle only group.
***Conclusion/Significance:** These results suggest that the delivery of hPSC-derived trophic factors in a controlled manner may contribute to efficient kidney repair from renal tissue injury.

381 - Porous Pcl Scaffold Seeded With Mesenchymal Stem Cells Transfected With Osteogenic Inducing Mirnas For Bone Tissue Engineering

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***Purpose/Objectives:** Bone is a structural component of the human body and has significant physiological functions. Bone allows for the protection of organs, skeletal muscle movement, support and anchorage of organs and muscles, blood cell production, pH regulation, housing of multiple progenitor cells and mineral homeostasis. Bone is capable of regenerating by itself when the injury is small such as in stable fractures. However, when the defect is large or if there is insufficient vascularity, aid is needed to heal the defect. Extensive research has focused on delivering growth factors for bone tissue engineering. Shortcomings associated with delivering growth factors in the protein form, such as the short half-life of protein and cost and difficulty of manufacturing these recombinant proteins, have led to the search of other methods of delivering bioactive factors to aid in bone regeneration. By controlling the production of proteins capable of inducing osteogenesis *in vivo*, the proteins can be delivered in a more biologically active form with more precise post-translational modification and tertiary structure formation compared to the exogenous growth factors that may be altered through the delivery process. The overall goal of this project is to contribute towards the development of novel strategies to aid bone tissue regeneration by delivering mesenchymal stem cells (MSCs) expressing microRNAs that induce expression of the runt-related transcription factor 2 (RUNX2) in a porous PCL scaffold. In this study, microRNAs that can induce the expression of RUNX2 which promotes osteogenesis were identified from the literature and evaluated side by side *in vitro* in MSCs to determine which miRNA most effectively induce osteogenesis by evaluating gene expression of osteogenic markers and calcium deposition.
***Methodology:** Porous PCL scaffolds with different porogen amount and size range were fabricated to evaluate the pore properties that will result in enhanced cell attachment and proliferation using an MTT assay. MSCs transfected with these miRNAs were then seeded on the optimal porous PCL scaffolds to evaluate the ability of these biomaterial composites to induce osteogenesis. All the miRNA tested resulted in a significantly higher amount of *RUNX2* compared to the negative control miRNA, however the levels for miRNA-26a, miRNA-196 and miRNA-218 were more significantly higher than the control. miRNA-196, miRNA-335 and miRNA-218 resulted in an overexpression of ALP. Enhanced Alizarin red S calcium deposit staining was observed for MSCs overexpressed with all the miRNA tested compared to the negative control.
***Results:** Overexpression of miRNA-26a, miRNA-196 and miRNA 218 demonstrated a more significant enhancement of staining compared to the negative control and other miRNAs tested. Scaffolds fabricated with 80 vol. %/300-500 µm porogen were optimal for cell colonization as higher absorbance of metabolized MTT was detected compared to other scaffold types. Porous PCL scaffolds fabricated with 80 vol. %/300-500 µm porogen and seeded with MSCs transfected with the miRNAs resulted in higher Alizarin red S calcium deposit staining compared to the control.
***Conclusion/Significance:** In conclusion, our results suggest that porous PCL scaffolds seeded with miRNA-26a, miRNA-196 and miRNA 218 can be potentially used for bone tissue engineering.

382 - Potential Of Epithelial Differentiation In Situ And Ex Vivo Of Mesenchymal Stem Cells Of The Apical Papilla

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***Purpose/Objectives: Purpose:** Human apical papilla stem cells have inherent differentiation capabilities and potential usefulness in regenerative medicine. However, the epithelial differentiation capability and the heterogeneity of these cells have not been fully explored to the date. **Objective:** The aim of the present study was to analyze the expression of different epithelial markers in native tissue of the apical papilla and cell culture of stem cells of the apical papilla by histological analysis and immunofluorescence.
***Methodology:** Four normal humans impacted third molar were selected with immature roots from healthy patients (2 donors aged 14-16 years). The procedures were performed in the dentistry service of the Antonio Nariño University. Bogota Colombia. Briefly, we characterize by flow cytometry the cells of the apical papilla with markers CD90, CD45, CD105 and CD34. Additionally, we evaluate the osteogenic and chondrogenic differentiation by alizarin red and Alcian blue stains. We analyzed the expression of several undifferentiation and epithelial markers (cytokeratin 4, 7, 19 and desmoplakin) in cells located *in situ* in the tissue of apical papilla and *ex vivo* cell cultures of apical papilla stem cells by histological analysis and immunofluorescence.
***Results:** The results found in this study demonstrated the heterogeneity and intrinsic capacity in cell culture of apical papilla cells to differentiate into osteogenic, chondrogenic and epithelial lineage. Apical papilla cells showed the ability to express *in situ and ex vivo* stem cell markers, CD90 and CD105 and not express the CD34 and CD45 markers. On the other hand, the epithelial markers cytokeratin 4, 7, 19 and desmoplakin were expressed in the native tissue of the apical papilla and under *ex vivo* cell culture conditions. However, important differences were detected among some cell types in the apical papilla *in situ vs ex vivo* cell culture showing the highest epithelial markers.
***Conclusion/Significance:** These results suggest that apical papilla stem cells have intrinsic potential to express relevant epithelial markers and support the idea that they could be used as alternative cell sources for epithelial tissue engineering.

383 - Using Microrna-122 Mimics Or Microrna-451 Inhibitors To Prevent The Osteoarthritis Process

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***Purpose/Objectives:** MicroRNAs (miR) are short, non-coding segments of RNA that play a vital role in post-transcriptional gene regulation by binding to mRNA and preventing translation. OA is characterized by increased production of inflammatory cytokines and matrix-degrading enzymes such as matrix metalloproteinase 13 (MMP-13). miR-122 stimulates proliferation whereas miR-451 inhibits proliferation and stimulates the production of the catabolic enzyme, MMP-13 in rat articular chondrocytes (rArCs). Catabolic cytokines, most notably interleukin-1 beta (IL-1β), stimulate their own production and the production of catabolic pathway mediators. Interrupting these pathways through miR regulation could provide a novel approach that targets the underlying mechanism driving the disease progression. The aim of this study was to determine if adding miR-122 or inhibiting miR-451

would have a protective effect on the production of inflammatory mediators and matrix-degrading enzymes in an in vitro model of OA and to examine endogenous levels of these miRNA in OA in vivo.
***Methodology:** rArCs were isolated from 100-125g male Sprague Dawley rats. At 60% confluence, first passage cells were transfected with the transfection vehicle lipofectamine (Lipo, negative control), miR-122 or miR-451. Twenty-four hours following transfection, cells were treated with or without 10 ng/mL IL-1β. Conditioned media were collected and MMP-13, nitric oxide (NO) and prostaglandin E2 (PGE2) were measured. A multiplex protein assay was run on groups treated with IL-1β. Cultures were treated with locked nucleic acid (LNA) inhibitors to inhibit the action of miR-122 and miR-451. Endogenous levels of miR-122 and miR-451 were measured in healthy cartilage and OA cartilage. Osteoarthritic cartilage was harvested 70 days after performing a bilateral ACLT on male Sprague Dawley rats (n=8 rats).
***Results:** Treatment of rArCs with IL-1β increased MMP-13, NO, and PGE2, as expected. MiR-122 prevented the increase in these molecules in the presence of IL-1β in MMP-13, NO, and PGE2 and significantly increased DNA. The presence of miR-451 mimic exacerbated the inflammatory response in these same molecules. Multiplex data on proteins indicated that miR-122 in the presence of IL-1β significantly decreased IL-1α, IL-2, Il-4, IL-6, GM-CSF, MIP-1A, RANTES, and VEGF while miR-451 significantly increased these factors with the exception of IL-1α, RANTES and VEGF. LNA inhibitor experiments revealed a dose-dependent increase in MMP-13 in the presence of IL-1β + miR-122 inhibitor. Using IL-1β + miR-451 LNA inhibitor restored MMP-13 levels back to control levels. In vivo expression of miR-122 demonstrated a 1-fold increase in OA tissue, whereas expression of miR-451 was 2 fold higher.
***Conclusion/Significance:** MiR-122 exhibits a marked ability to prevent the IL-1β dependent increase in pro-inflammatory and catabolic molecules. MiR-451 exacerbates the inflammatory response and matrix-degrading enzymes in the presence of IL-1β in this model. Treatment with these microRNAs may modulate the uncontrolled inflammatory response present in OA.

384 - Regulation Of Growth Plate Chondrocytes By MicroRNA

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***Purpose/Objectives:** Local cell communication regulates the proliferation and differentiation of chondrocytes within growth plate cartilage during endochondral bone formation, culminating in extracellular matrix (ECM) mineralization. A key player in the mineralization process is matrix vesicles (MVs) which are selectively enriched with enzymes and growth factors. We recently found that MVs are also selectively enriched with microRNAs (miRNA). The aim of this study was to determine whether specific miRNA that are enriched in MVs regulate growth zone (GC) chondrocytes.
***Methodology:** GC cells were isolated by enzymatic digestion from costochondral growth zone cartilage harvested from 5-week-old male Sprague Dawley rats (under approval of VCU's IACUC). GC cells were cultured and fourth passage cells were transfected for 24 hours with miRNA mimics (miR-22, miR-122, miR-223, miR-451); scrambled miRNA mimic and no treatment groups served as controls. Cell layers and conditioned media were harvested 48 hours post transfection completion. DNA (PicoGreen), alkaline phosphatase specific activity (pNPP), sulfated glycosaminoglycan content (DMMB), apoptosis (TUNEL; Bax/Bcl2; p53), proliferation (EdU) and protein levels for collagen 2, osteoprotegerin, p53, RANKL, and VEGF were measured by ELISA. All experiment groups had an n of 6 cultures per variable; results were

validated in repeat experiments. A one-way analysis of variance with Tukey's multiple comparison test was carried out. All significant differences have a $p < 0.05$ and were determined using R v 3.4.3.
***Results:** Cell proliferation as measured by total DNA and EdU incorporation increased in GC cultures transfected with miR-122 while other miRNA had no effect. Examined more closely (every 12 hours from 0 to 48) active proliferation was increased by miR-122 at 12 and 24 hours while total DNA was increased at all time-points. Apoptosis markers were unaffected for all treatment groups. Cell layer alkaline phosphatase specific activity was reduced by miR-122 and miR-451, whereas sulfated glycosaminoglycan content was increased only by miR-122. Osteoprotegerin production was increased by miR-122 and miR-451; RANKL was undetected for all groups; and VEGF production was increased by miR-22. Collagen 2 was increased by miR-22, miR-122 and miR-451.
***Conclusion/Significance:** The selective packaging of microRNA within the MVs indicates that, in addition to a key component in the mineralization of the growth plate, MVs are functioning in the role of cell signaling, likely in an autocrine and paracrine fashion. Taken together, our data indicate that miRNAs enriched in MVs are active components in the regulation of chondrocyte proliferation, differentiation, and ECM synthesis. Specific microRNA may be valid candidates for modulating ECM production and turnover within the growth plate.

385 - The Role Of Caveolin-1 In Mediating The Response Of MSCs To Rigidity Within A Biomaterial Scaffold

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***Purpose/Objectives:** Although the response of mesenchymal stem cells (MSCs) to substrate stiffness has been widely investigated since 2006, most of the studies were conducted on two-dimension (2D) culture. For tissue engineering purposes, MSCs are usually subjected to a 3D culture environment. Therefore, we aim to investigate the effect of substrate stiffness on MSC differentiation in a 3D context and explore the underlying mechanism. To generate soft and hard scaffolds with the same ligand density, we encapsulated MSC within a photocrosslinked gelatin hydrogel developed in our laboratory. By adjusting the light exposure time, we were able to generate MSCs-encapsulated gelatin scaffolds with different stiffnesses. We then examined the osteogenesis and neurogenesis of MSCs within these soft and hard scaffolds. We hypothesized that MSCs within soft scaffolds would display higher neurogenic differentiation, while MSCs within hard scaffolds would induce higher osteogenesis.
***Methodology:** Human MSCs (hMSCs) were isolated from human femoral heads with IRB approval. For 3D culture, hMSCs were mixed within a 15% gelatin solution (containing 0.15% photoinitiator LAP) and then subjected to either 8 seconds or 2 minutes of visible light illumination, to generate soft or hard constructs, respectively. The mechanical properties of the constructs were first measured with AFM. Then, the constructs were cultured in either a growth medium, an osteogenic medium, or a neurogenic medium. The cell morphology was examined with fluorophore-conjugated phalloidin staining. The neurogenic and osteogenic differentiations were assessed by immunofluorescence and real-time PCR. In particular, we introduced siRNA to suppress the expression of caveolin-1, to determine its role in MSCs sensitivity to stiffness under 3D culture condition.
***Results:** We have successfully fabricated photocrosslinked 3D scaffolds with different elastic moduli (0.1 to 30kPa). MSCs in stiff scaffolds displayed a round morphology with very limited extension. Contrarily, MSCs in soft scaffolds were bigger with more branching structures. Real-time PCR results

indicated that MSCs displayed higher osteogenic marker gene expression in stiff scaffolds, and higher neurogenic marker gene expression in soft scaffolds, which was further confirmed by immunostaining and western blot assays. Interestingly, caveolin-1 expression in MSCs within soft scaffolds was significantly higher than that of MSCs within hard scaffolds. In addition, the inhibition of caveolin-1 promoted robust osteogenesis of MSCs within soft scaffolds, indicating its critical role in mediating the response of MSCs to stiffness.
***Conclusion/Significance:** This study was conducted within a biomaterial scaffold, so the findings from 3D culture are more informative to tissue engineering than the traditional 2D culture. In addition, the mechanistic study determined some key pathway components, such as caveolin-1, that are responsible for mechanotransduction and differentiation. Through modulating these pathways, we are able to control cell differentiation toward a desired lineage, thus decoupling the restriction from scaffold stiffness.

386 - Dynamic Organoid Cultures Of Mouse Embryonic Stem Cells In Modeled Microgravity Enhance Directed Endodermal Differentiation

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***Purpose/Objectives:** Despite extensive research on embryonic stem cells, the ability to precisely control and predict their differentiation is still limited. Specifically, induction of definitive endoderm (DE) offers great potential in tissue engineering models and regenerative medicine, since these cells can further give rise to cells of pancreatic, hepatic, intestinal and pulmonary lineages. Prior studies suggested that culture of pluripotent cells in simulated microgravity may promote and enhance specific differentiation pathways. Here we characterized directed differentiation of mouse embryonic stem cell (mESC) organoids into definitive endoderm under modeled microgravity (MMG) conditions using rotating wall vessel (RWV) bioreactors, and compared the outcomes to culturing the cells in 2D monolayers and 3D embryoid bodies (EBs).
***Methodology:** Murine embryonic stem cells (mESC - E14) were maintained in an undifferentiated state in DMEM supplemented with fetal bovine serum, β-mercaptoethanol, leukemia inhibitory factor (LIF), L-glutamine, and pen/strep. EBs were generated by plating 3000-6000 cells into each well of ultra-low attachment, round-bottom 96-well plates containing either maintenance media or DE induction media, which included low-serum media supplemented with Activin A (AA), L-glutamine, and pen/strep. EBs were then seeded into a RWV bioreactor (hereafter referred to as organoids). At different time points, the cells from 2D, EBs, and organoids from RWV culture were analyzed using a live/dead viability assay, confocal microscopy, histology, and immuno-histochemistry. Cells were harvested from 2D culture or digested into single cells from the EBs and organoids, and characterized by flow cytometry using markers for pluripotency and DE. Finally, the cells were analyzed with a battery of markers of pluripotency, endoderm, ectoderm, and mesoderm markers using RT-PCR.
***Results:** Examination of EBs and organoids from microgravity culture showed that under both conditions, aggregates were roughly spherical, and several hundred micrometers in diameter. EBs and Organoids that were cultured in DE induction media were smaller in size than those cultured in maintenance media. Most of the cells in the outer layers were viable. Flow cytometric analysis and RT-PCR results revealed marked differences between cells in 2D, EBs, and organoids, and indicated distinct

cellular heterogeneity within the organoids and EBs. Both EBs and organoids grown in the presence of a differentiation media featured decreased expression of pluripotency markers, while revealing high expression of endoderm markers. Results also showed more substantial downregulation of pluripotency and higher expression of endoderm markers upon AA-induced, directed differentiation in MMG.
***Conclusion/Significance:** The protocol developed in this research can be used to study the effects of MMG on the differentiation of pluripotent stem cells. This method can potentially induce specific lineage differentiation towards endoderm.

387 - Correlation Between Immunomodulatory Properties Of Human MSCs

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***Purpose/Objectives:** Certain clinical applications of human mesenchymal stem/stromal cells (hMSCs) revolve around the ability to modulate the immune response. A number of mechanisms of hMSC immunomodulation have been identified including the secretion of soluble factors, expression of cell surface molecules, and regulation of immune cell proliferation and phenotype. Most hMSCs are well-characterized for phenotype and differentiation potential, with much more limited analysis of the immunomodulatory properties. Furthermore, there is little understanding of the correlations of the disparate immunomodulatory properties. This study expands bone marrow-, adipose tissue-, and umbilical cord-derived cells cultured using multiple media types and evaluates for multiple immunomodulatory properties, exploring possible correlations between attributes.
***Methodology:** hMSCs secrete a number of soluble proteins that have known immunomodulatory effects. These soluble factors include indoleamine 2,3-dioxygenase (IDO), prostaglandin E-2 (PGE-2), programmed death ligands 1 and 2 (PD-L1/2), TNF-α stimulated gene/protein 6 (TSG-6) and transforming growth factor β (TGF-β). Expression of these soluble factors can be increased when hMSCs are stimulated with inflammatory cytokines such as interferon γ (IFN-γ) and tumor necrosis factor α (TNF-α). Multiple studies have proposed "priming" hMSCs with IFN-γ and/or TNF-α before implantation to enhance hMSC immunosuppression for various clinical applications. We will analyze hMSC expression of IDO, PGE-2, PD-L1/2, TSG-6, and TGF-β in the absence and presence of IFN-γ and/or TNF-α using quantitative ELISAs to evaluate both the innate and "primed" immunomodulatory potential of hMSCs.
The ability of hMSCs to modulate the immune response may also be enhanced by the ability to persist at the target site by evading immune detection. hMSC immune evasion is likely due to a combination of the local immunosuppressive environment created by the secretion of soluble factors as well as the expression/non-expression of specific immune-associated surface markers. We will use a flow cytometry panel to quantify hMSC expression of surface markers associated with co-stimulatory markers (CD40, CD80, and CD86), natural killer (NK) cell inhibition (HLA-E and HLA-G), macrophage recognition (CD47), and complement regulation (CD46, CD55, and CD59).
Suppression of T cell proliferation is a hallmark of immunomodulatory function, and co-culture of hMSCs with T cells has been shown to inhibit T cell proliferation. Moreover, co-culture of hMSCs with naïve CD4+ T cells has also been shown to increase the number of regulatory T cells (Tregs), a small subset of CD4+ T helper cells that play an important role in suppression of the auto-immune response and are a popular target for the development of therapies for auto-immune diseases. We will develop a hMSC/T cell co-culture assay that will analyze the ability of hMSCs to suppress T cell proliferation as well as induce the formation of Tregs.

***Results:**
***Conclusion/Significance:** By extensively characterizing the immunomodulatory properties of hMSCs in multiple donors and tissue types to establish correlations between the various attributes, this study will further our understanding of the mechanisms by which hMSCs may modulate the immune system in vivo.

388 - Characterization Of Chicken Neonatal Bone Marrow Cells; Cellular Properties And Differentiation Potential

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This study was conducted to characterize chicken bone marrow-derived cells retrieved from neonatal chicks. Femurs were retrieved from either hatched or Four-day-old white leghorn chicks. The bone matrix consisted of various cells involved in the endochondral ossification of mature bone marrow. Four distinct regions (zone of resting, zone of hypertrophy, zone of proliferation, and erosion zone) were visible, while the epiphyseal line was not formed at this stage. Four-day-old bone marrow cells became cuboidal at the end of primary culture (P0), and vigorous proliferation was detected in P2 and P3 cells. RT-PCR analysis showed that the expression of *Col X* and *Aggrecan* was prominent in P0 cells. *Col I, Sox9, Sox2, CD44, CD45*, and *CD105* were also expressed, while *Col II, PouV, Nanog, CD31*, and *CD34* expression was weak or undetectable. Similar expression patterns were detected in P5 cells, but all osteochondrogenesis-related genes (*Sox9, Col I, Col II, Col X*, and *Aggrecan*) and *CD31* became strongly expressed. Alkaline phosphatase, an osteogenesis marker, was also detected, and most (82%) cells were Sox9-positive. P5 cells induced both osteogenic and chondrogenic differentiation, but not adipogenesis. In conclusion, the femurs of neonatal chicks contain both osteochondro-progenitor cells and mature bone marrow cells. Sox9 can be used as a marker for these cells that tend to induce osteogenesis and chondrogenesis in vitro.

389 - The Role Of Intermittent Hypoxia On The Osteogenic Differentiation Of Human Mesenchymal Stem Cells

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Studies have shown intermittent hypoxia (IH) could be a potential nonpharmacological strategy for treating diseases. Most of these studies have focused on hypoxic paracrine activation, but few studies have evaluated its autocrine outcome in osteogenic differentiation. Consequently, this systematic review sought to elucidate the following question: can the IH improve the differentiation of hMSCs in bone progenitor cells? This review was registered on PROSPERO (CRD42017069084) and reported by the guideline of PRISMA Statement. Two reviewers independently conducted a search in the literature. The inclusion criteria considered: sound human cells; at least 7 days of differentiation using IH. After screening a total of 1901 potentially relevant documents by title and abstract reading, only 20 studies were selected after qualitative analysis: one in vivo (5.3%) e nineteen in vitro studies (94.7%). Regarding

the in vivo study, the osteogenic differentiation improved using IH when compared to normoxia condition. In contrast, in vitro studies demonstrated a better osteogenic differentiation in normoxia in eight studies (42.1%). Both conditions obtained a similar outcome in six studies (31.6%), and IH obtained a better outcome in only five studies (26.3%). In conclusion, it was not possible to confirm the role of IH on the bone differentiation once the benefit was only in one in vivo study. Thereby, other studies are needed since we should not absolutely believe in in vitro studies which are designed in normoxia since in our body the stem cell niches are in physiological hypoxia. This work was supported by the FAPERGS and CNPQ/BR.

Poster Session 3
Wednesday, December 4, 2019, 4:30 PM - 6:00 PM

390 - Serum-Free And Size-Controlled Adipose-Derived Stromal/stem Cells Spheroids Robotic Biofabrication

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***Purpose/Objectives:** Spheroids 3D culture have been reported to improve the stemness, secretion capacity and angiogenic effectiveness of adipose derived stem/stromal cells (ASCs). Considering the broad range applicability of homogeneous spheroids with specific size in several fields (e.g. 3D bioprinting, therapeutics, as alternative methods for animal testing, drug development, cancer research, cell biology, nanoparticles exposure, etc), it is mandatory to develop automated platforms using robotics technology able to scale-up the spheroids biofabrication process in a serum free environment. Our aim was to develop an automated platform for ASC seeding to biofabricate size-controlled spheroids. We also inestigated whether different sizes of spheroids have implications in their morpho-functional properties.
***Methodology:** ASC spheroids were formed using the technique of micromolded non-adhesive hydrogel (3D Petri Dish; MicroTissues, Inc.). The Epmotion 5070 pipetting platform was used to seed ASC suspension into the micromolded agarose (biofabrication). Three different concentrations of ASC suspension were used: $2.7x10^5$, $6.5x10^5$ e $2x10^6$. At day 7 of culture the following analysis were performed: cell viability by fluorescent microscopy and flow cytometry, ultrastructure by scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM), morphology by phase contrast microscopy and histology. Secretory profile was assessed by Cytometric Bead Array (CBA kit) and Multiplex. Cell cycle was assessed by flow cytometry. Biomechanical properties of ASC spheroids was measured using the equipment microsquisher (Cell Scale, Canada).
***Results:** ASC spheroids were successfully biofabricated showing a cell viability of 90%. It was possible to achieve three distinct diameters of ASC spheroids seeding $2.7x10^5$, $6.5x10^5$ and $2x10^6$ corresponding to 200, 300 and 400µm respectively ($p<0.0001$). ASC spheroids showing 400µm of diameter had higher secretory levels of VEGF ($p<0.0001$), IL-6 ($p=0.0002$), IFNγ ($p=0.0022$), IL-8 ($p<0.0001$), IL-10 ($p<0.0001$), IL-15 ($p=0.0005$) and IL-12P70 ($p=0.0002$). Histological and ultrastructural analysis revealed that ASCs located in the surface of spheroids have a fibroblastic morphology when compared with those located inside. Evaluation of biomechanical properties showed that spheroids showing 200µm of diameter had lower force of compression than spheroids showing 400µm ($p<0.0001$). Cell cycle analysis are in progress.
***Conclusion/Significance:** The stage of ASC seeding for spheroids formation was successfully automated. Biofabricated ASC spheroids showed controlled-size and high cell viability. Farther, spheroids with different diameters have distinct morpho-functional properties.

391 - Cad/cam Design And 3d Print-based Fabrication Of An Automatable Perfusion Machine For Engineering Soft Tissue

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***Purpose/Objectives:** Current methods for tissue decellularization are abundant and reliable, but also laborious and rigid. A challenge is to develop a single, modular system that allows the versatility to decellularize tissues in the same device that they will be subsequently recellularized and transported to the point of care. The objective of this work is to fabricate a sharable platform capable of automated soft tissue engineering. A prototype of an integrated perfusion machine has been designed, constructed and tested for capacity of decellularization, with the relevant components for recellularization integrated including heating feedback, humidity, and CO2 sensing.
***Methodology:** Scaffolding for the perfusion machine were designed in CAD/CAM and then 3D printed in polylactic acid (PLA) using fused filament fabrication. Electronics are integrated to support decellularization and, prospectively, recellularization, which include two peristaltic pumps governing flow rates, two 3-way valves for fluid exchange, and control of a sterilizable chamber in which the tissue is contained throughout the process. Each are integrated by a customized printed circuit board serving as an Arduino shield, which is an open-source platform. Control and automation of the system is governed by LabVIEW, which also serves as a data acquisition system for exporting and saving parameters throughout a process. Soft tissues are capable of decellularization either through direct infusion, such as with skeletal muscle, or by perfusion as in kidney or heart, where vascular access through a pedicle can facilitate exposure to surfactants and disinfection solution.
***Results:** Using the embedded controls, the innovative perfusion machine is capable of controlling fluid flow and temperature, while simultaneously monitoring for flow rate, intra-scaffold pressure, chamber temperature, humidity, and gaseous CO2 within the tissue chamber. Tissues without vascular pedicles can be decellularized by infusion and vascularized composite tissues can be decellularized by perfusion in the same system. Significant reduction in DNA content was quantified while the overall macrostructure was preserved, as observed histologically.
***Conclusion/Significance:** The perfusion machine designed is a compact, integrated system capable of successful decellularization of multiple tissue types with the relevant components integrated for recellularization and long-term culture. 3D printed design of the components allow rapid fabrication and adjustment of parts in the event of changing needs or part failure. Further, this platform is constructed on open-source electronics, allowing researchers to expand and customize the system as necessary for applications in tissue decellularization, recellularization, ex vivo modeling of whole tissue systems, or dynamic cell culture systems.

393 - Establishment Of The Ex Vivo Cornea Model For A Drug Toxicity Evaluation

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***Purpose/Objectives:** To evaluate the toxicity of the ophthalmic drug, the Draize test commonly used (OECD, test guideline 405). The various animals have been utilized for irritation test to determine the efficacy of a drug. Experimental animals were under stress and pain due to long-term exposure to the

drug. Regarding physiological functions, experimental animals do not properly reflect a human eye condition. Thus, cornea *ex-vivo* models such as EpiOcular™ (MatTek, USA) and HCE (SkinEthic, France) were presented as alternative evaluation methods of animal testing. However, these commercial eye test *ex-vivo* models did not mimic the original tissue structure. In this sense, the cornea tissue mimicked structure was fabricated to realize the *ex-vivo* toxicity model in this study.
***Methodology:** The corneal epithelial cells (CECs) and keratocytes (CKs) isolated from rabbit eyeball were seeded on non-patterned silk film (SF) and patterned silk film (pSF) at 32,500 cells/cm^2 and 6,500 cells/cm^2. Sequentially, pSF were stacked to mimic a multi-layered stroma structure. The thickness of films was about 15.63 µm and the distance of patterns was about 3 µm. A and B stain were performed to confirm the cell proliferation on each layer and cytoskeletal alignment on pSF. To assess the barrier function, the permeation test of the cornea model was conducted with high-performance liquid chromatography (HPLC) using Franz diffusion cell compared with the original cornea. Cell viability was also investigated to assess drug toxicity.
***Results:** The CECs and CKs showed good cell attachment on the SFs and pSFs. Proliferated cells expressed the specific phenotype of corneal epithelium and stroma. Additionally, cytoskeletal alignment was confirmed along with patterns of pSF. The barrier function was verified by similar permeation between the rabbit cornea and bio-engineered cornea model. The evaluating efficacy of the suggested model for drug toxicity was confirmed by decreasing cell viability after treatment of medication.
***Conclusion/Significance:** We successfully established the *ex-vivo* cornea toxicity model to replace the eye irritation animal tests. In further studies, we will set up the *ex-vivo* cornea toxicity model using human cells and then will evaluate the drug screening efficacy.

394 - Flow Perfusion Flowrate Influence On MSC Oxygen Consumption Rate Cultured In 3D Porous Scaffolds.

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***Purpose/Objectives:**
Bone grafts comprise a multibillion dollar industry. These grafts are commonly autologous or allogenic. Autologous grafts are taken from the patient, and are sourced from a donor site from the patient's body. Allogenic grafts are derived from cadavers. Each of these graft types are associated with issues such as donor site morbidity in autologous grafts and immunological response in allogenic grafts. Bone tissue engineered constructs are a logical approach to combat the issues commonly encountered with current autologous and allogenic bone grafting techniques. While it is possible to grow bone tissue engineered constructs *in vitro,* it is necessary to destroy the construct to determine the number and type of cells present. Recent work has led to the development of models that have the potential to predict the number and types of cells within the construct through metabolite monitoring. The metabolites, such as glucose and oxygen, are used to monitor cell growth while the construct remains in the bioreactor, eliminating the need to lyse cells and destroy the scaffold. This allows for the development of quality assurance methodologies that are utilized on the specific graft to be used on a patient. These models, however, neglect to account for factors affecting the construct, such as flow rate and scaffold geometry.
***Methodology:**

This study aimed to determine the flow characteristics present within the bioreactor system, and hoped to quantify the effects of these characteristics on oxygen uptake rates of mesenchymal stem cells. The work done utilized a residence time distribution analysis using an easily monitored dye to develop residence time distribution functions, and associated these functions with literature values to characterize the flow patterns and residence times of media moving through the construct. Oxygen concentration was measured using the NeoFox Kit with RedEye® oxygen sensing patches.
***Results:** Findings indicate that flow within the bioreactor system is well approximated by linear tubular flow reactors, associated with gradients in the radial direction at low flow rates. These radial gradients, though previously undetected, are likely indicative of varied oxygen consumption rates by the stem cells throughout the construct. Additionally, oxygen uptake rates of stem cells in these perfusion reactors have a strong linear correlation with residence times of media in the cassette, providing the data needed to develop a predictive model for oxygen uptake rates based on flow rate. This data shows that as residence time decreases, mesenchymal stem cells increase their oxygen uptake rates. The correlation between these two variables could potentially be caused by the switch from diffusive mass transfer to convective mass transfer as flow rate increases, since increasing flow is associated with decreasing residence time. A theoretical maximum oxygen uptake rate by mesenchymal stem cells could also be inferred by assuming a residence time of zero. The relationship found suggests that continued replenishment of oxygen will lead to a maximum oxygen uptake rate by the cells of approximately 3.9 picomoles O_2/hr/cell.
***Conclusion/Significance:** These models show promise for providing corrective functions for on-line oxygen and glucose monitoring systems in current bioreactor designs.

395 - Microfluidic System Enables Ex Vivo Culture Of Pancreatic Islet Clusters In A Bio-inert Matrix Under Continuous Flow

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***Purpose/Objectives:** There is a growing interest in utilizing 3D cell culture systems for the application of drug discovery and tissue engineering with the aim to better mimic *in vivo* microphysiology. To create suitable microenvironments, particularly to support highly metabolically active cell clusters, such as the pancreatic islets of Langerhans, key challenges revolve around engineering a system that provides an oxygen-rich microenvironment and a supportive (3D) microarchitecture. Microfluidic platforms are advantageous, compared to the traditional static culture systems, in creating dynamic microenvironments that decrease diffusion gradients. In this regard, we have engineered a unique PDMS-free fluidic platform to investigate 3D clusters[1]. This benchtop microphysiological system (MPS) permits dynamic flow, simple cell loading, spatio-temporal tracking, and characterization of cell clusters within 3D matrices without inhibiting readouts. We have incorporated an oxygen permeable membrane at the base of the islet MPS niche to allow for local oxygen diffusion to the islets in culture. Moreover, we have optimized and demonstrated the capacity of our islet MPS to maintain and monitor the longevity of isolated islets embedded within a bio-inert alginate matrix.
***Methodology:** Rodent islets were embedded in 1.6% alginate hydrogels at a density of 75 islets per 25µL of alginate and cultured in traditional static culture dishes or in our microfluidic MPS, with or without an oxygen permeable membrane for up to 72h.

***Results:** Islets continuously perfused for 24h showed retention in cell viability, up to 97%, contrary to significantly reduced viability observed in static culture, at 89%. Spatio-temporal tracking of individual islet clusters was carried out by continuously perfusing fluorescent substrate reporter for live cells undergoing caspase 3/7 pathways that eventually results in apoptotic cell death. Percent apoptotic cells at 48h were significantly lower in islets cultured in our MPS compared to islets in static culture. Furthermore, rodent islets were unresponsive to a dynamic glucose stimulated insulin secretion (dGSIS) challenge after only 24h, while dynamically cultured islets demonstrated preserved functionality, as assessed by a sequential dGSIS challenge at 24, 42, and 72h. These trends were validated using human islets where the total insulin secretion during the high glucose stimulation phase for dynamically perfused islets within the MPS for 72h was found to have a 25-fold increase, when compared to islets in static culture.
***Conclusion/Significance:** Overall, we demonstrated a use of microfluidic MPS for *ex vivo* stabilization of pancreatic islet viability and functionality. With this ability to support highly metabolically active 3D clusters, basic research and translational studies, reflective of physiologically relevant information in a dynamic and 3D fashion, can be performed more efficiently. Future work is focused on expanding these MPS platforms to provide additional biomimetic features.
References: 1. Lenguito, G. et al. Resealable, optically accessible, PDMS-free fluidic platform for ex vivo interrogation of pancreatic islets. Lab Chip 17, 772-781 (2017).

396 - IPSCs-Derived Osteochondral Tissue Chip To Model Joint Physiology And Osteoarthritis Pathology

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***Purpose/Objectives:** In this study, we aim to develop an osteochondral tissue chip (OC-TC) to model osteoarthritis (OA) pathology and screen disease-modifying OA drugs (DMOADs). Due to their theoretically indefinite proliferation capacity, induced pluripotent stem cells (iPSCs) from humans were used in this study to generate the biphasic osteochondral tissues within a customized dual-flow bioreactor (patent pending). After the characterization of tissue phenotype, an OA-mimicking condition was created on OC-TCs. Finally, we validated this novel drug testing system by investigating the tissue response to known OA managing drugs.
***Methodology:** iPSCs were first differentiated into mesenchymal progenitor cells (iMPCs). iMPCs-derived OC-TCs were generated using a protocol developed in our laboratory (1). Briefly, in this novel dual-flow bioreactor, chondrogenic medium was perfused through the top part of the construct, in order to generate cartilage on one side. Osteogenic medium was perfused through the bottom part, inducing bone formation on the other side. The phenotype of the osteochondral tissue was characterized through real-time PCR and histology staining. To create an OA model in OC-TCs, 1ng/ml interleukin-1β (IL-1β) was applied to either the top or bottom flow to challenge cartilage or bone. Osteochondral tissue without IL-1β treatment served as a control. In addition, to study the tissue crosstalk under physiological or OA conditions, we engineered tissue chips that only contained cartilage or bone. Lastly, the potential DMOADs such as celecoxib were applied to this OA model to validate the novel system. Real-time PCR, histology staining, immunofluorescence, Western Blot, ELISA, and micro-

CT assays were conducted to analyze the tissue's phenotype and crosstalk between cartilage and bone.
***Results:** PCR and histology results indicated the generation of two distinct phases in the newly formed tissue, with cartilage on the top part, bone on the bottom, and a transient zone in between, suggesting the successful development of an iPSCs-derived osteochondral tissue chip. After stimulation with IL-1β, the chondral and osseous parts of the osteochondral tissue showed much stronger inflammatory and catabolic responses than the chondral and osseous tissues alone in the single-phase constructs, clearly indicating the bone-cartilage crosstalk in the OA progression. The application of celecoxib, a nonsteroidal anti-inflammatory drug (NSAIDs), attenuated the inflammatory effect caused by IL-1β. Interestingly, we also found that celecoxib might enhance the expression levels of chondrogenic marker genes, thus reducing/restoring the cartilage damage caused by IL-1β.
***Conclusion/Significance:** The human iPSCs-derived microphysiological osteochondral tissue chip developed here realized the ambitions of generating a robust *in vitro* platform for high-throughput screening of DMOADs, which eliminates or reduces the use of live animals. The biphasic tissues also allow the study of molecular and cellular crosstalk between cartilage and bone during OA progression.

397 - Optimized Combination Of Photoinitiator And UV-absorber In Digital Light Processing-based 3D Bioprinting

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***Purpose/Objectives:** 3D bioprinting technologies have been widely used as a fabrication tool that can incorporate living cells and biomaterials into the target 3D design. Among the various printing methods, digital light processing (DLP) printing enables predominant speed, resolution, and adaptability for fabricating complex 3D tissue structures. For DLP printing, photoinitiators (PIs) and UV-absorber (UAs) both play a critical role during the polymerization of photo-sensitive polymers that contain cells. PI produces free radicals which initiate the photopolymerization of bioink. Therefore, the light wavelength should be matched with the excitation wavelength of PI. Too low amount of PI cannot fully cure the hydrogel and too much PI results in cell cytotoxicity. On the other hand, UA blocks UV light, preventing over-curing of layers beyond the focal plane. Without UA, it is impossible to print the open channel structure; however, over the amount of UA increases the printing time and restricts the proper photo-curing process. In this study, we aimed to optimize a combination of PI and UA for DLP-based 3D bioprinting, which provides high cell viability and printing resolution.
***Methodology:** We have chosen multi-arm poly(ethylene glycol) (PEG) as a base material for the bioink and modified it to utilize the double bond for photo-crosslinking. Commercially available water-soluble PIs and UAs were prepared and tested for their feasibility in DLP bioprinting.
***Results:** The PI conveys the electromagnetic energy of light to chemical energy by generating free radicals which initiate the photo-crosslinking. We first tested the photocuring ability of PIs with high and low intensity of UV light (λ = 365 nm). Among 11 candidates of PIs, lithium phenyl(2,4,6-trimethyl benzoyl) phosphinate (LAP) showed the most stable photo-crosslinking behavior and used for the further screening of UAs. The main role of UA is to block the scattering UV light inside the hydrogel and confine the photo-crosslinking to the focal projected layer. This function not only increases the resolution but is also critical for printing open channel structures. Therefore, the UV block function of UA candidates was tested and printability of open microchannel structure (D = 2 mm) was observed.
***Conclusion/Significance:** LAP was the most optimal candidates among the tested PIs and three

candidates of UA (R1800, R1888 and UV377A) were shown to be feasible through initial screening tests. In addition, cell cytotoxicity of the UA candidates is being tested using cells. The selected PI/UA combination has the potential to be used for future DLP bioprinting applications.
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398 - 3D In Vitro Models For Cancer Cell / T-cell Interactions

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***Purpose/Objectives:** 3D in vitro testing for oncological research is significantly underdeveloped in the literature. The transition from 2D to 3D in vitro models elicits microenvironmental changes that affect expression, morphology, and cell communication. A reliable platform to test immunological influences of cancerous components and environments in 3D will provide an ideal in vitro system to isolate their effects. Our first objective is to create a well-characterized system to immobilize CD8+ T-Cells while maintaining their activation state. The second objective is to test whether the same techniques translate to 3D printed scaffolds for use in bioreactor systems.
***Methodology:** 2D poly-(L)-lactic acid (PLLA) polymer films have been used to test T-cell viability before 3D printed scaffolds could be used. PLLA films were made using well-characterized 98.5% L PLLA pellets dissolved in chloroform and evaporated overnight. The films were then incubated with varying concentrations of poly-lysine in a 7:3 solution of acetone and water for 12 hours. The films were then incubated with the same concentration of SPDP for 1 hour, and then incubated with the same concentration of RGD for 1 hour. The prepared scaffolds were then seeded with CD8+ T-Cells and incubated for 12 hours with 10% FBS-1% AA RPMI solution. The T-Cells were then inspected visually for attachment and cell-spreading before stimulation using PMA for 4 hours. An ELISA was used to collect cytokine release. 3D scaffolds were printed using self-made, extruded polymer fiber using the same PLLA pellets and a MakerBot 3D printer.
***Results:** 2D 8mm scaffolds showed that increasing RGD densities increased CD8+ T-Cell attachment by 1200±100% compared to poly-lysine modified scaffolds and unmodified scaffolds while remaining activated. The 2D films modified with RGD showed an increase in T-Cell immobilization compared to films using only poly-K and a substantial increase compared to unmodified films, where no immobilization was present. 151±101 nmoles RGD scaffolds allowed for 3000±100 cells with decreasing T-Cell attachment when decreasing RGD densities. The PMA stimulation tested for T-Cell activation as well as presented a reliable method to quantify immobilized T-Cells. Interestingly, increased RGD density decreased overall T-Cell spreading present on each scaffold. 3D printed surface modified PLLA scaffolds show similar results while maintaining CD8+ T-Cell activation.
***Conclusion/Significance:** The 2D films showed a viable method for T-Cell attachment and activation and denote successful 3D results. Future studies include bioreactor use with differing shear rates to determine detachment as well as incubation with cancerous exosomes to test their effects on immunosuppression and decreased activation in 3D environments.

399 - Reconstruction Of Orbital Wall Using Standardized, Commercialized Three-dimensional Printing Implant

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***Purpose/Objectives:** For the reconstruction of orbital fracture, it is essential to obtain the accurate shape and position of the implant. Manual-bending implants did not achieve correctly, and pre-bent implants made by rapid prototyping (RP) generated additional cost and time to produce a contour model before surgery. We standardized Korean orbital cavity data to fabricate orbital implants using three-dimensional (3D) printing and apply it to simulation model and patient.
***Methodology:** After averaging the CT data of 100 Korean adult cadavers, we extracted the inferomedial orbital strut that migrated from the medial wall to the floor and created a tangent sphere using CAD program. The curves were compared with the CT data of 20 adult patients. Based on this data, standardized 3D implants for Korean were created and applied to complex orbital fracture.
***Results:** The radius of the sphere in contact with the orbit was measured as 33.54mm. It was confirmed to be well covered in the simulation model. Ratios of the preoperative (Vpre) and postoperative (Vpost) volume to the normal volume (V0) of the orbital wall were compared. The mean Vpre/V0 was 112.59%, and the mean Vpost/V0 was 103.72%, thus the postoperative orbital volume was not statistically different from the normal orbital volume.
***Conclusion/Significance:** Standardized 3D implants for Korean provide anatomically precise restorations in comparison with manual-bending implants. Compared to pre-bent implant using RP, it shows an acceptable result while reducing cost and time. It can be manufactured as ready-made commercialized product. Moreover, it has good results for complex orbital fracture.

401 - A Software Tool For Automated Generation Of Customized Organ-on-chip Devices

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***Purpose/Objectives:** Biofabrication is increasingly seen as the ideal solution for generating *in vitro* models. Not only it allows to accurately deposit and assemble specific materials (including cells) into complex 3D constructs but also allows to culture, stimulate, and analyze them into highly controlled and dynamic environments. Given the complexity of the human body, it becomes necessary to develop tools which are able to automatically generate body-mimicking architectures and environments, particularly in a body-on-chip fashion. The purpose of this work was to demonstrate the limitless ability of newly developed software tools to generate body-like structures and environments and in this way democratize the access to organ-on-chip technologies.
***Methodology:** Parametric design methodologies were employed in order to enable automated generation of 3D device designs upon insertion of user-defined settings. Resulting parametrically designed shapes were then physically materialized by employing additive manufacturing and bioreactor know-how in order to produce fully functioning organ-on-chip devices which may be composed of an array of customizable designs and features.
***Results:** It was possible to demonstrate that parametric design methodologies combined with additive manufacturing and bioreactor technologies may allow to easily, intuitively and automatically generate organ-on-chip devices with endless degrees of complexity, which may be employed in a wide array of

applications.
***Conclusion/Significance:** The combination of "smart" software tools with highly automated technologies such as additive manufacturing and bioreactors may lead the way to democratizing the access to organ-on-chip technologies. Furthermore, it may as well open new avenues towards a deeper understanding of the human body by means of a greater ability to mimic its physiology and morphology under *in vitro* controlled environments. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 798014.

402 - Discovering Macrophage Role In Ocular Pathologies Using 3d Bioprinted Rpe/choroid

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The purpose of this study was to investigate the role of mature macrophages on choroid development and pathology. We previously developed on a 3D-Bioprinted *in-vitro* outer blood retina barrier that contains an intact, confluent RPE monolayer and a "choroid" with a dense capillary network. In this model, we're able to study ocular pathologies such as choroidal neovascularization (CNV) and degenerative conditions in the back of the eye. By adding these components, we aim to increase the accuracy of our *in vitro* models. Methods: A collagen-derived gel is used for encapsulation of endothelial cells, choroidal fibroblasts, and ocular pericytes for bioprinting to provide microenvironments conducive for microvascular network formation. We bioprinted a vascularized tissue on the basal side of a degradable PLGA scaffolds. We then seeded IPSC-RPE on the apical side at 7 days post printing. Primary M1 and M2 polarized macrophages were added to the tissues. A combination of confocal microscopy, cytokine analysis, trans-epithelial resistance measurements, and flow cytometry were used to evaluate the health of these constructs. Results: We observed M1 and M2 specific vascular phenotypes at various time points. M2 macrophages promoted tissue growth. In contrast, M1 macrophages supported vasculogenesis only when added on day 0. When added to the tissue at later timepoints, M1 polarized macrophages curtailed angiogenesis. Conclusions: We have successfully created an *in-vitro* construct which allows for investigation of immune mediated development and pathologies of the eye. Future studies will attempt to expand on the mechanisms of macrophage subpopulations in the choroid and RPE.

403 - 3d Printing Of Bio-compatible Adhesive And Conductive Ink For Skeletal Tissue Engineering

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The conductive and adhesive behavior of the material synthesized enable their use as a bio-ink for 3D printing various shapes, the biocompatibility, rheological properties and the ability to alter the properties based on the percentage of Ionic liquid provides a wide range of application. When incorporated with choline acrylate, these structures constitute biomaterial for bioprinting and 3D cell culture applications. Although recent studies have successfully fabricated stretchable electronics consisting entirely of soft materials, conductive hydrogels and dielectric elastomers, thus far these

fabrication techniques have relied on casting or a combination of extrusion printing with other methods to improve the desirable properties. In this study, we aimed to address these limitations by engineering an adhesive and conductive bio-ink based on visible light photo-curable Ionic liquid functionalized hydrogel. We explore the potential application of this printed structure as biomaterials for 3D cell culture through studies biocompatibility. We found that the percentage viability of cells cultured is 98%±1% with conductivity and adhesive strength of 0.279±0.02 S/cm and 34.3±1.9 KPa for the highest percentage of GelMA anf Choline Acrylate. Therefore, the proposed bio-ink with high processing capabilities are promising materials for the development of biodegradable adhesive and conductive substrates, particularly 3D scaffolds for possible use in tissue engineering.

404 - Design, Manufacture And In Silico Assessment Of A Novel Bioreactor System For Evaluation Of SIS-based Vascular Grafts

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Since commercially available vascular grafts do not have regenerative capabilities and eventually require surgical replacement, it is of interest to develop regenerative vascular grafts (RVG). Decellularized SIS is an attractive material for RVG, however, the performance evaluation of these grafts is challenging due to the absence of devices that mimic the conditions found *in vivo*. Therefore, the objective of this study is to design, manufacture and validate *in silico* and *in vitro* a novel bioreactor system for evaluation of human umbilical vein endothelial cells (HUVECs) proliferation on SIS-based RVG, under dynamical conditions as those found *in vivo*. Our perfusion and rotational bioreactor system was designed with the aid of Autodesk Inventor 2018. *In silico* validation of the system was carried out using ANSYS Fluent software for three dynamical conditions (perfusion, rotation and both simultaneously). Mechanical and biological parameters such as flow regime, pressure gradient, wall shear stress, sterility and indirect cell viability (MTT assay) were also evaluated. Cell adhesion was assessed by H&E stain and SEM. The fluid flow regime within the system remains laminar and exhibits pressure losses below 1 mmHg. During operation, the system maintained sterility and showed low cytotoxicity levels. HUVECs were successfully cultured on SIS-based RVG under both perfusion and rotation conditions. *In silico* analysis agreed well with our experimental results and recent reports on *ex-vivo* cell culture. The system presented is a tool for evaluating RVG and represents an alternative to develop new methods and protocols for a more comprehensive study of regenerative cardiovascular devices.

405 - Gene Expression Studies For Analyzing Effects Of Space Radiation In Tissues

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***Purpose/Objectives:** Ionizing radiation causes alteration in gene expression of cells [1]. Therefore, it is important to identify these changes in gene expression to analyze the effect of space radiation in cells and tissues. Previous studies have employed real-time PCR to assess the effect of ionizing radiation on gene expression in embryos that were irradiated *in-vivo* after fertilization and allowed to develop for 16 weeks [2]. The goal of the current study is to analyze the effects of ionizing radiation in heart tissue by

quantifying its impact on the expression of genes that correlate with chronic inflammatory responses and indicative of the presence of Reactive oxygen species (ROS).
***Methodology:** Heart tissue samples were obtained from irradiated 900 c57bl6 breed mice. The tissue samples were crushed using tissue pulverizer and RNA was isolated using TRIzol method. RNA was reverse transcribed to synthesize cDNA and real-time PCR run was performed by using TaqMan Gene Expression assays for interleukin 6 (IL6) and superoxide dismutase 2 (Sod2). Target gene expression was quantified using $2^{-deltadeltaCt}$ method by normalizing the expression of target gene with house-keeping genes (Actb and Hprt) and relative to their expression on control non-irradiated samples.
***Results:** A total of five irradiated samples and three control samples were assessed. The quantity and purity of isolated RNA was confirmed by spectrophotometric analysis, which showed that the ratio of absorbance at 260 nm versus 280 nm was between 1.8 and 2.0 for all samples. Results showed that expression of IL6 was upregulated in two samples while downregulated in three samples. For Sod2, slight upregulation was observed in three samples while downregulation was observed in the other two samples. These results suggest that ionizing radiation differentially impacts each experimental sample by modulating gene expression. A parametric study of different irradiation doses (15 to 200 cGy) and different sources of radiation (Iron, Oxygen, and Gamma) will need to be performed for more in depth analyses of the effect of radiation on gene expression.
***Conclusion/Significance:** In conclusion, alterations in the gene expression may result in severe damage to the cells causing cellular mutations and cell destruction. In this study it is observed that ionizing radiation alters the gene expression of IL6 and Sod2. This alteration in gene expression may result in inflammation and is indicative of the presence of oxidative stress. IL6 is a pro-inflammatory cytokine while Sod2 is produced in response to limit the effects of reactive oxygen species. Future studies will focus on investigating the impact of irradiation on the expression of anti-oxidative response genes to better understand the changes in gene expression pattern, and develop ways to prevent these biological changes. **Acknowledgements:** This work was supported by NASA Florida Space Grant Research Program.
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406 - Potential Of Induced Pluripotent-derived Mesenchymal Stem Cells To Generate Tissue-engineered Heart Valves

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***Purpose/Objectives:** The durability of the native heart valve is maintained by valve interstitial cells, which synthesize and remodel the extracellular matrix. Identifying the ideal cell source for generating tissue-engineered heart valves is still crucial. In cardiovascular research, MSCs have been evaluated as promising cell sources due to their ability to differentiate into the various cell types and their self-renewal capacity. Induced pluripotent stem cells (iPSCs) have a high medical potential, which can be used to generate autologous mesenchymal stem cells (iMSCs). iMSCs, in addition, acquire a rejuvenation gene signature circumventing aging-associated drawbacks of primary MSCs. Here, we compared the in vitro seeding capacity of iMSCs on biological and synthetic materials to generate tissue-engineered

heart valves.
***Methodology:** Human iPSCs were generated from the skin biopsy and cultured on a basement membrane-like matrix in special cell culture medium for stem cells. Differentiation of iPSC into iMSCs via the induction of mesoderm cells was confirmed by trilineage differentiation and flow cytometry. iMSCs were seeded on decellularized leaflets and sheets made of PCU (polycarbonate urethane) and biodegradable, randomly oriented electrospun PCL (polycaprolactone) nanofibers. Analysis of surface colonization, DNA content, histology and surface topography quantified the seeding success.
***Results:** Successful differentiation of iPSCs into iMSCs was confirmed by differentiation into osteocytes, adipocytes and chondrocytes. Derived iMSCs had $CD34^-$, $CD44^+$, $CD45^-$, $CD90^+$, $CD146^+$ and $CD166^+$ surface expression. Histological analysis of decellularized porcine pulmonary leaflets revealed complete removal of all cellular components. Seeding of iMSCs on biological and synthetic scaffolds resulted in efficient cell attachment on all surfaces. Gelatin coating of PCL nanofibers improved cell attachment. Increased DNA content of decellularized leaflets, histology and topographic analysis of biological and synthetic material confirmed efficient seeding results.
***Conclusion/Significance:** This study provided a proof of concept that iMSCs generated by a skin biopsy of the patient efficiently recolonized the surface of biological and synthetic material. Further investigations will focus on the ingrowth of cells, formation of an extracellular matrix and the mechanical properties to quantify the functionality.
Acknowledgements: Financial support was received from the German Centre for Cardiovascular Research (DZHK) and the German Heart Research Foundation (DSHF).

408 - Response Of Human Mesenchymal Stem Cells To Physiologically Modeled Mechanical Stimuli In Tissue-engineered Vascular Grafts

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***Purpose/Objectives:** Typical approaches to reengineer small diameter vascular grafts result in cell populations persisting in a wound state that ultimately drives a range of occlusive pathologies such as thrombosis, neointimal hyperplasia or atherosclerosis. As such, methodologies to drive cells from a wound state toward a functional, less reactive, state may limit the aforementioned pathologies. However, transport limitations typically inhibit cell wound state phenotype and results in poor construct development. The aim of present work was to develop an approach to rapidly repopulate the scaffold by regulating transmural hydraulic conductivity and apply a novel pressure/pulse condition (physiological modeled dynamic stimulation (PMDS)) to drive cells toward a functional state that mimics the typical non-proliferative, anti-thrombotic and anti-inflammatory state of healthy vessels.
***Methodology:** Using a model *ex vivo*scaffold derived from the human umbilical vein (HUV) and duel circuit perfusion bioreactors the effects of constant pulse frequencies (CF) and PMDS on changes in human mesenchymal stem cell (MSC) phenotype. Human Wharton's jelly MSC (hMSC) were isolated from birth tissues, expanded to <P5 and used in all experiments. Prior to MSC seeding, scaffolds were incubated in a cocktail of growth factors derived from human placenta cotyledons (human placental matrix (hPM)). MSC were seeded on the abluminal surface of the HUV and cultured at low lumenal pressure (~30mmHg) for 5 days with the goal of maximizing hMSC to SMC differentiation and cell migration into the vessel wall. The lumenal surface was then seeded with hMSC in endothelial growth media (ECGM) for a further 5 days. CF(10dynes/cm^2at 1Hz) was compared to PMDS (7.2-18.4dynes/cm^2

with frequencies set between 0.8-2.6 Hz). PMDS pulse/frequency were simulated from real-time temporal fluctuations observed in healthy blood flow[1].
***Results:** Scaffolds incubated in hPM in combination with low lumenal pressure displayed enhanced cell adhesion and migration, where the directed nutrient gradient encouraged cell migration toward the lumenal nutrient source. MSC adhesion and proliferation by 30% and 55% respectively as compared to control samples. Furthermore, co-cultured grafts exposed to PMDS conditioning displayed extensive tissue remodeling displaying multiple layers of smooth muscle myosin heavy chain (SMMHC) expression on the medial/adventitial boundary, and von Willebrand factor (vWF) -positive cells lining in the lumen. While overall metabolic activity remained consistent, PMDS flow conditioned cells released higher levels nitric oxide (NO) (32.45 ± 7.39 vs. 68.32 ± 8.13nmol/10^5cells, $p<0.05$) than those exposed to CF after 5-days perfusion. While the mechanism by which hPM modulates cell function is yet to be resolved, the cooperative effect of hPM and PMDS to drive MSC toward phenotypically discrete cell types (EC, SMC and stromal cells) is a clear. These data suggest that more complex cocktails of growth factors are required to drive cells toward these discrete cell functionalities.
***Conclusion/Significance:** In this present study, we have successfully differentiated in situ human primary MSC into two different vascular cells lineage. The feasibility of rapid fabrication of MSC-seeded TEVG suggest that functional vascular cells can be manipulated by modulating environmental factors, including complex growth factor cocktails to advance our understanding, and control, or vascular regeneration.

409 - Self-assembly Of Endothelial Cells Into 3D Microvascular-like Networks Under Defined Conditions

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***Purpose/Objectives:** Non-vascularized tissue-engineered constructs are restricted in size due to the limitation for nutritional supply of the integrated cells by diffusion. Therefore, as a minimal requirement a vascularization needs to be implemented into 3D tissue engineered constructs. In this study a self-assembly strategy of endothelial cells in co-culture with mural cells was employed addressing: **a)** hydrogel generation from xeno-free components, **b)** culture of construct under defined serum-free conditions and **c)** the characterization of the generated construct.
***Methodology:** GFP labeled human umbilical vein endothelial cells (EC) were combined with human adipose-tissue derived stromal cells in a hydrogel cast onto decellularized small intestine submucosa and cultured in either endothelial cell growth medium-2 or serum free medium[1,2]. The hydrogel contained either Matrigel/rat tail collagen I or human collagen I derived from fibroblasts[2].
***Results:** The generation of stable EC networks is observed under all conditions[2]. The 3D quantification of the network revealed a slightly thicker construct for the matrigel/rat collagen I-based hydrogel compared to human collagen I-based constructs[2]. Additionally, the EC within the formed network were able to produce their own extracellular matrix proteins (e.g. Collagen IV). A staining against -smooth muscle actin (α-SMA) revealed the presence of α-SMA positive cells along the EC cords which seem to be

in physical contact with EC[2]. Moreover, Texas red-labeled dextran was detected in structures encircled by EC indicating the presence of hollow structures within the EC network[2].
***Conclusion/Significance:** Stable EC networks are generated in a xeno-free hydrogel in serum-free medium. The retrieved EC structures are hollow and enwrapped with α-SMA positive cells indicative of a microvascular EC network stabilized by pericytes[2]. This achievement represents a fundamental step towards a clinical applicable vascularized construct.
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410 - Isolated Microvessels For The Vascularization Of Implantable Tissues And Tissue Models

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***Purpose/Objectives:** The ability to build or manipulate microvasculatures is critically important for both *in vivo* vascular regeneration and *in vitro* tissue fabrication. In the repair or construction of a functional microvasculature, it's important to consider that an effective microcirculation critically depends on the proper organization of stable microvessels into a perfusion-competent network. Therefore, successful neovascularizing strategies must address the construction of individual microvessel elements, and also post-angiogenesis assembly of these new elements into an organized and functionally matched vascular network. Microvascular form and function depend on a dynamic interplay between multiple vascular and perivascular cell types. It has become clear that angiogenesis, vascular remodeling, and vascular stability depend not only endothelial cells, but proper vessel architecture, mature matrix elements, and perivascular cells including smooth muscle cells, pericytes and vascular-niche stromal cells. These cells not only establish proper structure and stability, they impart an intrinsic phenotypic plasticity that enables the microvasculature to meet tissue-specific needs. Furthermore, the cellular complexity of a microvessel drives the microvessel remodeling and adaptation necessary for the evolution of an effective perfusion network.
***Methodology:** With these considerations in mind, we developed a versatile, enabling platform for vascularizing tissues and tissue models (for *in vitro* and *in vivo* applications) utilizing intact, isolated human microvessels. Harvested from discarded adipose, the microvessel isolate is a collection of intact arterioles, capillaries, and venules. Importantly, the microvessels contain all the varied cell types normally comprising the microvessels, including vascular niche cells such as MSCs. When cultured in 3D collagen gels, the microvessels spontaneously give rise to an interconnected network of immature neovessels with formed lumens (or neovasculature). Upon implantation, this neovasculature forms a mature microcirculation and spontaneously inosculates with the host circulation, perfusing of the implant.
***Results:** We highlighted the utility of this platform by accurately recapitulating native angiogenic sprouting and neovessel growth from isolated, intact parent microvessels that retain all intrinsic vascular and perivascular cells within a 3-D matrix environment. This system is uniquely positioned to enable the assessment of an integrated angiogenesis response. This model has been effectively used to identify and characterize angiogenic factors and inhibitors, evaluate microvascular instability, and build functional tissue models (e.g. liver, pancreas, muscle, etc.). Importantly, this versatile microvessel system is compatible with high-content analysis modalities, 3D bioprinting, and sacrificial molding approaches. Finally, in a 3D tissue model involving astrocyte precursors, important in establishing the blood brain

barrier, we show that organotypic tissue environments induce phenotypic changes in the isolated microvessel-derived vasculatures to match tissue function.
***Conclusion/Significance:** By isolating intact microvessels, instead of single cells, we retain the full complement of cells intrinsic to the native microcirculation. Consequently, the isolated microvessels exhibit the native-like plasticity and adaptability necessary to form effective, organotypic microvasculatures. We envision this tissue vascularizing system, based on isolates of native, human microvessels, will prove essential in fabricating tissues and tissue models for research, pharmaceutical, and autologous therapeutic applications.

411 - Engineering An Adipose Microtissue With A Functional Microvascular Network

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***Purpose/Objectives:** A vascularized adipose tissue model that mimics *in vivo* adipose tissue could be used for *in vitro* drug screening for therapeutics in the treatment of obesity, metabolic disease, and diabetes. In this work, we engineered a 3D adipose microtissue with a perfusable and functional vascular network within a microfluidic system.
***Methodology:** The microfluidic device consisted of cell culture chambers flanked by two side channels. Human adipose-derived stem cells (ADSCs) were first induced toward adipogenesis for 10 days prior to mixing with human umbilical vein endothelial cells (HUVECs) and normal human lung fibroblasts (NHLF) in fibrinogen and thrombin solution. The mixture was loaded into the microfluidic chambers and cultured at 37°C in a humidified incubator with 5% CO_2.
***Results:** Pre-induced ADSCs continued to differentiate into adipocytes and showed increased intracellular lipid loading. An interconnected vascular network was established within 2 weeks and formed anastomoses with the side channels. Perfusion of fluorescent dextran confirmed the functional connections of the vascular network. Perfusion of fluorescently labeled fatty acid analog through vessels resulted in the accumulation of the fatty acid in adipocytes, confirming the functionality of both the vascular network and adipocytes.
***Conclusion/Significance:** Overall, this work presented a vascularized adipose tissue model within a microfluidic device that can potentially be utilized for on-chip drug screening, as well as provide insights into potential engineering of larger volume vascularized adipose tissue.

412 - Promotion Of Anti-thrombogenicity Via Loading Resveratrol In A Polyurethane Nanofibrous Scaffold

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INTRODUCTION: Resveratrol (trans-3,5,4-trihydroxystilbene, RES) is a natural polyphenol with a wide range of beneficial properties such as a platelet antiaggregation[1], anticancer, antioxidant and anti-inflammatory[2]. Recent studies showed the beneficial effect of RES in the treatment of thrombosis[3]. Here, we investigated blood and cyto-compatibility in nanofibrous polyurethane scaffolds loaded with resveratrol. **METHODS:** Polyurethane scaffolds loaded with resveratrol drug were fabricated by electrospining technique. Mechanical properties, resveratrol release rate and anti-thrombogenicity of scaffolds were evaluated. The endothelail cell compatibility on scaffolds also were assayed. **RESULTS:** Tensile strength and Young's modulus were significantly higher in resveratrol-loaded nanofibrous polyurethane scaffolds compared to nanofibrous polyurethane scaffold alone ($p<0.001$). The resveratrol release from the scaffolds was in a sustained manner. Anti-thrombogenicity of resveratrol-loaded nanofibers increased compared to polyurethane alone ($p<0.05$). The viability of endothelial cells on resveratrol-loaded scaffolds was confirmed after 6 days ($p<0.05$). **DISCUSSION & CONCLUSIONS:** Our findings demonstrated the beneficial effect of resveratrol-loaded nanofibers not only on antithrombotic properties but also on endothelial cell compatibility of scaffolds. These resveratrol-loaded nanofibers are suggested as potential scaffolds for acellular small-caliber tissue engineered vascular grafts. **ACKNOWLEDGEMENTS:** We would like to acknowledge the financial support presented by Tehran University of Medical Sciences and Health Services (grant number: 93-01-87-24919). **Conflict of interest** The authors have no conflicts of interest to declare. **REFERENCES** [1] Lin K.H et al. Cardiovascular research. 83(3), 575-585, 2009. [2] Gambini J et al. Oxidative medicine and cellular longevity. 2015 [3] Bonnefont-Rousselot d et al. Nutrients. 8(5), 250, 2016.

413 - Multilayered Scaffolds Made From Aliphatic Polyesters For Applications In The Rational Engineering Of Cardiovascular Grafts: Manufacture And Preliminary Biocompatibility Assessment

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Aliphatic biodegradable polyesters such as polycaprolactone (PCL) and polyhydroxyalkanoates (PHAs) have attracted attention due their availability and versatility for development of tridimensional scaffolds in tissue engineering and regenerative medicine [1, 2]. A main obstacle for their implementation are their inert nature and immiscible properties with other polymers reducing their regenerative potential [3, 4]. We aimed to fabricate novel cardiovascular grafts based on PCL and PHAs. We introduced chemical modifications to their backbones so their biological, physicochemical and mechanical properties fulfill the requirements of this application. Briefly, PCL and PHAs were chemically oxidized to overcome miscibility issues and enhance biomolecular interactions. The functionalization of the materials was qualitatively confirmed via Fourier-transformed infrared (FTIR) spectroscopy. Thermal stability was analyzed via thermogravimetric analysis (TGA). Multilayered tridimensional constructs were manufactured via spin coating. The construct longitudinal tensile strength and burst strength were determined through mechanical tests. Metabolic activity (MTT) and hemolysis assays were performed to validate biocompatibility. Results demonstrated that oxidated materials exhibited carboxyl and epoxy groups, in agreement with previous reports [5, 6]. Thermal and mechanical stability of the multilayered constructs was compromised after oxidation, which was overcome by manufacturing the construct with both pristine and oxidized PCL and PHAs. Mechanical testing and burst strength of the constructs

showed properties similar to native cardiovascular tissue. Cytotoxicity of the constructs was below 20% and the hemolysis below 5% suggesting high biocompatibility and hemocompatibility. Future work will assess cell proliferation and platelet activation and aggregation as well as the assembly of constructs with cylindrical topology.

414 - Ready-to-use Bioink Formulations For Extrusion Based 3D Bioprinting

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***Purpose/Objectives:** 3D bioprinting is a rapidly growing biomanufacturing technique that combines precise positioning of living cells, biomaterials and extracellular matrix (ECM) components for layer-by-layer fabrication of 3D tissue constructs. Bioink, a critical component to 3D bioprinting living cells, contains biomaterials, ECM components, and crosslinkers in a solution form. Currently there is a shortage of commercially available ready-to-use bioink formulations on the market. Therefore, availability of ready-to-use bioinks to enable reproducible fabrication of 3D constructs is critical for supporting the accelerated growth of the 3D bioprinting field.
***Methodology:** Gelatin is one of the most widely used bioink material due to its biodegradability, biocompatibility, limited antigenicity, integrin recognition, low cost, and ease of handling. Although gelatin closely mimics the native extracellular matrix, unmodified gelatin exhibits low mechanical strength. Chemical modification of gelatin is necessary to introduce photocrosslinkable groups to provide uniform and fast curing bioinks that can be controlled to obtain different physical properties of the printed material. Therefore, we modified gelatin with different degrees of methacrylate substitution groups to enable photocrosslinking of the 3D printed structure.
***Results:** We developed and optimized Gelatin Methacrylate (GelMA) formulations to obtain suitable mechanical properties for printing at high resolution, with high cell viability and in vitro functionality. We combined GelMA and alginate, a widely used biocompatible natural polymer, to facilitate 3D printing at elevated temperature and to allow multiple modes of crosslinking for better structural stability. In addition, photoinitiator concentrations were optimized to enable photocrosslinking using ultra violet and visible light, conducive to high cell viability and functionality. Formulations were validated for cell viability, metabolic activity and ability to express muscle specific proteins using a C2C12 mouse myoblast cell line.
***Conclusion/Significance:** This study offers tangible steps forward for further research and development of novel formulations and materials for 3D bioprinting applications.

415 - Biomimetic Design Strategy For Craniomaxillofacial Reconstruction Using 3D Bioprinting Technologies

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***Purpose/Objectives:** Three-dimensional (3D) bioprinting is emerging as a promising enabling technology for tissue engineering applications. Previously, we developed a workflow to take 3D medical images and utilized them for biomimetic bone construct design, bioprinting, and *in vivo* validation [1].

Medical imaging and 3D bioprinting strategy converged to allow the fabrication of a structure with complex shape and inner architecture based on patient anatomy and biomimicry [2]. In this study, we tested a biomimetic design based on the architectural arrangement of bone, which provides a porous core for bone in-growth and a dense external layer resisting fibrous tissue in-growth.
***Methodology:** Biomimetic bone constructs were made of a composition of poly(ε-caprolactone) and β-tricalcium phosphate (PCL/TCP) and fabricated on the Integrated Tissue-Organ Printing (ITOP) system [2]. The osteoconductivity of the printed PCL/TCP bone constructs were examined by simulated body fluid (SBF) immersion test and *in vitro* osteogenic differentiation study using human placental stem cells (hPSCs). We also examined the 3D bioprinted biomimetic bone constructs in a critically sized defect of rabbits at 4 and 12 weeks.
***Results:** Testing mechanical and surface characteristics and osteoconductive properties, we found 50% TCP/PCL composite construct to be the best candidate for bone tissue engineering applications with 3D bioprinting technology. *In vivo* rabbit study showed the increased bone density and volume and new bone formation and maturation with time. Our finding suggests that bone regeneration could be enhanced in a graft designed to minimize competing for fibrotic tissue forming in the bony defect.
***Conclusion/Significance:** We demonstrated that the 3D printed bone constructs were able to organize into mature tissues of their specific characteristics *in vitro* and *in vivo*. We validated the concept that patient-specific anatomy could be translated to 3D bioprinting strategy through medical imaging and image processing software with strong clinical relevance.
Acknowledgments: This work was supported by the Army, Navy, NIH, Air Force, VA, and Health Affairs to support the AFIRM II effort under Award No. W81XWH-14-2-0004. The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office. Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the Department of Defense.
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416 - Enhanced Craniofacial Bone Repair By DBM With Noggin Suppression In Polymer Hydrogels

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***Purpose/Objectives:** Demineralized bone matrix (DBM) as a promising alternative to autologous bone graft has been increasingly used for craniofacial bone repair. However, the application of DBM in larger bone defect areas was not successful partly due to rapid dispersion of DBM particles with blood and relatively lower osteogenic activity of DBM. Therefore, there is a need to develop alternative strategies that can effectively complement the osteoinductivity of DBM.
***Methodology:** Here, we report a novel approach by suppressing BMP antagonists like noggin using RNAi strategy, as well as introducing a 3D hydrogel scaffold to house the DBM and localize its effects.
***Results:** Combined treatment of DBM + noggin suppression showed robust osteogenic differentiation of human mesenchymal stem cells (hMSCs) in a photopolymerizable MeGC hydrogel *in vitro*. Moreover, the complex of DBM+noggin suppression that was encapsulated with a MeGC hydrogel was implanted in mouse critical sized calvarial defects, displaying the significant bone repair after 6 weeks

postoperatively.
***Conclusion/Significance:** These results demonstrate that incorporation of DBM with noggin suppression in MeGC hydrogel can significantly enhance osteoinductivity of DBM, potentially broadening application of DBM-mediated craniofacial bone repair.

417 - Decellularised Bovine Dental Pulp As A Scaffold For Regenerative Endodontics: In Vitro And In Vivo Studies

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***Purpose/Objectives:** Regenerative endodontics (REs) is a tissue engineering-based therapy for managing pulp necrosis in immature permanent teeth. Outcomes are currently unpredictable and are affected by the lack of an appropriate scaffold that mimics the pulp extracellular matrix (ECM). The aim of this work was to develop a scaffold from bovine pulp ECM through the process of decellularization and to assess its suitability for human dental pulp regeneration both *in vitro* and *in vivo*.
***Methodology:** Bovine pulp tissues were decellularized using a detergent-based protocol using modified Wilshaw et al (2017) and Matoug-Elwerfelli et al (2016).The success of decellularization was assessed using histological analysis and DNA quantification assay. The resulting scaffolds were then characterised for the retention of essential pulp ECM proteins and growth factors using immunohistochemistry. Scaffolds were then repopulated with human dental pulp stem cells (hDPSCs). The cells' viability, proliferation and differentiation were then assessed using live/dead cell assay, DNA quantification assay and quantitative RT-PCR, respectively. An *in vivo* study was then conducted in which scaffolds were repopulated with hDPSCs, implanted in pulpless tooth slices, and then transplanted into immunodeficient mice. The resulting tissues were then analysed using histology and immunohistochemistry.
***Results:** The decellularization protocol generated acellular scaffolds with more than 98% reduction in total DNA content, and retention of essential pulp ECM proteins and growth factors. Scaffolds were capable of supporting the growth, maintaining the viability and inducing the odontoblastic and angiogenic differentiation of hDPSCs. The *in vivo* study revealed the formation of a well-organised vascularised connective tissue in the root canal space with a layer of cells lining the dentine walls and resembling odontoblast morphology. The majority of the cells in the regenerated tissues reacted positively to anti-human nuclei antibody, indicating their human origin.
***Conclusion/Significance:** Bovine dental pulp was shown to be a rich source for decellularized scaffolds. The scaffolds were capable of supporting hDPSCs' growth and differentiation *in vitro* and guided the formation of a well-organised pulp-like tissue *in vivo*. The future application of these scaffolds in REs may fulfil a yet unmet need for an appropriate scaffold and help to improve the clinical outcomes and the survival of teeth with otherwise poor prognosis.

419 - Evaluation Of Polycaprolactone-associated Human Nasal Chondrocytes As A Therapeutic Agent For Cartilage Repair

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BACKGROUND: In this study, we manufactured a complex of human nasal septal cartilage (hNC) with polycaprolactone (PCL) for transplantation into cartilaginous skeletal defects and evaluated their characteristics. **METHODS:** Nasal septum tissue was obtained from five patients aged ≥ 20 years who were undergoing septoplasty. hNCs were isolated and subcultured for three passages *in vitro*. To formulate the cell-PCL complex, we used type I collagen as an adhesive between chondrocyte and PCL. Immunofluorescence staining, cell viability and growth in the hNC-PCL complex, and mycoplasma contamination were assessed.**RESULTS:** hNCs in PCL showed viability ≥ 70% and remained at these levels for 9 h of incubation at 4°C. Immunostaining of the hNC-PCL complex also showed high expression levels of chondrocyte-specific protein, COL2A1, SOX9, and aggrecan during 24 h of clinically applicable conditions. **CONCLUSION:** The hNC-PCL complex may be a valuable therapeutic agent for implantation into injured cartilage tissue, and can be used clinically to repair cartilaginous skeletal defects. From a clinical perspective, it is important to set the short duration of the implantation process to achieve effective functional implantation.

420 - Mineralization Of Ridge Preservation Products May Negatively Impact Cellular Activity

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Ridge preservation is often indicated following tooth extraction by inserting a biocompatible product. Current treatments for ridge preservation do not always maintain the level of bone required for implant placement. Variations in surgical technique, as well as graft material used play a critical role in determining clinical outcomes. Choosing the appropriate graft material is a challenge due to the wide variety of grafting materials available. To investigate how differences in commercially available ridge preservation products may affect their intended function: Bio-Oss Collagen, OsteoGen Plug, and J-Bone were evaluated.
Scaffolds were previously physically and chemically characterized. Biological characterization was carried out using Saos-2 human osteosarcoma cells to evaluate scaffold biocompatibility and osteoconductivity. Results indicated that these products vary not only in percent porosity, but also in degrees of pore interconnectivity when compared to J-Bone. Although FTIR showed that mineralization of Bio-Oss Collagen was chemically similar to native bone, OsteoGen Plug demonstrated significantly better biocompatibility with Saos-2 cells.
These products advertise their ability to maintain the ridge and facilitate bone fill, *in vitro* results demonstrated high variability when compared to native bone. OsteoGen Plug demonstrated superior osteoconductive and biocompatibility properties compared to Bio-Oss Collagen, perhaps due to its lower degree of mineralization, suggesting that a product mimicking native bone mineralization levels may not be ideal. Future work will include translating these findings into the development of advanced ridge preservation scaffolds to improve clinical outcomes.

422 - Automated In-process Characterization And Selection Of Ips Clones For Quality And Efficient Ips Production

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***Purpose/Objectives:** Induced pluripotent stem (iPS) cells are being developed for a broad range of research and therapeutic applications. However, reprogrammed iPS clones are inevitably heterogeneous. Current value judgements are made by visual inspection and manual selection. These methods are prone to large variation and lack the standardization or scalability needed for clinical translation. The goal of this work is to develop and validate Cell X™, a robust robotic platform that will enable automated, and standardized iPS cell manufacturing.
***Methodology:** We used DF6-9-9T.B hiPSC cell line, reprogrammed skin fibroblasts and peripheral blood mononuclear cells to develop our image processing parameters and picking and weeding protocols. To define and quantify critical quality attributes (CQAs) of "completely" reprogrammed iPS cells in systematic repeatable and reproducible manner, the Cell X™ Platform is integrated with automated quantitative cell and colony analysis software. Quantitative image analysis can be performed on individual images or can be batch-processed, with final data exported to spreadsheet. The most important quantitative metrics analyzed include colony area, cell density, cell morphology, and cell surface markers. The principles and nomenclature enabling this approach are outlined in ASTM Standard F2944-12. To automate manipulation of iPS cells in rapid, precise, repeatable and rigorously documented manner, the Cell X™ device integrates imaging, cell-manipulation, fluid handling, and motion control systems. The device is placed in a fully enclosed HEPA filtered laminar flow Biospherix Xvivo™ system that can be maintained at cell-culture conditions - 35-37^{o}C, 80% relative-humidity, 5-21% O_2 and 1-10% CO_2.
***Results:** Using Cell X™, picking operations were effective, and transplanted cells continued to proliferate effectively with morphology that is unchanged from that of the original colony. Automated image processing displayed output metrics (colony area, the x,y,z-centroid for each colony, colony density, roundness, perimeter etc), as well as highlighted the periphery of individual colonies or cell boundaries in the montage image. Based on these metrics, we can quantitatively define the cells or colonies of interest and selectively pick and transfer the cells for future experiments. The iPS expansion module includes automated detection of regions of differentiation. This can be used to objectively compare the performance of iPS clones and lines, and/or to guide "picking" and "weeding" interventions.
***Conclusion/Significance:** The Cell X™ Platform provides an integrated system of automated quantitative cell and colony imaging and analysis as well as tools for precision manipulation (biopsy, picking and weeding) driven by quantitative protocols that objectively determine the CQAs for repeatable, reproducible and quality iPS cell manufacturing. This enables the development of fully automated cell processing and fabrication methods to be developed in a manner consistent with the demands of a GMP environment that is entirely free of manual manipulation and subjective decision making. In essence, the Cell X™ platform capabilities enable a user to "See what you have. Pick what you want. Show what you've done. Know how to do it again."

423 - Tissue-engineered Electronic Nerve Interface (teeni) Histological Examination With Clarity

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***Purpose/Objectives:** The Tissue-Engineered Electronic Nerve Interface (TEENI) is a regenerative peripheral nerve interface made from stimulating and recording sites microfabricated on a planar polyimide substrate. This structure is surrounded by a tissue-engineered hydrogel wrapped in small intestinal submucosa (SIS). A nerve is transected and then the proximal and distal nerve stumps are sutured to the open ends of the SIS; the proximal axons then regenerate through the hydrogel towards the distal nerve stump and putatively nearby the electrode sites on the polyimide structure. This project's purpose focuses on significantly increasing the information bandwidth between neural tissue and digital processing units for the purpose of natural control of and sensory feedback from prosthetic devices. These improved prosthetic devices aim to significantly improve the quality of life for people living with amputated limbs. An important aspect of developing these devices is understanding the chronic tissue response both inside the hydrogel and surrounding the polyimide structure. In the process of traditional cryosectioning, histological information can be lost or distorted over the course of several sections. Here we report a modified CLARITY protocol where explanted devices were cleared, immunolabeled, and imaged using a light sheet microscope to examine the tissue-device interface and the regenerating tissue inside they hydrogel.
***Methodology:** TEENI devices were implanted into Lewis rats for 4 months (or until euthanasia criteria were met). Upon euthanasia, animals underwent standard perfusion protocol with phosphate buffered saline (PBS) and buffered 4% paraformaldehyde. Tissue was kept overnight at 4 degrees Celsius and washed for 1 day in PBS. TEENI samples were then run through a modified CLARITY protocol and labeled with antibodies targeting proteins of interest. After labeling and clearing, samples were imaged in a Zeiss Z.1 Lightsheet microscope.
***Results:** Histological evidence shows TEENI implants are readily vascularized by the regenerating tissue. Basal lamina is seen surrounding regenerating axons growing through the tissue-engineered hydrogel and in close proximity to TEENI devices. Over the course of weeks, the hydrogel continues to be digested and a foreign body response forms that thinly encapsulates the device.
***Conclusion/Significance:** In conclusion, the work here shows the proof-of-concept of the TEENI design with a potential in the future for expanding the number of polyimide structures inside the hydrogel by stacking them with some gap between each. The overall results suggest future TEENI device designs not limited to a specific structural shape and customized to interface with nerves of different sizes while maximizing the 3-dimensional distribution of stimulating and recording sites to achieve a high degree of selectivity. Finally, the incorporation of the tissue-engineered hydrogel offers the potential of adding in specific guidance cues to direct axon growth to help separate motor and sensory information pathways to obtain a high degree of specificity.

424 - Synthetic Presentation Of Noncanonical Wnt5a Motif Promotes Mechanosensing-dependent Differentiation Of Stem Cells And Regeneration

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***Purpose/Objectives:** Noncanonical Wnt signaling in stem cells is essential to numerous developmental events. However, no much prior studies have capitalized on the osteoinductive potential of noncanonical Wnt ligands to functionalize biomaterials in enhancing the osteogenesis and associated skeleton formation (Zhang K. et al. Adv Sci. Dec;5(12):1800875). Herein, we investigated the efficacy of the functionalization of biomaterials with a synthetic Wnt5a mimetic ligand (Foxy5) to promote the mechanosensing and osteogenesis of human mesenchymal stem cells by activating noncanonical Wnt signaling. Our findings showed that the immobilized Wnt5a mimetic ligand activated noncanonical Wnt signaling via the upregulation of Disheveled 2 and downstream RhoA/ROCK signaling, leading to enhanced intracellular calcium level, F-actin stability, actomyosin contractility, and cell adhesion structure development. Such enhanced mechanotransduction in stem cells promoted the in vitro osteogenic lineage commitment and the in vivo healing of rat calvarial defects. Our work provides valuable guidance for the developmentally inspired design of biomaterials for a wide array of therapeutic applications.
***Methodology:** The Foxy5 peptide and RGD peptide-functionalized MeHA (degree of methacryloyl substitution or methacrylation degree = 100% or 30%) was crosslinked to fabricate a 3D porous hydrogel scaffold or a 2D hydrogel substrate for subsequent experiments Growth medium (50 µL) containing 5×10^5 hMSCs (10^8 cells mL^{-1}) was injected into one semidry, porous Foxy5+RGD hydrogel, which was incubated at 37 °C for 4 hours to allow cell attachment to the hydrogels. Samples were collected on day 7 and day 14 to evaluate the degree of osteogenesis in vitro with immunofluorescence staining, IHC staining, RT-qPCR, and Western Blotting. The samples for calvarial bone defects regeneration were collected after 8 weeks to evaluate the in situ regenerative degree in vivo.
***Results:** RT-qPCR and Western Blotting analyses showed significant increase in RhoA/ROCK2 signaling in the presence of Foxy5 peptide at early time points. Immunofluorescence staining revealed a significant increase of focal adhesion formation and mechanotransduction in the hMSCs in the presence of conjugated Foxy5 peptides. RT-qPCR and IHC staining analyses showed significant elevation in the expression of osteogenic marker genes and bone matrix formation. Functionalization of biomaterial scaffolds with the Wnt5a mimetic peptide substantially enhances the in-situ regeneration of integrated and mature bone tissues, which is on average 73% of the level in native calvarial bones.
***Conclusion/Significance:** Collectively, these data systematically exhibit the promoting effects of non-canonical Wnt5a activation in hMSCs in the osteogenesis and in-situ bone regeneration.

425 - Engineering Senescent Muscle Tissues To Assess Reversal Of Muscle Regeneration Upon NANOG Expression.

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***Purpose/Objectives:** Skeletal muscle and smooth muscle loss due to aging are major medical problem facing elderlies. Adult muscle regeneration relies on the activity of resident progenitors in the muscle niche. However, systemic and intrinsic factors decrease the myogenic differentiation potential of senescent progenitors. Our lab has shown that expression of an embryonic transcription factor, NANOG, in senescent progenitors reversed their senescent phenotype and ultimately restored the myogenic differentiation potential which was impaired due to cellular senescence. To prove the improvement in myogenic differentiation we employed 3D tissue engineered constructs that mimic the structure of the muscles in our body.
***Methodology:** The micro scale 3D tissue engineered constructs were synthesized by embedding undifferentiated progenitors in the extracellular matrix composed of 2mg/ml collagen type I and 2mg/ml matrigel. Then, the mixture was neutralized by 1M NaOH and seeded on the arrays of mictowells made by polydimethylsiloxane (PDMS) and incubated at 37˚C for 10 min to allow formation of microtissues between two micro-pillars. These 3D tissue engineered constructs recapitulated the features of skeletal muscle and smooth muscle environment such as special confinement and mechanical tension. Using this tissue engineered constructs we assessed the recovery of myogenic differentiation in senescent myoblasts toward skeletal muscle and senescent mesenchymal stem cells (MSC) toward smooth muscle cells (SMC).
***Results:** Skeletal muscle regeneration was effective in 3D skeletal microtissues mimicking the skeletal muscle niche. The presence of NANOG was required for at least 15 days to reverse the impaired differentiation potential of senescent myoblasts. Upon induction of injury using cardiotoxin, senescent tissues failed to regenerate the injured muscle, while expression of NANOG boosted their regeneration capacity to form new myofibers.
In the senescent smooth muscle tissues, we show that expression of NANOG fortified the actin cytoskeleton and restored contractile function that was impaired in senescent MSCs. NANOG increased the expression of smooth muscle α-actin (ACTA2) as well as the contractile force generated by cells in smooth muscle microtissues.
***Conclusion/Significance:** Overall, our results demonstrate the synthesis of tissue engineered muscle constructs that can recapitulate sarcopenic muscle and can be used as a platform to test therapeutics that might improve regeneration and function of muscle after aging, dystrophic disorders, or cancer cahexia. Using this system, we showed that ectopic expression of the embryonic transcription factor NANOG in the senescent progenitors of skeletal muscle and smooth muscle cells restore muscle regeneration and contractile function.

426 - Bioconjugated Cytokine Ligand Dosing on 3D Printed Poly(propylene fumarate) Bone Tissue Engineering Scaffolds is Controlled by Bioglass Concentration

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***Purpose/Objectives:** Autologous bone transplantation is the gold standard treatment for segmental bone defects occurring secondary to trauma, cancer resection, bone infections, and orthopedic revision surgery. Currently, there is no commercially available tissue engineered bone substitute that can

routinely address patient-specific, critical size or larger, load-bearing bone defects. Some current devices utilize cell-signaling molecule delivery vehicles based on a drug-release paradigm. These devices release whole cytokines, highly mobile proteins, that have been associated with pleiotropic effects not limited to the cells that could be responsible for bringing about a healing response following a surgical intervention. An example of the drug delivery paradigm is Medtronic's (Minneapolis, MN) Infuse™ product which releases BMP-2 from a collagen sponge placed in an intervertebral fusion cage. This device has been associated with a wide range of deleterious effects, including nerve damage, spinal cord inflammation, and an elevated risk of cancer. We propose a paradigmatic change from the free release of powerful cytokines, to the presentation of a growth factor's active site, the ligand (i.e., a short peptide), by its conjugation to the surface of a defect-filling, resorbable, polymer scaffold. This strategy should eliminate the unwanted side effects of drug-release bone tissue engineering strategies.
***Methodology:** We have developed a method to bioconjugate growth factor ligands (i.e., RGD, bFGF, BMP-2, and OGP) in tunable concentrations on bone scaffold surfaces. These ligands were chosen to provide specific augmentation of bone defect healing by recruiting bone progenitor cells to attach, proliferate, and mature in a defect site that has been filled with a 3D printed, resorbable, poly(propylene fumarate) (PPF) porous scaffold. We tether these ligands to the scaffold through a ceramic-catechol-dendron moiety. The goal of the present study is to test our hypothesis that we can control the concentration and dose of each ligand, singly or in combination, by the amount of ceramic in the 3D printed polymer resin. Our method of tethering ligands (Xu Y., et al. Biomacromolecules, 18(10), 3168-3177) requires a bioactive ceramic (e.g., 45S5 Bioglass) be incorporated into our PPF resins. The catechol-dendron moieties that link micrometer scale Bioglass crystals to the desired ligand proteins have been shown, *in vitro*, to spur bone precursor cell attachment, proliferation, and differentiation leading to bone extracellular matrix formation. SEM and Quantitative Alizarin Red S imaging were used to measure Bioglass surface concentration on 3D printed scaffolds.
***Results:** SEM imaging shows that Bioglass is available at the surface of 3D printed parts in proportion to the amount loaded in the 3D printed PPF resin. Alizarin Red S imaging shows that catechol functionalization can be modulated directly based on the loading concentration of Bioglass in the PPF resin that is 3D printed.
***Conclusion/Significance:** This study demonstrates that the Bioglass concentration at the surface of 3D printed PPF scaffolds can be modulated and thus future studies should be able to validate whether optimized ligand doses will be effective in stimulating cells *in vitro* and/or *in vivo* for the repair of critical size or larger bone defects.

427 - Runx-1 Messenger RNA Delivered By PEGylated Nanocarrier Mitigate Disk Degeneration Scenario In A Rat Model

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***Purpose/Objectives:** Intervertebral disk (IVD) degeneration is often associated with severity of lower back pain. IVD core is an avascular, highly hydrated tissue composed of type II collagen, glycosaminoglycans, and proteoglycans. The disk degeneration is not only a destruction of IVD structure, but is also related to a disorder of the turnover of the disk matrix, leading the jelly-like IVD core to be replaced by fibrous components.
***Methodology:** Here we present a disease-modifying strategy for IVD degenerative diseases by direct

regulation of the cells in the IVD using mRNA medicine, to alter the misbalanced homeostasis during disk degeneration.
***Results:** When mRNA encoding a cartilage-anabolic transcription factor, runt-related transcription factor-1, was administered to a rat model of coccygeal disk degeneration using a polyplex nanomicelle composed of polyethylene glycol - polyamino acid block copolymers and mRNA, the disk height was maintained to a significantly higher extent (~ 81%) compared to saline control (69%), with prevention of fibrosis in the disk tissue. In addition, the use of nanomicelles effectively prevented inflammation, which was observed by injection of naked mRNA into the disk.
***Conclusion/Significance:** This proof of concept study revealed that mRNA medicine has a potential for treating IVD degenerative diseases by introducing a cartilage-anabolic factor into the host cells, proposing a new therapeutic strategy using mRNA medicine.

429 - Human Adipose Stem/Stromal Cells Spheroids To Mimic Stable Cartilage

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***Purpose/Objectives:** We aim to develop a scaffold- and serum-free method to mimic human stable cartilage from induced ASC spheroids. An overview of chondrogenesis was obtained by comparative secretome analysis. Induced ASC spheroids seeded into nanofibers of polycaprolactone (PCL) were implanted in the subcutaneous of mice.
***Methodology:** ASC suspension was seeded in a micromolded agarose hydrogel for spheroids formation. For chondrogenic induction, ASC spheroids were maintained under hypoxia (10% O_2 and 5% CO_2) in culture medium supplemented with 10 ng/ml TGF-β3 and 10^{-8} M dexamethasone for until 21 days. Induced and Non-induced ASC spheroids were characterized by size, immunohistochemistry and biomechanical properties. After cell dissociation, they were characterized for cell viability, and quantitative real-time polymerase chain reaction. Nontargeted (mass spectrometry) analyses were conducted on the culture supernatants.
***Results:** Induced ASC spheroids (ø = 350 mm) showed high cell viability. The gene expression for SOX-9, a master gene for chondrogenesis increased throughout induced ASC spheroid culture together with a significant decrease of Runx-2 and ALPL. Strong collagen type II in situ was associated with gradual decrease of collagen type X and a lower COLXA1 gene expression at day 14 compared with day 7($p = 0.0352$). Induced ASC spheroids showed a significantly high force module at day 7 ($p = 0.0003$), 14 ($p < 0.0001$) and 21 ($p = 0.0007$) compared to non-induced ASC spheroids evaluated by Microsquiser equipment. Secretome analysis identified 138 proteins directly relevant to chondrogenesis of 704 proteins in total, including and anti-hypertrophy protein, TSP-1. Histological analysis from mice subcutaneous tissue showed an engraftment of induced ASC spheroids seeded into PCL nanofibers for until 28 days.
***Conclusion/Significance:** Induced ASC spheroids revealed as a promising delivery strategy for cartilage tissue engineering.

430 - Modular, Click Functionalized Hydrogels For Cartilage Tissue Engineering

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***Purpose/Objectives:** Articular cartilage repair remains a significant clinical need, with poor patient outcomes from trauma and degenerative disease due to cartilage's poor capacity for self-regeneration. Furthermore, articular cartilage resides within a heterogeneous milieu of tissue phenotypes and biochemical cues in the osteochondral unit, necessitating the presentation of multiple tissue-specific cues in order to produce effective tissue repair.

To address these challenges, we have developed a **modular, mesenchymal stem cell (MSC)-encapsulated hydrogel system** that can be functionalized with *in situ* cues for chondrogenesis ranging from small peptides to large biomolecular components of the cartilage matrix, simply by click functionalizing the hydrogel crosslinker, poly(glycolic acid)-poly(ethylene glycol)-poly(glycolic acid)-di(but-2-yne-1,4-dithiol) (PdBT),[1] in a mild, aqueous alkyne-azide cycloaddition reaction.

***Methodology:** In this work, we have generated MSC-encapsulated hydrogels clicked with cartilage-specific cues including a synthetic **N-cadherin (NC) peptide** mimicking the cell-cell junction protein involved in early chondrogenesis, as well as the biologically derived macromolecule, **chondroitin sulfate (CS)** found in cartilage matrix during mid- to late-stage chondrogenesis.

To generate hydrogels, CS- and NC-clicked crosslinkers of varying concentrations were mixed with thermoresponsive poly(*N*-isopropylacrylamide-co-glycidyl methacrylate) and rabbit bone marrow-derived MSCs in phosphate buffered saline at 4 °C. Upon exposure to physiological temperature, hydrogels spontaneously solidified and crosslinked within 90 min to produce cell-encapsulated hydrogels with *in situ* chondrogenic cues. The hydrogels were then cultured in chondrogenic medium for 14 days, with assessment of MSC viability, glycosaminoglycan (GAG) synthesis as a measure of chondrogenic activity, and mineralization as a measure of unwanted hypertrophic calcification. All results were normalized to acellular controls.

***Results:** CS- and NC-clicked hydrogels at all concentrations promoted significantly greater GAG synthesis compared to non-clicked controls by day 14, with NC-clicked hydrogels producing the greatest amount of GAG synthesis at day 3 and CS-clicked hydrogels producing the greatest amount at day 14. Furthermore, both CS- and NC-clicked hydrogels retained cell viability more effectively than controls, with NC-clicked hydrogels in particular experiencing zero viability loss from day 0 to day 14. Neither CS- nor NC-clicked hydrogels produced significant differences in mineralization compared to controls by day 14.

***Conclusion/Significance:** Ultimately, this customizable hydrogel system was shown to strongly promote early to mid-stage chondrogenic activity and/or enhance cell viability depending on the selection of clicked components, while avoiding hypertrophic calcification in all cases. These findings demonstrate strong potential for the usage of this modular hydrogel system for cartilage tissue engineering. **References:** (1) Guo, J. L. et al. Modular, Tissue-Specific, and Biodegradable Hydrogel Cross-Linkers for Tissue Engineering. Science Advances 2019, 5 (6). **Acknowledgments:** We acknowledge support by the National Institutes of Health (R01 AR068073, P41 EB023833). B.T.S., E.W., and H.A.P. acknowledge support from the National Institute of Arthritis and Musculoskeletal and Skin Diseases (F30 AR071258), National Institute of Dental and Craniofacial Research (F31 DE027586), and National Science Foundation Graduate Research Fellowship Program, respectively. This work reflects authors' views and should not be construed to represent the FDA's views or policies.

432 - Dysregulation Of TGFß Signaling Pathway Contributing To Aging-associated Decline In Chondrogenic Potential Of MSCs

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***Purpose/Objectives:** Focal chondral defect is a high incidence joint disease, with articular cartilage(AC) loss as its main characteristic. Over the last 20 years, attempts have been made to regenerate articular cartilage to overcome the limitations of conventional treatments. In particular, the use of autologous and allogeneic mesenchymal stem cells(MSCs) have rapidly attracted tremendous attention. Previous studies showed that aging may negatively impact the differentiation potential of MSCs. However, the underlying mechanism has not been fully understood. In addition, a comprehensive study of cartilage formation from MSCs, isolated from donors at different ages, has not been conducted. Here, we hypothesized that natural aging declines the chondrogenic potential of MSCs, which may be due to the dysregulation of the TGF-β pathway and enhanced activation of SMAD1/5/8 at the expense of SMAD 2/3.
***Methodology:** MSCs were isolated from healthy young(<35 years Ymix) and old(>70 years Omix) donors with Institutional Review Board approval (University of Pittsburgh and University of Washington). We pooled cells from 6 donors to generate a young(Ymix) and old(Omix) population. Stemness of MSCs was validated via flow cytometry, trilineage differentiation and colony-forming unit assay. Then, Ymix and Omix MSCs at passage 4(P4) were subjected to standard pellet culture in chondrogenic medium supplemented with 1ng/ml, 3ng/ml, 10 ng/mL, 20 ng/mL, or 60 ng/mL TGF-β3. After 21 days of culture, the chondrogenesis was assessed using real-time PCR, histology, and GAG quantification. At different time points, we also used western blots to determine the expression level of TGF-β receptors and downstream signaling factors(TGFβRII, ALK1, 2, 3, 4, 5, 6, Smad1/5/8, Smad2/3).
***Results:** Compared to those from Ymix MSCs(Cartilage-Y), cartilage derived from Omix MSCs(Cartilage-O) displayed lower chondrogenic but higher hypertrophic gene expression, with significantly less deposition of GAG, which was revealed by real-time PCR and histology. In addition, higher expression of P21, P16, and SASP-associated genes was observed in Cartilage-O, suggesting the presence of an inferior senescent phenotype. Through western blots, we found that the TGF-β signaling pathway was dysregulated in Omix MSCs, in particular, the enhanced ALK1/5 ratio. Consequently, higher SMAD 1/5/8 was observed in Cartilage-O than Cartilage-Y. Interestingly, the combinatorial treatment of ALK1 inhibitor and a high dose of TGF-β(> or = 20ng/ml) significantly reduced the expression of hypertrophy and SASP-associated genes in Cartilage-O, opening a novel avenue to enhance the utility of old MSCs for articular cartilage repair.
***Conclusion/Significance:** Our findings suggested that MSCs from young donors display a better cartilage regeneration capacity than those from old. When it comes to autologous MSCs implication in older patients, we may need to manipulate the TGF-β signaling pathway to achieve robust cartilage regeneration with high quality.

433 - Enhancing The Property Of Mscs-derived Cartilage Through Hypoxic Culture: Inhibition Of Wnt/β-catenin

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***Purpose/Objectives:** Oxygen concentration gradients in the developing limb bud are known to contribute to the zonal differentiation of mesenchymal cells to mature chondrocytes. It has been also shown that hypoxic culture may not only enhance the expression level of chondrogenic genes, but also reduce the level of hypertrophy characterized by a loss of collagen network integrity and increased collagen type X content. A previous study demonstrated that hypoxia downregulates β-catenin in human mesenchymal stem cells (hMSCs) within 3D hydrogels. However, it should be noted that the hypertrophy inhibition effect of hypoxia is still debatable, as such effect is highly dependent on the properties of the biomaterial used for cell encapsulation. The mechanism by which hypoxic cultures suppress hypertrophy has not been reported. Recently, we constructed a developmentally informed technology to form cartilage using MSCs within their own ECM (MECM). We hypothesize that the hypertrophy suppression effects of hypoxic culture function through β-catenin, and that enhancing β-catenin will eliminate such effects.
***Methodology:** Human MSCs were harvested from femoral heads, femoral condyles, and the tibial plateaus of patients undergoing total joint arthroplasties. To replicate the environment needed for MSC chondrogenic differentiation, we generated MECMs through long-term confluent MSC culturing under normoxic conditions (20% oxygen). After, MECM constructs were subjected to chondrogenic culturing for up to 4 weeks under hypoxic conditions (5% oxygen). The constructs were also cultured under normoxic conditions for comparison purposes. Finally, we activated β-catenin with an agonist during hypoxic culturing to determine the functionality of hypoxic cultures in combination with β-catenin to suppress hypertrophy in the ECM. Real-time polymerase chain reaction (RT-PCR), Safranin O/Fast Green staining, and a glycosaminoglycans (GAGs) assay were performed to assess the quality of chondrogenesis. The suppression of hypertrophy was further evaluated with RT-PCR, western blotting, and immunohistochemistry (IHC). The gene expression and protein levels of β-catenin were assessed with RT-PCR and western blotting. Two-way ANOVA was employed for data analysis.
***Results:** Gene expression data showed that MSCs cultured under hypoxic conditions have higher expression levels of chondrogenic marker genes, like collagen II (*COL2*), Aggrecan (*ACAN*), and SRY-Box9 (*SOX9*) in comparison to those subjected to normoxic conditions. Additionally, these conditions resulted in lower expression levels of hypertrophy genes, like collagen type X (*COL10*), alkaline phosphatase (*ALP*), and matrix metallopeptidase 13 (*MMP13*). Western blotting and IHC results were consistent with those of RT-PCR. Safranin O/Fast Green staining and a GAGs assay also confirmed that hypoxic culturing yields more GAGs. Interestingly, the groups under hypoxic conditions in ECMs upregulated the hypoxia-inducible factor-1α (HIF-1α) at both the gene and protein level, while downregulating β-catenin at the protein level. However, the effect caused by hypertrophic suppression disappeared when β-catenin was activated with an agonist during hypoxia, suggesting that the hypertrophy inhibition effect of hypoxic culture may function through the Wnt/β-catenin pathway.
***Conclusion/Significance:** Our study found that in MECM cultures normoxic culture followed by hypoxia treatment yields significantly lower hypertrophy than normoxic culture alone through the inhibition of Wnt/β-catenin signaling.

435 - Cell-laden, Poly(l-lysine)-incorporated Injectable Hydrogels For Cartilage Tissue Engineering

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***Purpose/Objectives:** Efforts to regenerate damaged articular cartilage have been repeatedly thwarted by the tissue's avascularity and low cell density. Recently, tissue engineers have started to investigate methods of programming the developmental process into tissue engineered constructs, with the ultimate objective of harnessing long-term regenerative potential. One of the molecules that have been investigated for this purpose is poly(L-lysine) (PLL), a polycationic molecule that has shown to enhance the expression of N-cadherin from mesenchymal stem cells (MSCs). This up-regulation is a prerequisite for mesenchymal condensation to occur during skeletal development, eventually leading to chondrogenesis. The objective of this study was to characterize the physicochemical properties of PLL-incorporated hydrogel, as well as to observe encapsulated MSC behavior within these constructs.
***Methodology:** An injectable, dual-gelling hydrogel system composed of a poly(N-isopropylacrylamide)-based thermogelling macromer (TGM) and a modified chondroitin sulfate (CS) network-forming macromer was used for this study. The macromers underwent thermoresponsive gelation followed by chemical crosslinking between epoxy groups on the TGM and hydrazide/N-hydroxysuccinimide (NHS) ester groups on CS. PLL was incorporated into the hydrogel by mixing into the precursor solution. The effects of PLL molecular weight (50, 225 kDa) and dosage (100, 1000 µg/ml) on the physicochemical properties of the hydrogel were investigated by measuring the swelling behavior of the hydrogel. In addition, MSCs were encapsulated into PLL-loaded hydrogels (10, 100 µg/ml) and cultured for 7 days *in vitro* to measure the effect of PLL on cell viability and proliferation.
***Results:** When compared to non-PLL loaded controls, neither PLL molecular weight nor concentration significantly affected the degree of swelling. These results indicate that the amine groups present along the surface of PLL do not impact the amine-epoxy or amine-NHS ester reactions between the macromers, and thus the physical properties of the hydrogel post-crosslinking. Viability and proliferation of encapsulated MSCs were also not significantly different among the groups that were tested at 7 days *in vitro*, demonstrating that the introduction of polycationic PLL did not result in the increase in cytotoxicity.
***Conclusion/Significance:** Altogether, these results highlight a promising approach to developing a platform that can be used to deliver MSCs with signaling molecules creating a microenvironment conducive towards the regeneration of cartilage tissue.

436 - Stem Cell Sheets Containing Growth Factor-loaded Gelatin Microspheres For Repair Of Rabbit Osteochondral Defects

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***Purpose/Objectives:** Osteochondral (OC) defects, which affect both cartilage and underlying subchondral bone, are a common injury (1). Since these defects don't heal naturally and their clinical management remains challenging, there is a strong interest in developing strategies to engineer

osteochondral tissue with the same functionality and durability as native. Scaffold-free cellular constructs are a promising strategy for cartilage regeneration due to their high density of cell-cell interactions, which are present during natural cartilage formation, and unrestricted space for cell proliferation and ECM production (2). Their capacity to repair OC defects in large animals and humans has recently been explored (2-3). Incorporation of polymer microspheres (MSs) within high-density cell constructs permits controlled presentation of chondrogenic bioactive-molecules while maintaining abundant cell-cell interactions. For example, TGF-β1-loaded MSs within high-density hMSC sheets have been shown to improve chondrogenesis of hMSCs after 3 weeks of culture (4). The purpose of this study was to evaluate the capacity of hMSC sheets with incorporated TGF-β1-loaded gelatin MSs to repair of OC defects in a rabbit model.
***Methodology:** hMSC sheets (A) containing no MSs with exogenous TGF-β1 treatment, (B) incorporated with empty-MSs with exogenous TGF-β1 treatment, or (C) incorporated with TGF-β1-loaded-MSs without exogenous TGF-β1 treatment were cultured *in vitro* for 3 weeks followed by hMSC chondrogenesis evaluation. 2-week pre-cultured hMSC sheets from conditions (B) and (C) were then implanted into rabbit femoral condyle OC defects to evaluate cartilage repair after 1 and 3 months *in vivo*. Empty OC defects served as controls (Sham). Cartilage repair was assessed by morphological and histological healing scores and by Fourier transform infrared imaging spectroscopy (FT-IRIS) of histological tissue samples.
***Results:** After 2 weeks of *in vitro* culture, MS-incorporated hMSC sheets were significantly thicker than cell-only sheets, and after 3 weeks TGF-β1-loaded-MS containing sheets exhibited significantly higher glycosaminoglycan content compared to the other groups. After 3 months of implantation in rabbit OC defects, there was no significant difference in morphological and histological scores for knees implanted with MS containing sheets. However, both MS-incorporated sheet conditions had higher average scores than Sham defects, with significance reached with the histological scores of empty MS-incorporated sheets. Histological and immunohistochemical staining for GAG and collagen type II demonstrated expression of these ECM molecules in the majority of the cartilage portion of the OC defects with implanted MS-incorporated sheets. FT-IRIS analysis showed that average proteoglycan and collagen contents were higher in MS-incorporated sheets at 3 months compared to 1 month and in TGF-β1-loaded-MS-incorporated sheets compared to empty-MS-contained sheets at both time points, but significance was not reached.
***Conclusion/Significance:** Our findings demonstrate that gelatin MS-incorporated hMSC sheets implanted after only 2 weeks of *in vitro* culture enhance *in vivo* cartilage repair by 3 months of implantation, and this strategy may lead to a promising therapeutic treatment for osteochondral defects. **References:** 1)Aroen A, *Am J Sports Med* 2004,32,211; 2)Yano F, *Biomaterials* 2013,34,5581; 3)Shimomura K, *Am J Sports Med* 2018,46,2384; 4)Solorio LD, *J Control Release* 2012,158,224.
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437 - Non-destructive Evaluation Of Cellular Differentiation By Assessing The Composition Of The Secreted Extracellular Matrix During In Vitro Chondrogenesis

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***Purpose/Objectives:** Cartilage is a unique tissue in which a relatively small population of cells produces an extensive extracellular matrix (ECM) composed of proteoglycans (PGs) and collagens[1]. PGs are heterogeneous molecules consisting of a core protein with sulfate glycosaminoglycan (GAG) chains. PGs in cartilage are located on chondrocyte cell surfaces, and in pericellular, territorial, and interterritorial domains[2, 3]. Each stage of cartilage development is marked by the appearance of specific GAGs that form structural PGs, or modifications of existing ones. Assessing these changes provides an opportunity to assess the quality of the engineered tissue during the fabrication process, given that it provides indirect information regarding the status of cell differentiation. The composition of the cartilaginous ECM determines not only the type of cartilage generated (hyaline, fibrocartilage, elastic), but it can also predict its mechanical performance and durability. Because current assessments of engineered cartilage rely on destructive methodologies, it is impossible to predict failures early in the process. Therefore, we aim to develop a dynamic, non-destructive technology to monitor cell differentiation in real-time based on the analysis of the presence of particular GAGs and gene expression profiles. Central to this approach is the observed fact that immature cartilage ECM does not incorporate all of the secreted products of chondrocytes[3]. Thus, both the maturity of the ECM and the chondrocyte's synthetic program can be assessed by sampling the conditioned medium (CM).
***Methodology:** We have developed a panel of monoclonal antibodies, which can detect sulfated PGs in CM. These antibodies detect carbohydrate motifs that have been shown to be developmentally regulated. In combination with a panel of anti-collagens antibodies and previously-obtained sequencing data from differentiating cells, an assay to determine the quality of the ECM will be developed. Adult human mesenchymal stem
cells (hMSCs) were induced to differentiate to chondrocytes as previously described[3]. CM and aggregates were collected for ELISA, histology and IHC at different time-points so that early, mid, and late-stages are analyzed.
***Results:** Using the panel of antibodies we found a correlation between the amounts of specific GAGs and collagens secreted into the CM, up to day 10 (when the amount released into the CM starts to decree) with the quality of the cartilage ECM. We assert that this reduction is due to the presence of a mature ECM capable of retaining (anchoring) secreted GAGs. The data supports the association between the status of ECM maturation inside the pellet and the presence of key molecules in the CM. The results also correlate with gene expression data and quality of ECM.
***Conclusion/Significance:** In summary, here we analyzed the kinetics of ECM production during hMSC chondrogenic differentiation. The results indicate the existence of a biphasic mode of differentiation and maturation during which matrix genes and molecules are differentially activated and secreted. These results have important implications for developing approaches for the creation of tissue engineered articular cartilage.
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438 - Multifactorial Scaffolds Direct Multilineage-inducible Adult Stem Cells Toward Hyaline-like Articular Chondrocytes In A Mechanorganotypic Model

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***Purpose/Objectives:** Articular cartilage defects are common among young and active adults. Current treatments (microfracture, autologous chondrocyte implantation with or without matrix) have several limitations including inadequate generation of hyaline-like articular cartilage, fibrocartilage formation and delamination. A tissue engineered product for cartilage repair should provide a potent chondro-inducing support for the articular hyaline tissue, and should be preferably injectable into the joint, thereby avoiding an open-knee surgery and reducing the patient's recovery time. Ideally, this product should be readily available as an off-the-shelf manufactured product.
***Methodology:** We developed an ex vivo mechanorganotypic model of osteochondral defects to evaluate the efficiency of hyaline cartilage repair strategies. 8-mm in diameter osteochondral plugs were made from the condyle of pigs. A 3-mm hole was then drilled in the cartilage surface, paying attention to drill down to the bone. These osteochondral plugs were then filled with cells alone or in combination with multifactorial scaffolds and placed in a dedicated bioreactor. The cells include marrow-isolated multilineage inducible adult (MIA) stem cells and the multifactorial scaffolds micronized human cadaveric articular cartilage (microcartilage) product and/or TGFβ3-loaded pharmacologically active microcarriers (PAMs-TGFβ3). The filled osteochondral plug samples were placed in culture and mechanically stimulated for one hour every day at a frequency of 1Hz to simulate post-surgical physical therapy.
***Results:** We analyzed the osteochondral plugs by immunohistochemistry and observed that mechanical stimulation had a dramatic effect on cell survival during cartilage repair as soon as two weeks. Cell survival was even more dramatic in the presence of the PAMs-TGFβ3. Interestingly, the hyaline specific marker expression (high Aggrecan and low Collagen-X) were also optimal in the presence of both microcartilage and PAMs-TGF-β3, confirming what we observed before in our micro-pellet system. Mechanical stimulation dramatically increased the MIA cell-mediated integration of the microcartilage into the osteochondral organotypic plug tissue structure as a function of the physiological-like TGFβ3 release from the PAMs.
***Conclusion/Significance:** The combination of these multifactorial scaffolds directs the phenotypic determination of MIA stem cells toward hyaline-like chondrocytes capable of synthesizing a hyaline-like extracellular matrix that appears to stimulate the integration of the newly formed regenerated tissue with the existing host osteochondral tissue. We expect to determine whether the developed multifactorial strategy directs newly formed tissue that exhibits the appropriate biomechanical properties to warrant clinical assessment of our novel injectable articular cartilage tissue engineered regenerative product.

439 - -omic Signatures Of Mesenchymal Stem Cell-derived Chondrocytes With High Vs. Low Chondrogenic Potential

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***Purpose/Objectives:** Mesenchymal stem cell (MSC)-based therapies have the potential to regenerate new cartilage by depositing chondrogenic matrix in vivo, stimulating resident chondrocyte matrix production, and inhibiting inflammation. At present, MSC-derived cartilage fails to retain functional

articular cartilage structural components, which critically impairs the function of the engineered tissue [1]. These outcomes are likely due to our incomplete understanding of the biology of MSC chondrogenesis and how it differs from cartilage formation during development or post-natal cartilage homeostasis. Currently, protocols to induce chondrogenesis in MSCs are based on extended treatment with growth factors and loading conditions known to have significant anabolic effects in articular cartilage. Recent studies, however, clearly demonstrate that MSC-derived cartilage transcriptome is very different from primary articular chondrocytes as they accumulate matrix in vitro [2]. The goal of this work was to establish the -omic signature during MSC chondrogenesis with "high" vs. "low" chondrogenic potential.

***Methodology:** Human MSCs were placed in pellet cultures with or without 10ng/ml TGF-β1 to simulate "high" or "low" chondrogenic potential for 28 days. Both groups demonstrated chondrogenesis with positive staining for sulfated glycosaminoglycans (sGAGs) and collagen type II with more intense staining in the TGF-β1 treated group. The real-time activity of functional promoters and gene expression of known biomarkers of chondrogenic differentiation markers (Sox9, Aggrecan, Col2a1, Col10a1, and Runx2) demonstrated a differential expression profile after 7 days of culture. The profile of sulfated glycosaminoglycan (sGAG) previously shown to correspond to the deposition of the pericellular, territorial, and inter-territorial matrix of articular cartilage was quantified in conditioned media collected throughout the chondrogenic program using enzyme-linked immunosorbent assays (ELISAs) [3]. The amino acid profiles in the medium, which indicates the uptake/secretion data of amino acids and how they related to protein content, was also quantified from the conditioned media.

***Results:** The outcomes indicated differences promoter activity, gene expression, and protein expression of chondrogenic markers as well as in the deposition of the territorial matrix throughout chondrogenic program with TGF-β1 treatment.

***Conclusion/Significance:** With this data, we are able to determine which early stem cell signatures robustly correlate with the production of cartilage-specific structural macromolecules (biochemical assays and histological staining) of cartilage tissues grown *in vitro*.

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440 - Effects of Cell-Adhesion Integrin Binding Peptides on 3D Culture of Mesenchymal Stem Cells

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Osteochondral (OC) defects contribute to progressive joint deterioration leading to osteoarthritis. Current clinical treatments do not address the underlying pathology which is challenging to treat as damage occurs in articular cartilage and subchondral bone. Encapsulation of mesenchymal stem cells (MSCs) in scaffolds has been one of the most sought out treatments for OC defect repair. MSCs express transient increases in integrin subunits during differentiation, the latter enhanced upon integrin-mediated adhesion to the RGD cell adhesion ligand in combination with other peptides. This suggests that MSC-laden scaffolds immobilized with combinations of integrin-specific peptide ligands that promote chondrocyte and osteoblast differentiation can be used as a minimally invasive treatment for

OC defect repair. Proteolytically degradable polyethylene glycol hydrogel scaffolds were therefore synthesized with varying molar ratios of immobilized laminin-derived (IKVAV) and fibronectin-derived RGD peptides to encapsulate human MSCs (hMSCs) as individual cell suspensions or as 3D cell spheroids. Cell viability and 3D spheroid invasion were quantified at days 1, 7 and 14 using florescence microscopy. At day 14, MSC viability for individual cell suspensions was 91%, 65% and 88% in gels immobilized with IKVAV, RGD and with both RGD and IKVAV peptide ligands, respectively. Furthermore, scaffolds immobilized with both peptide ligands were found to be favorable for MSC spheroid culture as they supported the highest growth, length and extent of 3D sprouting compared to either peptide alone. The impact of these findings not only holds potential for OC regeneration but also for other adhesion mediated cell differentiation and tissue regeneration applications.

441 - Fast-crosslinking Hyaluronic Acid And Gelatin Hydrogels As A Translational Platform For Treating Spinal Cord Injury

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***Purpose/Objectives:** A series of two *in vitro* studies were performed to assess cell adhesion and evaluate a fast-crosslinking hydrogel as a foundation for further studies of translational and bioactive materials for spinal cord regeneration. Spinal cord injury (SCI) causes permanent damage and clinical treatments do not result in complete functional recovery. There are numerous publications about SCI from the past decade, but the lack of new clinical treatments demonstrates the need to develop clinically translatable therapies. One of the most promising translational research areas utilizes injectable, *in situ*-forming hydrogels; however, many hydrogel precursors possess low viscosities and surgical placement within the cyst-like injury is difficult. We previously developed hydrogel precursors with a paste-like rheology using a pentenoate-functionalized hyaluronic acid (PHA) with thiol-ene click chemistry for faster photocrosslinking (~2 min) than traditional hydrogel chemistries. PHA hydrogels could overcome the challenges of delivering a treatment to SCI, so in these studies, we refined hydrogel formulations of PHA combined with pentenoate-functionalized gelatin (PGel) to support the greatest adhesion of rat neural stem cells (rNSCs).
***Methodology:** In the first study, hydrogels were formed from varying concentrations of PGel (i.e., 5, 10, and 15 wt%), with a crosslinker (dithiothreitol, DTT) and photoinitiator (Irgacure 2959, I2959). Hydrogels were seeded with rNSCs and evaluated after 1 and 14 days with the alamarBlue assay and Live/Dead staining. In the second study, hydrogels were formed from varying concentrations of PHA (i.e., 3, 4, and 5 wt%) and PGel (i.e., 5 and 10 wt%), with DTT and I2959. Hydrogels were seeded with rNSCs and were again assessed after 1 and 14 days with the alamarBlue assay.
***Results:** In preliminary studies, seeded rNSCs failed to adhere to PHA hydrogels, therefore in the first study, rNSCs were seeded on different concentrations of PGel hydrogels. After 1 day, rNSCs adhered the most to 5 wt% PGel hydrogels compared to 10 and 15 wt% PGel but after 14 days, the hydrogels had degraded. To prevent the rapid degradation of PGel and enhance the paste-like precursor properties, in a second study, different concentrations of PHA and PGel were assessed. After 1 day, decreasing concentrations of PHA had increasing cell adhesion and after 14 days, the 3 and 4 wt% PHA had similar amounts of cell survival, for both PGel concentrations. While all PHA+PGel groups had slower degradation compared to PGel alone, the 4 wt% PHA + 5 wt% PGel had a paste-like precursor while also

supporting sufficient rNSC adhesion and survival.
***Conclusion/Significance:** Overall, the addition of PHA to PGel generated a paste-like precursor for easier surgical application with slower degradation, which improved clinical translation. PHA+PGel formulations will be refined with peptides and growth factors to further enhance cell adhesion and neural differentiation to promote nerve regeneration and functional recovery after SCI.

442 - Development Of A 3D Blood Brain Barrier Model With Tissue Specific ECM Conditions

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***Purpose/Objectives:** Currently, there are few physiologically relevant models of the human brain that accurately model blood brain barrier (BBB) function. A system that mimics the *in vivo* selectivity of the human BBB is an essential tool that could be used to study neurological disorders, design new treatments, and study toxicity and bioavailability of existing drugs. Current models of the neurovascular unit (NVU), the functional unit of the brain where brain tissue interfaces with the BBB, have been made using 3D cell spheroids and microfluidic devices, but they fail to mimic the 3D structure, cellular composition, and extracellular matrix (ECM) components of the human brain. In this study, we develop a model using a hydrogel with physiologically relevant ECM proteins to create a functional 3D BBB.
***Methodology:** Brain endothelial cells (BEC) were derived from human induced pluripotent stem cell (hiPSC) according to a previously described procedure [Hollmann 2017]. Human astrocytes (AC, ScienCell) were grown on the bottom of a transwell culture system with a membrane pore size of 0.4µm and an area of 0.33cm^2, coated with poly-(L-lysine) and allowed to grow for 24hrs. Human brain vascular pericytes (hVPC, ScienCell) encapsulated in hydrogel at a concentration of 5 million cells per mL were seeded on top of the semipermeable membrane. The hydrogel was composed of modified hyaluronic acid, gelatin, and a PEG crosslinker was supplemented with thiolated fibronectin and laminin at a concentration of 0.25mg/mL, and it was crosslinked with UV light. Staining for tight junction marker ZO-1 and permeability testing using 5kDa dextran tagged with FITC was used to confirm BBB function. Trans-endothelial electrical resistance (TEER) was measured using an epithelial ohm meter.
***Results:** When a monolayer of BECs were grown on the hVPC and ECM hydrgel, ZO-1 staining reveals robust tight junction formation. TEER measurements show that the hydrogel produces stable resistance measurement over time. Permeability testing using FITC tagged dextran molecules also reveals that our *in vitro* BBB exhibits physiologically relevant permeability characteristics that are dependent on the ECM hydrogel composition. Further staining for transport proteins such as ATP sensitive potassium channels, and other permeability assays have also been conducted to confirm the presence of active transport mechanisms in our model. Charge selectivity can be tested using the drug MPTP. The drug is metabolized by astrocytes and forms the toxic molecule MPP+ which is positively charged which should not cross the BBB.
***Conclusion/Significance:** Our model mimics the permeability and active transport mechanisms present of the human BBB and demonstrates the importance of mimicking in vivo ECM conditions via the addition of proteins such as fibronectin and laminin in 3D tissue engineered models. The model will be combined with other work in our lab to create a functional NVU model with relevant vessel geometries. BECs can also be produced using hiPSCs derived from patients with specific neurological disorders in order to study a variety of neurodegenerative diseases including Alzheimer's disease. hiPSC derived

neurons, astrocytes, microglia, and pericytes can also be incorporated into the system to better model these diseases.

443 - An In Vitro Model Of Neurovascular Unit

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Stroke is the leading cause of adult disability with patients surviving their initial stroke, but not recovering fully. Stroke is particularly devastating due to the lack of spontaneous self-renewal in the brain. Regeneration approaches focus greatly on neural stem cell-based transplantation. However, lack of vascularisation and spatial support at the infract site result in poor survival of the transplanted cells and reduced integration with host tissue of the ones that survive. Neuronal and vascular cells communicate closely in the brain and organise in what is called neurovascular unit NVU. After stroke communication is disrupted. A hyaluronic acid-collagen (HA-Col) composite hydrogel will facilitate survival, growth and communication of human neural stem cells (NSCs) and endothelial cells (ECs) derived from induced pluripotent stem cells (iPSCs) to establish a neurovascular network (NVN) *in vitro* that recapitulate the brain NVU. For the NVN formation, iPSC-ECs and iPSC-NSCs were mixed in a HA-Col hydrogel at various cell ratios. Confocal microscopy and histological immunostaining were used to assess the formation of a 3D capillary-like network within the NVN with lumen like structures, expression of multipotency (Sox2/nestin), proliferation (ki67) and differentiation (MAP2/NeuN/GFAP/O4/NG2) markers. Evidence of the impact of the hydrogel on the coupling of angiogenesis and neurogenesis will also be presented. Our results demonstrate that the composite hydrogel provides the appropriate cues and support the formation of a 3D NVN; a promising *in vitro* model to study neural regeneration mechanisms in healthy and pathological environments.

444 - Omega-3 Effects The Behavior Of Muscle And Non-muscle Cells On A 2d Fibronectin And In A 3d Fibrin Construct

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Omega-3 Effects the Behavior of Muscle and Non-Muscle Cells on a 2D Fibronectin and in a 3D Fibrin Construct Simon Sozzi, Danilo Souza, Victor Garcia, Bill TawilPresenting Authors: Simon Sozzi simonsozzi9@gmail.comDanilo Souza danilofs22@gmail.comVictor Garcia victor.garcia745@myci.csuci.edu*M.S Biotechnology - Biomedical Engineering, California State University Channel Islands*
INTRODUCTION:HFF cells have proven to be efficient and easy to handle when doing research studies. Fibronectin are a simple and reliable source when dealing with proliferation of HFF cells. Fibronectin will be used to study the proliferation and adhesion of Human Foreskin Fibroblasts exposed to different concentrations of Omega 3. **METHODS:**Cells were seeded in 24 well plates, coated with 10ug/ml of Fibronectin at 4 different concentrations of Omega3; 0mg, 1mg, 5mg and 10mg. Media was then added to reach a total volume of 2mL. The wells were loaded with an initial density of cells per well and stored at 37 degrees C. After 0, 3, and 7 days ofincubation. Florescent samples were then quantifiedusing a FilterMax F5 Multimode Microplatereader. **RESULTS:**HFF cell adhesion is best in presence of 1mg

solution of Omega 3 and decreases as the Omega 3 concentration increases. The highest percent of proliferation of HFF cells are seen best in the presence of 5mg of Omega-3 on Day 7. **DISCUSSION & CONCLUSIONS:**Different concentrations of Omega-3 were used and the experiment was conducted twice over a 7-day period and the results were recorded on Day1, Day 3 & 7 (n=6). The results show that the higher concentrations of Omega-3 decreases the adhesion of HFF cells.

445 - 3d Printed, Macroporous Organosilicone Scaffolds For Cell-based Treatment Of Type 1 Diabetes

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***Purpose/Objectives:** Clinical islet transplantation is a potential cell-based treatment for Type 1 diabetes (T1D). The site of cell engraftment plays a major part in subsequent cell survival and capacity for glycemic control. Historically, the hepatic portal vein has served as a promising site due to its immediate access to blood supply. However, the combinatorial effect of the process of islet infusion, the unfavorable and unfamiliar environment of the liver, and early immune responses can result in poor engraftment. One alternative transplant site is the omental pouch. In our laboratory, we have previously engineered a highly porous polydimethylsiloxane (PDMS)-based scaffold platform to physically house islets in 3D in the omental pouch for localized drug release of anti-inflammatory agents. [1] Implantation studies found these scaffolds to be biocompatible and suitable for housing donor islets; however, the use of sodium chloride (NaCl) crystals in the generation of the porous structure results in an inherently variable structure and pore size distribution.
***Methodology:** To establish a more consistent fabrication process, address the variable pore size of the previous platform, and permit further personalization of scaffold geometry, we employed a reverse-cast 3D printing technique to generate PDMS scaffolds with consistent shapes and pore sizes. By utilizing commercial 3D printing to quickly model and fabricate molds using polyvinyl alcohol (PVA) filament, a multitude of geometries and pore sizes could be generated consistently to suit different applications.
***Results:** Fabrication precisely reproduces features created in 3D modeling, with an example printed construct having a porosity of 74.5% resulting in scaffolds with an experimentally measured porosity of 77.5 ± 2.1%. Scaffolds with an average pore size of 161 ± 17 µm can be generated from a 1.8 mm thick, 10 mm diameter PVA mold. *In vitro* screening of cytotoxicity using HUVEC cells observed no impacts of the final product on metabolic activity.
***Conclusion/Significance:** Methodology resulted in fabrication of porous, organosilicone scaffolds with consistent pore size and porosity. Future work will explore the potential of this scaffold platform to direct desirable vessel formation both before and after transplant, as well as exploring the platform as a potential base for protein conjugation testing and implementation into other applications in cell-based transplant.

446 - Methacrylated Gellan Gum Poly-l-lysine Complexes As Semipermeable Matrices For Cell-based Therapies

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PORTUGAL, [3]Immunology, Biomedical Research Center (CINBIO), Vigo - Pontevedra, SPAIN, [4]The Discoveries Centre for Regenerative and Precision Medicine, Guimarães, PORTUGAL.

***Purpose/Objectives:** Cell encapsulation is an alternative to the use of immunosuppressant drugs after cell transplantation. It shields cells from the host immune system, allowing the diffusion of nutrients and oxygen[1]. The alginate - poly-L-lysine - alginate system is the most well-studied method[2], but biocompatibility issues are still being reported[3]. Bearing this in mind, this work accounts the use of polyelectrolyte complex (PEC) formation to design semipermeable beads[4], that can be used on cell-based therapies.
***Methodology:** Using one-step protocol, methacrylated gellan gum (GG-MA, anionic polymer) is extruded by gravitational dripping into a poly-L-lysine (PLL, cationic polymer) bath. Due to the interaction between the carboxylic groups of the GG-MA and the charged PLL amines, a bead is formed comprising a GG-MA hydrogel core surrounded by a semi-permeable PEC membrane.
***Results:** Permeability was assessed using albumin-fluorescein isothiocyanate conjugate (BSA-FITC, 66 kDa),methylene Blue (MB, 319.85 Da), and Dextran-FITC with 4, 20 and 70kDa. While small molecules (MB and 4kDa Dextran-FITC) were rapidly released, the larger molecules had a hampered outflow and inflow. Therefore, it is expected that the designed beads will block the passage of antibodies but allow the free diffusion of nutrients, oxygen and therapeutic molecules. The immune response to PEC was also considered,by qualitative determination of total complement activation, macrophage proliferation and cytokine release. No significant adverse immune response was noticed in any of the aforementioned studies. Also, subcutaneous implantation of the GG-MA/PLL beads into the back of CD1 mice showed that the material does not elicit substantial fibroblastic overgrowth, over a period of 8 weeks. At last, human adipose stem cells were successfully encapsulated in the GG-MA/PLL beads. Live/dead quantification studies, as well as DNA quantification, showed that encapsulated cells remained viable up to 7 days of culture.
***Conclusion/Significance:** In summary, the designed PEC system showed stability over time, a semi-permeable behavior and biocompatibility, paving the way for its application on hydrogel-mediated cell therapies.
References:1 - L. Gasperini, J. F. Mano and R. L. Reis, J. R. Soc. Interface, 2014, **11**, 20140817-20140817.2 - S. V. Bhujbal, B. de Haan, S. P. Niclou and P. de Vos, Sci. Rep., 2014, **4**, 6856.3 - I. Ortega-Oller, M. Padial-Molina, P. Galindo-Moreno, et al., Biomed Res. Int., 2015, 1-18.4 - S. Vieira, J. Silva-Correia, J.M Oliveira, R.L. Reis, PCT/IB2016/057077. **Acknowledgments:** Sílvia Vieira was awarded an FCT PhD scholarship (SFRH/BD/102710/2014) and also acknowledges the IACOBUS scholarship (Eurorregión Galicia - Norte de Portugal, Agrupación Europea de Cooperación Territorial) and Award "Príncipe da Beira for Biomedical Sciences, 2017". The FCT distinctions attributed to J. Miguel Oliveira (IF/01285/2015) and J. Silva-Correia (IF/00115/2015) under the Investigator FCT program are also greatly acknowledged. This work was also partially supported by Xunta de Galicia (Grupo de referencia competitiva ED431C 2016/041) and the project FROnTHERA (No. NORTE-01-0145-FEDER-000023), supported by Norte Portugal Regional Operational Programme (No. NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

447 - Ultrathin Cerium Oxide Nanoparticle Coatings On Cell Clusters And Pancreatic Islets

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***Purpose/Objectives:** Cell transplantation is a promising therapeutic approach for diseases that involve the pathological loss of cells with low regenerative power, such as in diabetes, cardiovascular failure, and neurodegenerative diseases. However, cell replacement therapy is challenged by non-specific inflammatory reactions to the foreign graft, as well as antigen-specific immune rejection. The microencapsulation of cells within immune-isolating biomaterials can provide a physical barrier between the transplanted cells and the immune system. Although effective in preventing direct contact with the immune system, non-specific inflammatory reactions can still destabilize the encapsulated graft via soluble factors such as Reactive Oxygen Species (ROS). These soluble factors can permeate most cell-encapsulating biomaterials and cause direct degradation of cellular components (e.g., lipids, nucleic acids, and proteins). Also, ROS can assist in the formation of a fibrotic capsule that blocks free diffusion of nutrients, oxygen, and waste from the biomaterial. To mitigate the effect of ROS in graft destabilization, we have focused our work on developing antioxidant coatings that can dissipate ROS and protect encapsulated cells. In previous work, we have shown antioxidant protection using Cerium Oxide Nanoparticles (CONP), a highly effective antioxidant used in biomedical applications, due to its self-renewability and its ubiquitous free radical scavenging capabilities. Using layer-by-layer techniques, CONP coatings were formed onto biomaterial surfaces of different size and surface chemistry. CONP-coated alginate microbeads were able to dissipate physiologically relevant concentrations of H_2O_2 and protect the encapsulated cells from ROS-mediated damage. A challenge with this approach, however, is that microencapsulation can result in a high volume graft, which limits the site of transplantation and imparts diffusional delays. For that reason, our current efforts are focused on translating microbead surface layer-by-layer approaches to direct islets cell coatings. To test the feasibility of this concept, clusters of insulin-producing mouse insulinoma beta-cell spheroids (MIN6) and pancreatic rat islets of Langerhans were coated with nano-thin layers of CONP and sodium alginate (Na-Alg).
***Methodology:** β-cell clusters and pancreatic Lewis rat islets were coated with alternating layers of Na-Alg and CONP solutions, both at a concentration of 3 mg/mL. The uniformity and conformity of CONP/Alginate coatings on cells were verified using a fluorescently labeled alginate via confocal microscopy. The effect of CONP coatings on cell health was assessed via Live/Dead/Hoechst stains, MTT metabolic activity assay, and Glucose-Stimulated Insulin Release (GSIR).
***Results:** Uniform coatings were formed on the surface of β-cell clusters and the coatings did not impart a significant change in cell metabolic activity or glucose-stimulated insulin secretion. CONP and alginate coatings on pancreatic islets, however, resulted in a 30% decrease in islet viability, when compared to non-coated islets.
***Conclusion/Significance:** In this work, we show the feasibility of applying nanoscale coatings on islet and beta cell spheroids using alternating layers of CONP and alginate. Future work will focus on improving layering approaches to mitigate toxicity for primary pancreatic islets via chemical modifications.

448 - Scaffold-based 3d Model To Study Migratory Mechanisms Of Pancreatic Cancer Cells

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***Purpose/Objectives:** In 2018, pancreatic ductal adenocarcinoma (PDAC), ranked as the fourth cause of cancer-related deaths in Europe and its incidence rate is increasing [1]. Owing to its asymptomatic behavior, PDAC is not diagnosed at an early stage and metastases often occur to the surrounding organs. The epithelial-mesenchymal transition (EMT) mechanism has been hypothesized in recent years to underlie the migration of tumor cells to other organs [2]. EMT is regulated by several signaling pathways, therefore its study is complex and multifactorial. In recent years, new three-dimensional (3D) in vitro models have been developed to better understand cancer mechanisms and resemble tumor microenvironment (TME) [3]. Aim of this study was to obtain 3D in vitro models of PDAC to unveil important phenomena, such as cancer cell migration, TME composition, ECM remodeling and EMT.
***Methodology:** We fabricated and optimized spongy scaffolds made of PVA/Gelatin (G) (70/30 w/w) via emulsion of a 10% (w/v) solution of PVA (99% hydrolyzed, 85,000-146,000 Mw) with G, followed by 1 freezing cycle, and lyophilization. Primary PDAC cells were isolated from patient's specimens, characterized and seeded at 1×10^6 cells/scaffold. These constructs were cultured for 15 days and assayed 2, 5, 8, 15 day time-points (n =3). During the experiment, the AlamarBlue metabolic assay was performed. At each time-point, the constructs were processed for histology: cell morphology was investigated via hematoxylin and eosin staining, proteins involved in the EMT (i.e., EGFR, PAN-CK, TGF-β, MMP-9, actin and desmin) were evaluated via immunohistochemistry and cell migration via image analysis of histologic sections.
***Results:** The results showed that our scaffold-assisted 3D model: (1) is representative of cell migration and could also suggest a new study approach for the evaluation of molecular factors sensitive to therapy, associated with the invasion process; (2) showed high reproducibility of the EMT process, in particular TGF-β protein modulation, which could be the discriminant of the activation of the EMT process. The phenotypic expression of TGF-β and EGF tested in PDAC tissue specimens seems indeed to corroborate this observation. (3) EMT was clearly observed in this timeframe.
***Conclusion/Significance:** By mimicking the pancreatic tissue environment, PVA/G sponges can unveil cancerous mechanisms of PDAC and help the development of new and effective therapies for this dismal malignancy. **References** [1] Ferlay J, Colombet M, Soerjomataram I, Dyba T, Randi G, Bettio M, Cancer incidence and mortality patterns in Europe: Estimates for 40 countries and 25 major cancers in 2018. European Journal of Cancer. 103:356-87 2018. [2] Shuai W, Shuai H, Yu Ling S, Epithelial-Mesenchymal Transition in Pancreatic Cancer: A Review. BioMed Research Inter. Vol. 2017. [3] Ricci C, Moroni L. Danti S. Cancer tissue engineering-new perspectives in understanding the biology of solid tumors-a critical review. OA Tissue Engineering Apr01; 1(1):4. 2013.

449 - Development Of Gastrointestinal Patches Based On Bilayer Electrospun Membranes

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***Purpose/Objectives:** Intestinal tissue regeneration has been challenging due to the complexity of the gastrointestinal tract which is based on structured interaction between specialized epithelial, smooth muscle, endothelial, and nerve cells. In this study, a novel tissue engineering approach based on a graft-scaffold the system was developed to establish functional epithelial- smooth muscle cell cocultures based on an electrospun bilayer membrane. One side was prepared with aligned fibers and coated with Poly Ethylene Glycol Acrylate- Collagen I (PEG-AC-Collagen) to induce smooth muscle cell growth and the other side was prepared with random fibers and coated with PEG-Ac-Laminin to stimulate epithelial

polarization and cell growth.
***Methodology:** Polycaprolactone was spun randomly by electrospinning to achieve different diameters and the prepared mesh was attached to a drum and aligned PCL fibers were spun on top of random fibers. Samples were soaked in a photoinitiator solution overnight and then were exposed to ultraviolet light in baths containing PEG-Ac-Collagen and PEG-Ac-Laminin. The morphology of the samples by scanning electron microscopy (SEM), contact angle (n=6), mechanical properties (n=10) and permeability (n=4) of the membranes were also evaluated. *In vitro* studies were conducted by using IEC6 epithelial cells and rat primary smooth muscle cells over a 3 weeks period. Cell proliferation using Alamar Blue, immunofluorescence imaging (Actin, DAPI, α smooth muscle actin, ZO-1) and Apical Alkaline Phosphatase (ALP) staining were conducted on samples. Quantitative data were compared using one-way Analysis of Variance (ANOVA) followed by Tukey's test for *post hoc* determination of significant differences at $p<0.05$.
***Results:** The SEM images demonstrated that the bilayer was successfully fabricated with clearly distinguishable morphology. Pycnometry showed that aligned fibers had the lowest porosity (70%) which also affected the permeability. The aligned mat showed the lowest permeability over 24 hrs. The contact angle was significantly different after the coating in all groups which is due to the presence of PEG-Ac, Laminin, and Collagen in the coating. The cell staining revealed the presence of α smooth muscle actin in smooth muscle cells and tight junction formation between epithelial cells was also observed in all groups; however, only the 1 μm fibers or the membrane with Laminin coating showed epithelial polarization.
***Conclusion/Significance:** According to our data, we were able to design a membrane with two different morphologies while the system's integrity allows us to co-culture epithelial cells and smooth muscle cells indirectly. This system can be used as a membrane in gastrointestinal tissue regeneration as its unique structure enables us to control transport permeability morphology allows for tuning cellular functionality.

450 - Hypoxia-on-a-chip: A Controllable System To Investigate The Magnitude And Duration Of Hypoxia On Human Intestinal Epithelial Stem Cells

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Joint Department of Biomedical Engineering, North Carolina State University and University of North Carolina at Chapel Hill, Raleigh, NC.

***Purpose/Objectives:** Intestinal epithelial stem cells (ISC) reside at the base of the small intestine, in a crypt with a steep oxygen gradient created by the surrounding microvasculature. Tight regulation of ISC proliferation and differentiation is essential for homeostatic epithelial renewal and repair of the barrier in disease states. During intestinal ischemia, the microvasculature becomes blocked and the epithelium undergoes a severe hypoxic insult and eventual death if oxygen is not restored. Paradoxically, when oxygen is restored, additional reperfusion injury can occur due to damaging free radicals and oxidative stress. How ISC respond to IR-injury is not well understood in humans. Conventional hypoxic chambers are large, have static atmospheric parameters, and do not easily allow for modulation or monitorization oxygen. Without a system that continuously monitors and controls oxygen in vitro, it would be impossible to study the effects of the magnitude and duration of hypoxia on human ISC function.We have developed a hypoxic platform with integrated oxygen sensors to culture human ISC in a known oxygen environment and monitor oxygen at the air-liquid interface of the ISC during various periods of

hypoxia. Previous literature has shown that hypoxia causes other stem cell types, including breast cancer stem cells and embryonic stem cells, to increase proliferation and pluripotency.[1-3] Furthermore, ischemic preconditioning, or brief periods of ischemia with intermittent reperfusion, before intestinal injury has shown to increase murine ISC activity, as evident from increased expression of stem cell markers.[4] **Informed by these studies, we will test the hypothesis that hypoxia will promote human ISC proliferation.**

***Methodology:** Primary intestinal epithelial cells were isolated from the jejunum of human donor tissue and cultured in 6 well tissue culture plates on 3D collagen scaffolds containing stem expansion media in a normobaric incubator. ISCs were isolated and seeded within a polymethyl methacrylate (PMMA) microfluidic chip containing a hydrogel scaffold with an integrated phosphorescent lifetime detector oxygen sensor composed of poly(2-hydroxyethyl methacrylate) gels functionalized with palladium-benzoporphyrin derivatives (Pd-BPD) that responds to local oxygen concentrations via phosphorescence quenching. Hypoxia (0.3% O_2) was established using an oxygen sparaging technique via continuous nitrogen gas flow on-chip. Following hypoxic insult, ISC were dissociated to single cells, cultured, and organoid formation was quantified as a functional assay of stem cell activity.

***Results:** We found that human ISC respond to hypoxic insult by increasing proliferation, evident from an increase in organoid forming efficiency, as compared to normoxic human intestinal epithelium.

***Conclusion/Significance:** Our platform demonstrates precise control of the duration and magnitude of hypoxic environment, furthermore, the hypoxia-on-a-chip shows that 0.3% oxygen for 24 hours induced increased ISC proliferation. This platform provides a means to mimic IR-injury and monitor ISC regenerative behavior, additionally, the device can be used to address questions related to IR-injury in a broad range of tissue types.

References: [1] PMID: 27001847; [2] PMID: 26372732; [3] PMID: 19755485 [4] PMID: 24176207.

451 - Biofabrication Of Three-dimensional Multicellular Human Gastrointestinal Model For High Throughput Toxin Testing

J. D. Gaston[1], T. Picou[1], A. Melton-Celsa[2], V. B. Ho[2];
[1]The Geneva Foundation, Rockville, MD, [2]Uniformed Services University, Bethesda, MD.

***Purpose/Objectives:** 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-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} Shiga toxin producing *Escherichia coli* (STEC) and enteroaggregative *E. coli* (EAEC) are food- and water-borne pathogens responsible for many cases of diarrheal illness annually. Standard two dimensional monocellular tissue culture and animal models have revealed some aspects of STEC and EAEC pathogenesis but are inadequate for faithfully recapitulating the human gastrointestinal microenvironment. The ability to rapidly test and develop treatment methods and infection control with a human-derived GI model system is essential for optimal disease prevention and personnel readiness.

***Methodology:** 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-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} To this end, we have designed and fabricated a multilayer human *in vitro* hindgut model for testing with STEC and EAEC. The core of this colonic model system is a striated membrane comprised of induced pluripotent stem cell derived colonic epithelium and endothelium surrounding mesenchymal stromal cell derived myofibroblasts. The complex multilayered structure of the model is achieved through the culture and differentiation of each individual cell type followed by assembly into a single model.
***Results:** 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-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} Immunohistochemical analysis demonstrates that correct colonic architecture was achieved included crypts and mucin production. Furthermore, these *in vitro* tissue models can be rapidly fabricated using bioprinted techniques, resulting in high reproducibility, low variability, and the ability to conduct high throughput testing. Colonic tissue models could be successfully infected with Shiga toxin and was capable of supporting bacterial growth.
***Conclusion/Significance:** 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-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} Future studies will focus on co-culturing a commensal *E. Coli* strain above the gut epithelium and loosely adherent with the mucus layer. This model system will allow us to learn more about the Shiga toxin infection, pathogenesis, and biofilm formation and serve as a platform for countermeasure development to limit toxicity and morbidity.

452 - Tissue Engineered Human Intestine With Inflammation Responses

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Biomedical Engineering, Tufts University, Medford, MA.

***Purpose/Objectives:** The intestine presents a surface area of 250 m^3 to 400 m^3 to an external environment comprised of digesting food and a population of 10^{13} to 10^{14} bacteria known as the gut microbiota [1, 2]. The intestinal epithelium protects the host and coordinates the intestinal immune system to eliminate pathogens and to secrete factors contributing to epithelial renewal and remodeling

[3, 4]. Investigations of intestinal immune phenomena are usually conducted with *in vitro* 2D cell or tissue models or *in vivo* using mice models. In the present work, an *in vitro* 3D tissue engineered human intestinal model was used to assess colonic epithelium-immune responses using human LGR5+ colonic organoids (colonoids). The goal of this system was to mimic physiologic responses of the epithelium when co-cultured with a human primary monocyte-derived macrophage mimicking the epithelial tissue organization.
***Methodology:** To achieve 3D intestinal tissue organization, our previously reported small intestine epithelium tissue model cultured on lyophilized silk sponge matrices was modified by introducing a modular secondary macrophage laden 3D tissue layer [5]. This bilayer system enabled the replacement of the macrophage laden outer layer without disruption of the inner scaffold layer containing the epithelium in order to observe the migration of the macrophages towards the epithelial layer. To ensure physiologic responses of the intestinal epithelium, human colonoids were cultivated as LGR5+ epithelial stem cells derived from colonic crypts [6].
***Results:** Analysis of the epithelium of the inflamed 3D co-culture models showed a significant decrease in epithelial coverage and morphology in the presence of the macrophages, in addition to infiltration of macrophages towards the basal side of the epithelium. Cytokine analysis showed enhanced secretion of inflammatory and anti-inflammatory cytokines, reflective of profiles of *in vivo* inflammation.
***Conclusion/Significance:** This paper presents a novel bilayer system based on lyophilized silk sponges using human colonoids and human primary monocyte-derived macrophages cultured in a 3D spatial distribution mimicking *in vivo* intestinal epithelium.
References: (1) Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLOS Biology 14, 8, 2016. (2) Thursby E, Juge N. Introduction to the human gut microbiota. The Biochemical journal 474, 11, 2017. (3) Mowat AM, Agace WW. Regional specialization within the intestinal immune system. Nat Rev Immunol 14, 2014. (4) Davies LC, et al. Tissue-resident macrophages. Nat Immunol 14, 10, 2013. (5) Chen Y, et al. In vitro enteroid-derived three-dimensional tissue model of human small intestinal epithelium with innate immune responses. PLoS One 12, 11, 2017. (6) Sato T, et al. Long-term Expansion of Epithelial Organoids from Human Colon, Adenoma, Adenocarcinoma, and Barrett's Epithelium. Gastroenterology 141, 5, 2011.
Acknowledgements: Support from the NIH (U19-AI131126, P41EB002520) and the Air Force Office of Scientific Research, National Defense Science and Engineering Graduate (NDSEG) Fellowship is greatly appreciated.

453 - Expandable Lung Epithelial Progenitors Originating From Human Pluripotent Stem Cells Differentiate To Terminal Phenotypes

A. Hampl, H. Kotasova, V. Pelkova, M. Klouckova, J. Dumkova, B. Michalikova, S. Farkas, I. Koutna; Department of Histology and Embryology, Masaryk University, Faculty of Medicine, Brno, CZECH REPUBLIC.

***Purpose/Objectives:** In many airway diseases such as asthma, chronic obstructive pulmonary disease, obliterative bronchiolitis, and cystic fibrosis, the reparation capacity provided by endogenous lung epithelial progenitor cells is often insufficient. Therefore, it is of a great clinical interest to derive sufficient numbers of exogenous lung progenitor cells suitable for cell therapy and/or tissue engineering. A promising option is derivation of lung progenitors from human pluripotent cells, embryonic or induced, by recapitulating differentiation events similar to those occurring during

embryonic development.
***Methodology:** In this study we derived early lung epithelial progenitors (ELEP) through differentiation of human embryonic stem cells (hESC) into lung lineage. Our method does not rely on specialized sorting or purification protocols. Instead, we generated ELEP in a monolayer culture without formation of 3D organoids. We measured the expression of key regulators of lung epithelial lineage by quantitative real time PCR, western blotting and indirect immunofluorescence, and we studied the detailed morphology of ELEP by transmission electron microscopy. We assayed the differentiation capacity of ELEP by in vitro culture under 3D conditions in matrigel and also in mice upon transplantation under kidney capsule.
***Results:** Here we prepared cells differentiated *in vitro* from hESC that can be propagated for long-term in culture and most likely represent equivalent of early lung progenitors (ELEP) occurring in development. We have shown that these cells can be maintained in culture for a minimum of 65 passages without losing their key characteristics. ELEP maintain their population doubling time at an average of 26.5 hours and the activity of their telomerase holds at about 50% of that typical for undifferentiated hESC. ELEP express high levels of anterior foregut marker SOX2 (also typical for self-renewing cells), marker of definitive endoderm SOX17, and marker of early lung epithelial lineage, thyroid transcription factor-1. As found by transmission EM, ELEP also possess morphological features of cells differentiating towards airway epithelia, multivesicular and lamellar bodies. When induced to terminally differentiate, ELEP increase levels of FOXJ1 (ciliated cells), pro-surfactant protein B (alveolar epithelial cells), Club cell specific protein (Club cells), aquaporin A (type I pneumocytes), and surfactant proteins A and C (type I pneumocytes and Club cells). Under 3D conditions and *in vivo*, differentiating ELEP then develop morphologies of alveolar- and airway-like structures. ELEP also possess capacity to interact and grow on decellularized lung scaffolds as well as on nanofiber matrices.
***Conclusion/Significance:** We for the first time describe establishment of stable population of human cells possessing features of early lung epithelial progenitors. Since these cells can be unlimitedly grown and frozen stored without losing their differentiation potential, they offer an attractive cell source for wide variety of biomedical applications.

454 - Collagen Hydrogel Matrices Permit Formation Of Mature Intestinal Mucosal Features In Cultures Of Ipsc-derived Intestinal Mucosal Cells.

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People with inflammatory bowel disease have progressive ulcerating intestinal lesions which impair normal function by reducing surface area and depleting mucosal epithelial cells. Inflammation is sustained by lack of a mucosal barrier to intestinal flora, creating a vicious circle which impedes healing. Our goal is to use induced pluripotent stem cells (IPSC) to generate a personalised tissue graft, to patch over ulcerated areas to promote healing. Here we present results of our work to date in A) developing a reliable and reproducible protocol for generating intestinal mucosal cells from IPSC and B) using a collagen hydrogel matrix to support their growth for several weeks in an *in vivo-like in vitro* culture system. Our protocol is capable of generating intestinal endoderm (Cdx-2/Ecad/Villin positive) efficiently

(>80% of population) with a smaller remaining fraction of cells expressing mesenchymal markers (vimentin/SMA). The population has significantly lower expression of pluripotency markers (Oct-4/Nanog) after differentiation, compared to published methodologies. In a type 1 collagen hydrogel system, over several weeks these cells spread over the surface and mature as a polarised intestinal epithelial monolayer, with an underlying stroma containing mesenchymal cells capable of matrix remodelling. This interaction promotes spontaneous formation of intestinal crypt-like structures containing a range of mature intestinal cell types, which we demonstrate by histological techniques. In summary, we have developed a novel platform for generating intestinal mucosal structures from IPSCs. With further development and refinement of supporting biomaterials/scaffolds, this platform could be capable of generating personalised tissue grafts for people with inflammatory bowel disease.

455 - Impact Of Carbon Monoxide Dose From Electrospun Scaffolds On Vascular Endothelial Cells

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***Purpose/Objectives:** Local delivery of gasotransmitters - e.g., carbon monoxide (CO) - from scaffolds has the potential to improve endothelialization in tissue-engineered vascular grafts (TEVGs). CO has been delivered via CO releasing molecules (CORMs) for biomedical applications since it is anti-inflammatory at appropriate doses [1]. However, controlling CO dose and understanding its impacts is critical since CO has a biphasic effect, and seemingly conflicting results are reported [2]. Local delivery from scaffolds is particularly challenging due to mass transport considerations and, as we have found [3], the CORM's chemical structure can impact the ability to activation and release CO. Thus, the goals of this study were to develop a new photoactivatable CORM material / scaffold system and determine the impacts of dose on endothelial cells (ECs) both experimentally and with a computational simulation.
***Methodology:** DK4 was synthesized in a 5-step process and incorporated within poly(ε-caprolactone)(PCL) electrospun scaffolds and spincoated films. CO release profiles after activation with 470 mm light were assessed both directly (myoblobin assay) and indirectly (fluorescence, 490/520 nm). Other CORMs (DK1, DK3) were also included as controls. Scaffolds were ethylene oxide sterilized and cultured with human umbilical vein ECs (HUVECs). Biocompatibility testing was performed with spincoated films with different CORM loadings. The impact of CO was assessed for surfaces activated 1 day after seeding with a DNA assay, live/dead, and immunfluorescence. Additionally, a two-part model was developed and validated to simulate a microfibrous scaffold and CO availability to cells.
***Results:** The DK4-based CORM/scaffold system can be activated after an extended culture time. Spincoated films were biocompatible for the 0-1% (w/w) loadings tested, with no differences in cell number between loadings (n=3, one-way ANOVA with Tukey). For CO release from electrospun meshes, ECs stained for functional markers (vWF) after mesh activation, and preliminary DNA data (n=3) suggests that CORM activation can improve EC proliferation. However, there is question about CO release in cell culture that cannot be tracked experimentally, so we simulated possible CORM/scaffold systems with our computational model. This showed that CO would leave a well plate very quickly (majority out by 30 min). Further, we showed that scaffold properties (e.g., fiber diameter, volume fraction) impact the CO dose available to cells. One implication is that the more cell contact with the scaffold, the higher dose of CO that the cells will receive.
***Conclusion/Significance:** We demonstrate that photo-activatable CORM materials (e.g., DK4) can be

incorporated within fibrous scaffolds, are biocompatible, and allow controllable CO release from the scaffold. However, our simulations indicated that controlling the dose available to the cells is more difficult since only a fraction of the initial CO concentration released from fibers will be available to cells. In addition, scaffold parameters (e.g., compliance and topography) that impact the degree of cell spreading can have an important impact not just for traditional tissue engineering but also for drug delivery. **References:** [1] Motterlini et. al., Nature Drug Discovery, 2010. [2] Washington et. al., Frontiers in Pharmacology, 2017. [3] Michael et al., Biomedical Materials, 2016. **Acknowledgments:** NSF (CBET 1510003)

457 - Vibration Loading Induces Phenotypic Modifications And Increased Transgene Expression

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***Purpose/Objectives:** One major drawback of non-viral gene delivery means stands in the lack of suitable techniques to deliver the exogenous genetic material inside the cells.[1] Since their introduction, chemical vectors - i.e., cationic carriers able to self-assemble with nucleic acids (NAs) into nano- and micro-particles - and physical methods - i.e., the application of membrane-disruptive forces to ease the intracellular delivery of NAs - have made strides forward.[2] However, the poor efficiency of the former and the risk of potential cell damage of the latter are still hampering their widespread application. In this context, we propose a novel in vitro transfection strategy relying on the delivery of linear polyethylenimine (lPEI)-based polyplexes to mechanically vibrated cells, with the aim of easing the internalization of the genetic cargo, thus improving the transfection efficiency (TE) of the non-viral carrier.
***Methodology:** A vibrating platform consisting of a sine wave generator connected to a custom-made mechanical wave driver was used to stimulate two cell lines. Membrane morphology during and after the application of 5 min vibration loading at different frequencies from 10 to 1,000 Hz was inspected by means of Scanning Electron Microscopy (SEM). Cells were next transfected with 25 kDa lPEI/pGL3 complexes with an ammine-to-phosphate molar ratio (N/P) of 30 and stimulated for the same period at the above-mentioned frequencies. TE and cytotoxicity were assessed 24 hrs post-transfection.
***Results:** To shed light on the cell response to vibration stimuli, we evaluated the role of short stimulations at different vibration frequencies on cell membrane morphology. From a stimulation frequency of 100 Hz onward, cells displayed blisters and protrusions all over the surface, probably due to the blebbing phenomenon.[3] Moreover, when stimulation was released, cells were able to restore membrane smoothness within one hr. The same threshold (i.e., 100 Hz-vibrations for 5 min) was found to induce an increase in TE of lPEI-based polyplexes with respect to static unstimulated controls and to cells exposed to low-frequency vibrations of 10 and 50 Hz. Besides, viability of transfected cells was not affected by the mechanical stimulation.
***Conclusion/Significance:** We proposed a novel, cost-effective and non-toxic strategy to boost the transfection efficiency of polymer-based gene delivery vectors on different cell types by means of a short vibration loading. Further investigation on the mechanisms involved in the increase of polyplexes internalization should be performed. **References:** 1. Pezzoli D, Chiesa R, De Nardo L, Candiani G. We still have a Long Way to go to Effectively Deliver Genes! J Appl. Biomater. Function Mater. 10, 82, 2012. 2.

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458 - Biomimetic Hyaluronic Acid - Peptide Hydrogel To Model Liver Fibrosis

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***Purpose/Objectives:** The use of biomaterials and 3D culture environments for disease modeling has recently gathered attention. One such disease is liver fibrosis, which has a severe impact on patient quality of life and has been highly correlated to the development of cancer. However, the role of extracellular matrix (ECM) components in disease progression has not been well identified. Specifically, laminin, fibronectin and collagen adhesion motifs and their impact on hepatic stellate cell phenotype has not been defined. By developing a hydrogel that allows us to modulate these components, we will be able to understand their impact on activation of hepatic stellate cells and the fibrotic microenvironment.
***Methodology:** The hyaluronic acid (HA) based ECM peptide hydrogel was develop in two steps. Maleimide-hyaluronic acid (MaHA) polymer was initially synthesized using NHS and EDC chemistry and confirmed using h-NMR. Thiolated peptides SIKVAV (laminin), RGD (fibronectin), and GFOGER (collagen) were each mixed with MaHA, and thiol-maleimide reactions covalently immobilized the peptides to the HA-backbone material. The product was crosslinked using PEG di-thiol to yield the final hydrogel. The material was characterized for: stress/strain, pore size, and swelling ratio. Hepatic stellate cells (Lx2, $1x10^6$ cells per mL) were combined in the hydrogel, with or without the peptides, and 3D tissue constructs (organoids, 100µL) were formed via crosslinking as previously described. Organoids were assessed after 1, 4, and 7 days in culture using immunohistochemistry for integrins relevant to each of the peptides (laminin α5β1, collagen α3β1, fibronectin α2β1) and for cell phenotype to determine the levels of fibrosis/activation of the stellate cells.
***Results:** We were able to create a MaHA polymer using NHS and EDC chemistry, a method not previously documented in literature for this synthesis, and the product was confirmed by h-NMR. We were able to successfully add peptides to the hydrogel using maleimide-thiol chemistry. Materials testing indicated that there was no statistically significant difference in hydrogels with and without the addition of the peptides. Swelling and degradation testing showed that materials maintained properties from day 1 through day 7 and no variation was seen between groups. Hepatic stellate cells also grew well in the hydrogel supplemented with peptides for up to 7 days, demonstrating phenotypic differences, expression of β1 integrins and α-smooth muscle actin.
***Conclusion/Significance:** In conclusion, we have been able to synthesize a modified HA biomaterial which has not previously been described for this application. We were then able to functionalize the material with peptides to yield a hydrogel able to maintain its physical characteristics over one week and could support hepatic stellate cell culture. These results indicate that we have been able to successfully fabricate a hydrogel that induces fibrotic phenotypes in hepatic stellate cells encapsulated in the hydrogel, and create a model of liver fibrosis.

459 - Biomimetic Injectable Hydrogels With Tunable Mechanical And Biochemical Properties For Cell Transplantation In Peripheral Arterial Diseases (pad)

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***Purpose/Objectives:** Peripheral arterial disease (PAD) affects over 8 million people in the US. It is associated with narrowing the peripheral arteries and restricts the blood flow to peripheral limbs. Current treatments of PAD such as cytokine therapy have shown limited long-term angiogenic benefits in clinical trials. Cell-based approaches to enhance revascularization by delivery of therapeutic endothelial cells is promising. However, low transplantation viability hampers the therapeutic potential of this approach. Since extracellular matrix (ECM) interactions are critical to cell survival and function, the purpose of this study was to determine the optimal biochemical and biomechanical factors that modulate endothelial survival and angiogenic function.
***Methodology:** A family of genetically engineered ECMs (e-ECMs) termed elastin-like protein (ELP) was developed using recombinant protein technology. ELP-based e-ECM structure mimics many fundamental characteristics of natural ECM including elasticity and structural support through elastin-like domain as well as bioactive cell binding domains. The e-ECMs were fabricated by altering elastin-like structural sequences (VPGXG) and customizable bioactive peptide sequences (e.g., RGD). To develop three-dimensional (3D) hydrogels, ELP-polyethylene glycol (PEG) hydrogels were fabricated withinteracting hydrazine-modified ELP (ELP-HYD) with either aldehyde- or benzaldehyde-modified PEG (PEG-ALD or PEG-BZA), resulting in shear-thinninginjectable hydrogels with independently tunable mechanical stiffness and stress relaxation rates. We first characterized the elastic modulus and stress relaxation rates of RGD-ELP/PEG-BZA (2%/2%) and RGD-ELP/PEG-ALD (2%/2%) by dynamic oscillatory rheology experiments using frequency sweeps at 37°C at 1% constant strain. Afterwards, human umbilical vein endothelial cells (HUVECs) were encapsulated within 3D gels to assess cell viability and spreading at cell densities of $1x10^6$ cells/mLand $1x10^9$ cells/mL for 1 and 7 days using a Live/Dead Cytotoxicity assay and confocal fluorescence imaging.
***Results:** Rheology measurements of the RGD-ELP/PEG-BZA (2%/2%) and RGD-ELP/PEG-ALD (2%/2%) hydrogels demonstrated a storage modulus of 850 and 1300Pa, respectively. Measurement of stress relaxation rate showed significantly higher rate (>2x) in RGD-ELP/PEG-ALD compared to RGD-ELP/PEG-BZA. Live/Dead cell viability assay demonstrated that both hydrogels could support high cell viability (>90%) at both 1 and 7 days. For both initial seeding densities at day 1, no significant difference in cell spreading and morphology was observed. However, at day 7, cell spreading increased in RGD-ELP/PEG-BZA hydrogels and tubular networks appeared inside the gels. However, cells did not form elongated morphology in RGD-ELP/PEG-ALD hydrogels, suggesting that stress relaxation rate and mechanical stiffness are key characteristics in modulating endothelial cell behavior and lower stress relaxation rate and storage modulus (~850Pa) of ELP-PEG hydrogel system improve the formation of endothelial cell network.
***Conclusion/Significance:** Injectable ELP/PEG-ALD/BZA is transparent and is controllable in terms of its mechanical stiffness, stress relaxation rate and bioactivity, which all make this hydrogel system a promising candidate for cell transplantation in tissue regeneration.

460 - Chondrocyte Embedded In Collagen-agarose Scaffold

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***Purpose/Objectives:** Current treatments to repair damaged articular cartilages are not effective, so through tissue engineering techniques, it has been tried to manufacture cartilage with natural and synthetic polymers scaffolds with autologous cartilage cells that can be transplanted. One of the challenges to obtaining bioconstructed cartilaginous tissue is to expand the chondrocytes in vitro to obtain a sufficient number of cells, however, the expansion process is associated with loss of chondrocyte differentiation[1]. Thus, new 3D culture strategies for chondrocytes have been developed with two purposes: 1) that the state of differentiation is conserved and, 2) that the cells have a high chondrogenic capacity. In this sense, hydrogels have been used for the encapsulation of chondrocytes, which promote differentiation, proliferation, viability and cell adhesion[2]. Type I collagen is a natural polymer that provides adhesion sites for chondrocytes, while agarose may provide the stiffness to maintain the state of differentiation of chondrocytes[3]. Thus, we propose a scaffold that combines type I collagen and agarose that allows a suitable growth and differentiation of chondrocytes.
***Methodology:** Cartilaginous cells were isolated from femoral condyle of young pigs trough enzymatic digestion with 0.3% collagenase. The cells were cultured in DMEM containing 10% FBS, 1% penicillin-streptomycin. Cells were encapsulated in passage 3. Collagen and agarose solutions were combined to yield gel solutions containing 100 or 200 µl, and 0, 5, 10 or 15 mg mL^{-1}. Constructs were cast in polypropylene tubes with a final volume of 1 mL. To the hot agarose was added 400 µl 2X DMEM, collagen, 1 M NaOH, PBS and $1x10^6$ cells in 100 µl media were added. Constructs were allowed to polymerize at 37°C and 5% CO_2 for 30 min. Then transfer to 4°C for 5 min. Thin slices of constructs were cultured, the medium was changed every 2 days. Samples were analyzed for viability assay, histological analysis, SEM and immunohistochemical assay after 1, 7, 14, 21 and 28 days in culture.
***Results:** Cells in all scaffolds displayed a rounded morphology that reminds chondrocytes in their native matrix. By day 1 culture many death cells were observed, nonetheless, the number of living cells increased as the culture time augmented. It should be noted that the cells formed groups in a similar way to the isogenic nests of the cartilage and were positive for the Ki67 protein. Pericellular matrix dense shown GAGs and at the cells were positive to pro-Col II and sox9.
***Conclusion/Significance:** Mix-scaffolds of type I collagen and agarose are suitable to maintain the differentiation and proliferation of chondrocytes.

461 - In Vitro Co-culture Under Static And Vibratory Conditions To Study How Vocal Fold Lamina Propria Extracellular Matrix Hydrogel Modulates Macrophage Polarization And Fibroblast Activation

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***Purpose/Objectives:** Voice disorders represent a significant healthcare challenge that affect approximately 20 million Americans each year [1]. Due to their anatomical location, vocal folds (VFs) are susceptible to various injurious stimuli that can cause damage leading to irreversible changes in structure and ultimately in vibratory function. VF fibrosis represents a challenging therapeutic scenario

since it is associated with significant changes in composition and mechanical properties of the extracellular matrix (ECM). This outcome is likely due to interactions between macrophages within the most superficial layer and resident fibroblasts in the VFs. Naturally derived ECM-based biomaterials have been explored as bioactive scaffolds that promote tissue remodeling, reduce fibrosis, and are safe for clinical use. We have previously derived a porcine vocal fold lamina propria ECM (VFLP-ECM) scaffold [2] and have developed a hydrogel form of the ECM scaffold (VFLP-ECMh) that can be injected into the site of injury using minimally invasive methods. Our current study focuses on determining how VFLP-ECMh modulates the fibrotic response by assessing the paracrine effects of the VFLP-ECMh on macrophage polarization and fibroblast activation under static and vibratory conditions.
***Methodology:** Macrophages and human vocal fold fibroblasts (hVFFs) were co-cultured for 48 hours at 37°C under static or vibratory culture condition. TGFβ1 cytokine was used to induce fibrosis in hVFFs. Real time quantitative polymerase chain reaction (RT-qPCR) was used to evaluate macrophage polarization and to determine *ACTA2* expression in hVFFs.
***Results:** Our results show that VFLP-ECMh modulates the expression of *ACTA2* in hVFFs. In addition, culturing hVFFs with conditioned medium obtained during macrophage polarization resulted in changes in gene expression patterns typically associated with tissue fibrosis, highlighting the potential effects of macrophage polarization on resident fibroblasts.
***Conclusion/Significance:** In conclusion, we have developed a porcine-derived VFLP-ECMh and demonstrated its contribution to the paracrine activation of macrophages and vocal fold fibroblasts. The information learned from this study supports the potential use of VFLP-ECMh to modulate scar tissue formation in injured VFs.
Acknowledgments: We thank our funding sources: NIH R01DC017139, The UNC-Chapel Hill/NCSU Joint Department of Biomedical Engineering, and The Comparative Medicine Institute (CMI) at NC State University.
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462 - Bioactive Bilayered Chitosan-PCL Membranes As Scaffolds For Periosteal Tissue Regeneration

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***Purpose/Objectives:** The innate regenerative capacity of bone tissue is insufficient for the repair of large-bone defects; thus, their treatment remains a clinical challenge. To overcome this challenge, intensive research efforts have been directed towards regenerative approaches. Tissue engineered scaffolds able to mimic the periosteum, an important structure of the bone tissue, may improve bone regeneration. The periosteum consists of a membrane that covers the outer surface of cortical bone and is formed by two distinct layers: an outer fibrous layer, containing mainly fibroblasts and collagen fibers; and an inner cambium layer, which is highly cellularized and contains multipotent mesenchymal stem cells and osteoprogenitor cells. Bone healing induced by periosteum is advantageous in comparison to treatments involving direct bone or bone-substitute transplantation, since it provides essential biological cues for tissue repair.

***Methodology:** In this work, a bilayered engineered periosteum was developed by combining the synthetic polymer polycaprolactone (PCL) and the biopolymer chitosan (Ch). The hybrid material consists of a porous PCL layer that acts as long-term support for cell growth with slow biodegradation rate covered by a chitosan layer designed to be in direct contact with the bone. Chitosan is osteoconductive and able to enhance osteogenesis and angiogenic activity. Due to its bioactivity and ability to interact with both hard and soft tissues, bioactive glass (BG) was added to chitosan at different proportions. BG is a very attractive material for producing scaffolds directed to bone regeneration since they may enhance revascularization, osteoblast adhesion and differentiation of mesenchymal stem cells as well as osteoprogenitor cells. Human dental pulp mesenchymal stem cells (DPSC) were seeded on the chitosan layer surfaces and their viability was assessed by resazurin method.
***Results:** After 24, 48 and 72 hours, DPSC remained viable and proliferated well on all scaffold formulations.
***Conclusion/Significance:** Considering the intended application as periosteum substitutes, the scaffolds herein studied promote attractive biological response of seeded DPSC and, therefore, can be considered promising materials for periosteal tissue enginering.
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464 - In Vitro Assay For Screening The Efficacy Of Phosphate And Polyphosphate Loaded Nanoparticles In Attenuating Matrix Degradation By Pathogen Secreted Proteases

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***Purpose/Objectives:** Severe persistent inflammation in diseases such as ulcerative colitis and inflammatory bowel disease (IBD) are due to loss of the normal microbiome and its replacement by pathogenic microbes. While host-derived proteases are known to contribute to pathogenesis, the role of microbial-secreted proteases due to virulent phenotypes remains unclear. Following surgical removal of diseased intestinal tract, increased bacterial protease expression is a key phenotype involved in intestinal healing impairment. Antibiotic administration is an ineffective treatment as it inadvertently eliminates normal flora while allowing pathogenic bacteria to acquire antibiotic resistance. We have previously shown that intestinal phosphate depletion in the surgically stressed host triggers bacterial virulence which is suppressed under phosphate abundant conditions. Given the variation of secreted proteases and phosphate metabolism among microbes, we investigated the efficacy of monophosphate and polyphosphate loaded nanoparticles (NP-Pi and NP-PPi, respectively) in attenuation of protease production of Gram-positive (*E. faecalis*) and Gram-negative (*P. aeruginosa and S. marcescens*) pathogens expressing high proteolytic activity.
***Methodology:** A physiologically relevant *in vitro* assay was developed to screen the efficacy of Pi and PPi in attenuating bacterial protease-induced matrix degradation. NP-Pi or NP-PPi were entrapped in a non-degradable poly(ethylene) glycol (PEG) hydrogel matrix co-incubated with a proteolytically degradable collagenase-sensitive PEG hydrogel. Each pathogen was separately added to culture wells

containing both hydrogels (one serving as a reservoir for sustained release of phosphates and the other as degradable tissue surrogate). Degradable hydrogels were removed at pre-determined time points and their wet weights correlated to the concentration of bacterial enzyme secreted in the medium. As wet hydrogel weight is not the most accurate measure of gel degradation, an alternative assay was developed to quantify matrix degradation whereby hydrogels were stained with sirius red and the weight measured as a function of absorbance (540 nm).
***Results:** Using this in vitro platform and our degradation assay, complete degradation of protease-sensitive hydrogels occurred within 7h, 16h and 9h in the presence of 1:100 bacterial culture dilutions of *P. aeruginosa, S. marcescens and E. faecalis*, respectively, in the absence of NP-Pi or NP-PPi treatment. With the inclusion of NP-PPi, matrix degradation significantly reduced 55% and 80% for *P. aeruginosa* and *S. marcescens*, respectively, with no significant reduction observed in the case of *E. faecalis*. Significant reductions in matrix degradation, 25% and 70% to *P. aeruginosa* and *E. faecalis*, respectively, also occurred with NP-Pi treatment; however, this did not attenuate *S. marcescens*-related matrix degradation.
***Conclusion/Significance:** The results suggest that a combination of NP-Pi and NP-PPi may be used as a broad-spectrum treatment, which is currently being explored. Finally, to better elucidate the role of bacterial enzymes contributing to matrix degradation, elastase B (LasB), protease LasA and alkaline protease (AprA), key extracellular proteases expressed by *P. aeruginosa,* were overproduced and purified. Preliminary findings indicate that Las-B *(*10µg/ml) resulted in complete and rapid degradation of protease-sensitive hydrogels on the order of minutes. Current efforts focus on elucidating the role of LasA and AprA, as well as key enzymes secreted by *S. marcescens* and *E. faecalis* on matrix degradation.

465 - Adipose Stem Cells Respond To Borate Bioactive Glass By Altering Protein Secretion

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***Purpose/Objectives:** Adipose derived stem cells (ASCs) have been shown to repair diseased and damaged tissue through multiple mechanisms, but their regenerative capacity is susceptible to biologic aging, autoimmune disease, obesity, and overall health of patients. There is much interest in reprograming sub-therapeutic ASCs to increase their regenerative potential. Borate bioactive glass (B3) has recently: (i) helped speed the healing of dermal wounds in > 90% of elderly patients in the clinic, (ii) provided no signs of inflammation or infection surrounding the scaffolds, resulted in little to no scaring, demonstrated hair regrowth, and had complete healing of the dermatological wounds, and (iii) been administered safely in multiple applications.
***Methodology:** Because ASCs have a role in each step of the wound healing process, the goal of this work was to evaluate the effects of B3 on ASCs. We used ASCs from at least three different donors, all grown sub-confluent and used between passages 2-6, and evaluated the effects of B3 on ASC survival, migration, differentiation, and protein secretion *in vitro*.
***Results:** Under static culture conditions, when B3 was directly added to the standard cell culture media, concentrations ≤ 5 mg/ml did not affect ASC viability for up to a week. Using a transwell plate to evaluate chemotaxis over 5 hours, B3 was shown to attract ASCs. However, when ASCs were cultured with adipogenic or osteogenic media, the presence of B3 did not increase differentiation to fat or bone, respectively. Adipogenesis was measure by Oil Red O stain, while both Alizarin Red and an Alkaline

Phosphatase assay was used to measure osteogenesis. Using immunocytochemistry, B3 was shown to decreased ASC secretion of collagen I, but not collagen III, collagen IV, vitronectin, laminin, elastin, or fibronectin. As a high collagen III to collagen I ratio is associated with a lack of scarring, this may be one mechanism of why B3 prevents scaring in the clinic, even among the elderly and diabetic patients. Additionally, B3 decreased expression of PAI-1, MCP-1, DR6, DKK-1, Angiogenin, IL-1, IGFBP-6, VEGF, and TIMP-2; increased expression of IL-1R and E-selectin; had a transient decrease in IL-6 secretion; and had a transient increase in bFGF secretion.
***Conclusion/Significance:** These results shed light on multiple mechanisms that B3 regulates the epidermal/dermal architecture to quickly heal wounds without scarring. Additionally, it provides a method to change the protein secretion of ASCs.

466 - Evaluation Of The Activation Of Dendritic Cells By Means Of Tumor Lysates Encapsulated In Chitosan Nanoparticles

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***Purpose/Objectives:** Despite the great potential of the dendritic cells (DCs) as a tool for cancer therapy, it is noteworthy to say that one of the greatest challenges is the optimal activation of the DCs and the selection of the DCs lineage, in order to obtain an efficient antitumor response that overcomes the immunosuppressive tumor microenvironment. The most widely used source in the clinic for obtaining tumoral antigens are the tumor lysates (TL) from the same patients, however the antigens obtained are rapidly degraded and therefore, they are not adequately captured by the DCs. Consequently, if the tumor antigens are stabilized and encapsulated together with DCs-stimulating molecules in chitosan nanoparticles, then the anti-tumor immune responses mediated by the DCs could be more effective.
***Methodology:** TL obtained from melanomas of C57BL/6 mice inoculated with B16-F10 cells, Clec-9a, CD40-L and CpGs, were incorporated to chitosan nanoparticles (TLCH-NP). The TLCH-NP were analyzed with TEM and light scattering. Loading efficiency and release of TL were measured by the BCA protein assay. The TL CH-NPs cytotoxicity was assessed by flow cytometry. DCs were also cultured in the presence of TL CH-NP and the phenotype was analyzed.
***Results:** We were able to obtain chitosan nanoparticles that encapsulated TL and CpGs and incorporate the molecules Clec-9a and CD40-L in their surface. The average size of the CH-NPs alone or TL CH-NP was 290 nm (polydispersity index 0.294) and 408 nm (PDI 0.365), respectively. With TEM it was observed that the NPs had a spherical shape and a size between 300 and 800 nm. Also, it was observed that the TL release of the nanoparticles was higher at a low pH (4.5). Likewise, no cytotoxicity was observed when co-culturing CH-NP alone and loaded with mouse spleen cells. It is noteworthy that the DCs cultured in the presence of TL CH-NPs had a phenotype more activated than that of the DCs under control conditions.
***Conclusion/Significance:** Here, we characterized LT CH-NPs and demonstrated that they are suitable for activate DCs that could be use in antitumoral immunotherapy against melanoma. The final aim of this study is to probe this methodology in an *in vivo* animal and human models and analyze the tumor size, survival and induced immune response.

467 - A Comparison Of Decellularized Muscle And Gelatin Cryogels For Muscle Regeneration

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***Purpose/Objectives:** The aim of this research is to find a clinical therapy for volumetric muscle loss. Volumetric muscle loss or VML is a 10% or more loss of muscle mass, inhibiting natural regeneration. It is seen in traumatic injuries and typically requires invasive reconstructive surgeries leading to co-morbidity and infection. Adverse effects include chronic muscle weakness, impaired limb function leading to amputation, and lifetime disability. Myoblasts form skeletal muscle through myogenesis, forming multinucleated fibers or myotubes. Such structures are lost in VML prompting need for a tissue engineering solution. The proposed VML scaffold is a cryogel, a three dimensional macroporous scaffold that can provide the architecture for myogenesis. The high degree of porosity and interconnectivity is ideal for cell attachment, proliferation, and alignment for myotube formation. Cryogels have strong compressive properties able to withstand the high force and cyclic loading performed by muscle.
***Methodology:** Decellularized bovine muscle was used along with gelatin for the fabrication of cryogels. The decellularization process removes cellular components to limit immune response while maintaining essential structural proteins and growth factors. Myoblasts recognize the signals left in the decellularized muscle to attach and proliferate. Successful decellularization of bovine muscle was determined by H and E staining and double stranded DNA quantification. DECM and gelatin were crosslinked with glutaraldehyde for the three-dimensional formation under freezing temperatures (-20°C) to create large pores from ice crystals. 1% dECM, 1% gelatin, 1% dECM with 1% gelatin, 2% dECM, and 2% gelatin cryogels were fabricated and examined through structural and cellular analysis in this experiment. A thermal gradient was also used in the fabrication of 5% gelatin cryogels to create aligned pores within the cryogel to mimic fibrous muscle.
***Results:** Results from ultimate compression demonstrated that dECM cryogels were superior whereas the gelation cryogels presented slightly superior data under cyclic loading, indicating that a combination of the two materials is favorable. This was further exhibited in a swelling test where the two dECM scaffolds had a significantly lower swell ratio than gelatin counterparts and the dECM and gelatin cryogel. Porosity showed no significant difference between pore sizes of the dECM and dECM with gelatin cryogels. A 28-day degradation study revealed that the 1% dECM and 1% gelation had optimal long-term mechanical properties. The scaffolds were seeded with 50,000 C2C12 mouse myoblast cells and the 1% dECM with 1% gelatin had the most cell proliferation after 7 days according to an MTS cell proliferation assay. Alignment of cryogel pores using a thermal gradient during fabrication was confirmed via SEM imaging.
***Conclusion/Significance:** A cryogel fabricated from equal parts dECM and gelatin holds advantageous properties of each material. The dECM has many ideal topographical features for cell signaling, but gelatin encompasses many important mechanical properties needed for use in muscle. The alignment of pores in a cryogel for muscle should aid in the alignment of myoblasts once attached and proliferated. This should make an optimal scaffold for muscle regeneration.

468 - Development Of A Supercritical Carbon Dioxide Decellularization Method

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***Purpose/Objectives:** Supercritical carbon dioxide ($scCO_2$), commonly utilized in the food and pharmaceutical industry, benefits from liquid like density and solvation capability as well as gas like viscosity and diffusivity [1]. These unique properties enable $scCO_2$ to penetrate through mammalian tissue and make it a promising, non-toxic decellularization alternative.
***Methodology:** Porcine liver and porcine aorta (~1g), frozen at -20**°**C until used, were separately exposed to supercritical carbon dioxide ($scCO_2$; ~2900 psi, 37**°**C) for 0.25h, 1h, 2h, 5h and 72h (batch system). Detergent-based decellularization was applied under constant agitation: 0.02% Trypsin /0.05% EDTA (1h), 3% Triton-X-100 (1h), 4% Sodium Deoxycholate (1h) and 0.1% Peracetic acid (2h). Residual DNA content was used as a marker of decellularization. DNA was extracted using a phenol-chloroform extraction method and quantified using the Quant-iT Pico Green (Invitrogen) assay kit. Histological staining was performed to visualize removal of nuclei and cytoplasm (Haematoxylin & Eosin, H&E) as well as extracellular matrix (ECM) components such as glycosaminoglycans and collagen (Alcian Blue, AB; Picrosirius Red, PSR, respectively). Statistical analysis was performed using PRISM (GraphPad). Results were deemed significant if $p < 0.05$.
***Results:** Exposure to $scCO_2$ for 72h significantly decreased DNA content of liver and aorta by 74% ($p<0.0001$) and 75% ($p<0.0001$) respectively, whereas detergent-based decellularization method decreased DNA content by 95% ($p<0.0001$). This was confirmed by H&E staining which showed removal of cell nuclei by 72h $scCO_2$ exposure of both tissues tested and by the detergent treatment. Exposure to 5h $scCO_2$ was not sufficient to successfully remove cell nuclei in either of the tissues tested. Gross tissue morphology, as highlighted by H&E, AB and PSR staining, was different between treatment conditions; the detergent-based method showed very weak staining for glycosaminoglycan content as well as cytoplasm, which appeared more preserved in the $scCO_2$ exposed tissues.
***Conclusion/Significance:** Supercritical carbon dioxide removed cellular material from liver and aorta tissue whilst maintaining crucial extracellular matrix components, highlighting its potential use as an alternative to detergent-based decellularization.

469 - Channels In A Collagen Matrix Improve Graft Take In A Single Stage Procedure

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***Purpose/Objectives:** Soft tissue defects treated with dermal substitutes often require a two-stage procedure to achieve epidermal closure. The purpose of the first stage is to achieve an appropriate dermal bed which can support a thin autologous split thickness skin graft (STSG) applied in a second stage, typically 2-3 weeks after the initial stage. A single-stage procedure, in contrast, will save the patient a second operative procedure, minimize hospital stay and thereby reduce overall cost of care. However, single-stage procedures could result in graft loss due to lack of contact between the graft and wound bed leading to insufficient nutrient support, and therefore additional procedures are required to achieve epidermal closure. Previously, we demonstrated that macro channels in the dermal substitute facilitate rapid cellular migration and formation of well vascularized granulation tissue to support graft

viability. In this follow-up study, we evaluate the impact of channel density on graft survival.
***Methodology:** We investigate the effect of introducing 5, 9 or 18 channels into a collagen-chondroitin-6-sulfate matrix on STSG take and compared the findings to a matrix with no channels+STSG and STSG alone. Full-thickness wounds (4x4cm) were created on the dorsum of Yorkshire pigs. On Day 0, wounds received a collagen matrix with 0, 5, 9, or 18 equidistant 2mm diameter channels followed by a 0.012-inch-thick split thickness skin graft (STSG). Treatments were sutured to adjacent skin wound margins, then covered with moist bolster dressing. Dressings were changed every 3 or 4 days and gross observations, wound size/area measurements, and digital images were acquired. All pigs were euthanized on Day 14.
***Results:** At Day 14, in the group with no channels+STSG, the STSG became necrotic and sloughed. In contrast, the STSG was well integrated in all wounds that received matrix with channels. Wound healing characteristics, including re-epithelialization, matrix-take, granulation tissue traits, inflammatory cell types and numbers, were similar between matrix with 5, 9, or 18 channels. The highest density of channels did not diminish the ability of matrix to inhibit contraction. Wounds that were only STSG treated had more precontraction-type (scar) granulation tissue than the collagen matrix treated wounds.
***Conclusion/Significance:** In this single stage procedure, channels in the collagen-chondroitin-6-sulfate matrix increased autograft take. There were no differences in wound healing characteristics between matrix with 5, 9, or 18 channels.

470 - Electrospun Polycaprolactone/collagen Nanofibers Cross-linked With 1-ethyl-3-(3-dimethylaminopropyl) Carbodiimide/N-hydroxysuccinimide And Genipin Facilitate Endothelial Cell Regeneration And May Be A Promising Candidate For Vascular Scaffolds

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***Purpose/Objectives:** A promising vascular scaffold must possess satisfying mechanical properties, great hemocompatibility, and favorable tissue regeneration. Combining natural with synthetic materials is a popular method of creating/enhancing such scaffolds. However, the effect of additional modification on the materials requires further exploration.
***Methodology:** We selected polycaprolactone (PCL), which has excellent mechanical properties and biocompatibility and can be combined with collagen. Electrospun fibers created using a PCL/collagen solution were used to fashion mixed nanofibers, while separate syringes of PCL and collagen were used to create separated nanofibers, resulting in different pore sizes. Mixed and separated nanofibers were cross-linked with glutaraldehyde (GA), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), and genipin; hence, we named them as mixed GA, mixed EDC (ME), mixed genipin (MG), separated GA, separated EDC (SE), and separated genipin (SG).
***Results:** Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction showed that cross-linking did not affect the main functional groups of fibers in all groups. ME, MG, SE, and SG met the requisite

mechanical properties, and they also resisted collagenase degradation. In hemocompatibility assays, only ME and MG demonstrated ideal safety. Furthermore, ME and MG presented the greatest cytocompatibility. For vascular scaffolds, rapid endothelialization helps to prevent thrombosis. According to human umbilical vein endothelial cell migration on different nanofibers, ME and MG are also successful in promoting cell migration.
***Conclusion/Significance:** ME and MG may be promising candidates for vascular tissue engineering. The study suggests that collagen cross-linked by EDC/N-hydroxysuccinimide or genipin facilitates endothelial cell regeneration, which could be of great benefit in tissue engineering of vascular scaffolds.

471 - In Situ Remodeling Of An Off-the-shelf Bi-layered Small-caliber Vascular Scaffold Based On Poly(l-lactide-co-*ε*;-carprolactone)and Silk Fibroin

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***Purpose/Objectives:** Based on the poly(L-lactide-co-*ε*;-carprolactone) (PLCL) , silk fibroin (SF) and heparin (Hep), a bi-layered small-caliber vascular scaffold was prepared and in situ remodeling effect was evaluated.
***Methodology:** The PLCL/SF/Hep bi-layered small-caliber scaffold was prepared by electrospinning technology: the inner layer was a dense PLCL/SF/Hep nanofiber layer and the outer layer was a loose PLCL/SF nano-yarn layer. In vitro characterization including mechanical tests, hydrophilicity evaluation, heparin release and bio-compatibility evaluation. In situ remodeling evaluation was via orthotopic transplantation of unilateral carotid artery in New Zealand white rabbits.
***Results:** In vitro characterization showed the mechanical properties of the scaffold could meet the application requirements, the hydrophilicity of composite material was outstanding. Besides, the bio-compatibility showed the composite material could promote endothelial cells proliferation and had an active role in anti-platelet aggregation. Ultrasound results showed the scaffold remained unobstructed after 3 months of orthotopic transplantation. The blood inflammation index did not indicate immune rejection. Cell-specific stainings showed the remodeling of endothelium and smooth muscle layer were superior.
***Conclusion/Significance:** The PLCL/SF/Hep bi-layered small-caliber artificial vascular scaffold has preliminary clinical application prospects.

472 - Co-flow Microfluidic System For The Production Of Tuneable Elastic Gelatin Methacryloyl Microparticles

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***Purpose/Objectives:** In this study, a microfluidic system involving co-current flow for the production of Gelatin Methacryloyl (GelMA) microparticles has been designed as an approach for high-yield

production of microparticles showing consistent low polydispersity and mechanical properties, with the objective of creating a 3D environment for Mesenchymal Stem Cells able to sustain stiffness-driven differentiation, with the ultimate aim of cell delivery via injection.
***Methodology:** The experiments were designed using a Central Composite - Response Surface Methodology to evaluate effects of experimental factors on diameter and Young's modulus. Characterisation of microparticle size distribution was performed under phase contrast microscopy and confirmed with Dynamic Light Scattering. Morphology was evaluated with environmental Scanning Electron Microscopy, and assessment of Young's modulus via Atomic Force Microscopy. Culture of immortalised human mesenchymal stem cells across different microparticle sizes was carried to assess the biocompatibility of the scaffold.
***Results:** The microfluidic setup allowed the production of microparticles which diameters ranged from 170-760 µm with maximum Span and CV% of 0.35 and 20%, respectively. The porous macrostructure showed an increasingly ordered architecture in inverse proportion to the microparticle diameter. Furthermore, assessment of Young's modulus via force spectroscopy showed a tuneable surface modulus from 26-130 kPa, which is inversely related to the microparticle diameter. Cell culture confirmed approximately 80% cell viability after one week, thereby showing good biocompatibility of the material.
***Conclusion/Significance:** The co-flow microfluidic method is a reliable approach for the production of GelMA-based microparticles, which exhibit fine-tuned morphological and mechanical properties, in a high-throughput scale, highlighting its applicability in tissue engineering and regenerative medicine.

473 - Influence Of Lbl Nanocoatings Topography On Interaction With Cells

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***Purpose/Objectives:** The most useful systems are those presenting surfaces that interact with specific cells, promoting either adhesion, repellency or contact-killing. Materials with customized and designed properties to provide a specific response under certain conditions are possible to be built through innovative technologies, generated from the understanding of the mechanisms of action of these material surfaces. In the case of human cells, adhesion of circulating tumor cells can be interesting for screening experiments, for designing biosensors or to build constructs for tissue engineering.
***Methodology:** Several techniques can be applied to form these surfaces, being the layer-by-layer deposition method a promising technology to build coatings based on natural macromolecules with targeted properties. Natural macromolecules or natural polymers are particularly interesting as they are usually weak acids and bases that can be fine-tuned by pH and ionic strength. The topography is a key player for cell adhesion and recent studies in our group showed that nanoscale fractal roughness can provide interesting information about cell-surface interaction. Through a statistical description of the topography, we estimated some roughness parameters, such as interface width (w^2), lateral correlation length (ξ) and roughness exponent (α), from AFM surface images. Substrates were designed on glass and PDMS and Chitosan and HA were deposited for contact with prostate cancer cells.
***Results:** The results indicated a significant change of PC3 (type of prostate cancer cells) cell behavior on rougher surfaces. The adhesion changes depended on the shape and distribution of the surface pattern, being primarily influenced by the hidrophilicity of the surface.

***Conclusion/Significance:** Although further studies are still needed, the findings toward the fundamental understanding of the role of nanoscale fractal roughness in cell adhesion can contribute to the development of new biomaterials with applications in biomedical systems.

474 - Combination Of Poly-3-hydroxybutyrate-microfibers And Gelatin-nanofibers To Mimic The Natural Extracellular Matrix

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***Purpose/Objectives:** The natural extracellular matrix (ECM) is composed of randomly arranged fibers that provide mechanical support, regulates cellular activities and facilitates the transportation of nutrient and metabolic wastes. Electrospinning is an electrodynamic process used to produce ultrafine fibers of different polymers. Most studies report on the obtaining of fibers with constant and homogeneous diameter. However, this conformation might be not ideal for tissue engineering. Scaffolds should resemble the topography and properties of the ECM. Poly-3-hydroxybutyrate (PHB) is a biocompatible polymer suitable for biomedical applications; however, the biodegradation rate of PHB is low. Different studies have been developed combining PHB with gelatin (GE) which is a hydrolyzed of collagen highly used in tissue engineering that provides a better environment for cell attachment, growth, and proliferation. Therefore, the aim of our work was to implement an electrospinning protocol that allows simultaneous production of PHB-microfibers (PHB-MF) and Ge-nanofibers (Ge-NF) to mimic the ECM.
***Methodology:** Different electrospinning conditions were tested to obtain PHB-MF and Ge-NF, such as polymer concentration, voltage and flow rate. The fibers diameters were determined by FESEM using ImageJ software. Fibroblasts (500 cells) were seeded on the scaffolds (0.5x0.5 cm^2) and the viability evaluated with a standard MTS assay after incubation for 24, 48 and 72 h.
***Results:** Continuous and smooth PHB-MF were obtained at 8 %w/v, 25 kV and 0.5 mL/h with a diameter of 1.25±0.17 µm. Ge-NF were obtained at 30 %w/v, 25 kV and 0.1 mL/h with a diameter of 0.22±0.05 µm. The PHB-MF and the Ge-NF were successfully combined by simultaneous electrospinning of both polymers solutions from two separated needles, one by side to the other one.MTS assay demonstrated that the PHB-MF presented a good cytocompatibility with values of cell viability above 80 %, and there were no significant differences between the cells in contact with the PHB-MF and the control group. The fibroblast exposed to the Ge-NF and Ge-PHB scaffold showed similar growth at 72 h reaching 50 % of growth compared with the control.
***Conclusion/Significance:** For the first time PHB-MF and Ge-NF were successfully combined in a single electrospun mat with the conditions optimized for each polymer in separate. Effects on cell proliferation and differentiation will be further explored.
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475 - Assessing The Capacity Of A Novel Human- Derived Complex Growth-factor Cocktail In Inducing Angiogenesis And Osteogenesis

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***Purpose/Objectives:** Despite progress made in regenerative medicine and tissue engineering, such fields have limited success in translating experimental gains to the clinic. One challenge is associated with the inability to initiate, maintain and integrate angiogenesis to supply the construct with appropriate conditions for nutrient exchange. This is a multifactorial problem not limited to the choice of cell or biomaterial type, but it is likely driven by an inadequate biomolecular composition inhibiting nominal genetic regulation to induce and regulate these events. Such complexity has been evident when using murine tumor- derived basement membrane matrix assay which limits its application to*in vitro* testing making it clinically irrelevant. A novel approach to combat these challenges is using a growth factor rich human- based extracellular matrix that is derived from healthy placentas - human placental matrix (hPM). hPM is immunologically privileged tissue that provides a cocktail of growth factors that is hypothesized to enhance angio-inductive and osteo-inductive capacities, which we present in this study.
***Methodology:** Full- term placenta tissues were collected at the delivery suite in Shands Hospital at the University of Florida within 12 hours of birth; hPM was derived via series steps of centrifugation, homogenization and dialysis as described by Moore et al. [1]. In Angiogenesis Assay (AA), human Umbilical Vein Endothelial Cells (HUVECs) were isolated and seeded on hPM coated TC plates at 400 cells/ mm^2 cultured with endothelial cell culture (ECs) media. Tubule network formation was analyzed using Calcein AM and imaged at days 3 and 5 using an inverted Zeiss Axoivert 200 Fluorescence microscope. In Osteogenesis Assay (OA), Mesenchymal Stem Cells (MSCs), isolated from 1 mm^3pieces of dissected Umbilical Cord Wharton's Jelly [2], were seeded at 200 cells/ $mm^2$with osteogenic inductive differentiation cell culture media with and without hPM substrates. Quantification of extracellular calcium deposition on MSCs monolayers was performed using Alizarin Red S Staining.
***Results:** HUVEC seeded on hPM in the AA displayed an elongated morphology with interconnected tubal networks that is similar to the intussusceptive vascularization mechanism. MSCs cultured in osteogenesic differentiation media and seeded on hPM substrate resulted in a significant increase in calcium deposition compared to controls with Osteogenic Media only.
***Conclusion/Significance:** These results demonstrate the potent effect of this complex cocktail and growth factors in producing different responses with different cell types. This was evaluated in the context of cell behavior in angiogenesis and osteogenic differentiation by culturing ECs and osteoblasts, respectively on hPM substrates.
[1] M. Moore, V. Pandolfi et al., *Novel human- derived extracellular matrix induces in vitro and in vivo vascularization and inhibits fibrosis. Biomaterials*, 2015. 49: p. 37-46[2] R. Sarugaser, J. Ennis et al., *Isolation, propagation and characterization of human umbilical cord perivascular cells (HUCPVCs)*. Methods Mol Bio, 2009. 482: p. 269-79

476 - BMP-2-Delivering Nanoparticles To Stimulate Osteoblastic Differentiation In A Model Ligament Scaffold

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***Purpose/Objectives:** 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;} Due to its interarticular location and poor vascularity, the anterior cruciate ligament (ACL) is slow to heal following injury, and reconstructions are typically required following rupture. An engineered ACL tissue could be an alternative to an autologous or allogeneic tissue grafts, provided that it has a high tensile strength and possesses the biochemical cues to guide ligament tissue formation and bony integration. Toward the development of such an engineered tissue, we have developed anionic polycaprolactone(PCL)/heparin fiber scaffolds - to provide tensile strength and support tissue development - and cationic chitosan nanoparticles that can be displayed on the fibers to deliver bioactive factors to stimulate tissue formation and integration with bone. Importantly, these nanoparticles can be fabricated using small quantities of expensive bioactive factors and then be 3D printed onto the fiber scaffolds in a spatially controlled manner to create distinct ligamentous and osteogenic zones.
***Methodology:** In this work we focus on nanoparticles to stimulate integration of the engineered tissue with the host bone, and examine two types of bone morphogenetic protein (BMP)-2-presenting nanoparticles: 1) those displaying covalently conjugated BMP-2 on their surfaces, and 2) those containing encapsulated BMP-2 that can be released over 3-4 weeks. The first type of nanoparticles was fabricated by dropwise addition of tripolyphosphate (TPP) to chitosan/bovine serum albumin (BSA) blends. Subsequently, BMP-2 was covalently conjugated to the nanoparticle surfaces with sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexene-1-carboxylate to link the free thiol groups of BMP-2 to amine groups on the nanoparticles. The second type of nanoparticles were prepared by adding 200 ng/mL BMP-2 to the chitosan/BSA solution prior to addition of TPP.
***Results:** Dynamic light scattering indicated that nanoparticle sizes of 500-600 nm were achieved with both approaches. ELISA analysis revealed no release of BMP-2 from conjugated nanoparticles, but indicated sustained release of encapsulated BMP-2 over 3 weeks. At present, the osteogenic capacities of the nanoparticles are being examined by culturing bone marrow-derived mesenchymal stem cells (MSCs) on nanoparticle-coated PCL/heparin fiber surfaces. Analyses will include assays of ALP activity, mineral deposition, and mRNA expression of bone markers (e.g., collagen-I, Runx2, osteopontin and bone sialoprotein).
***Conclusion/Significance:** We anticipate that nanoparticles displaying covalently conjugated BMP-2 will prove more osteogenic than BMP-2 encapsulated nanoparticles, as covalent conjugation is expected to retain BMP-2 on the PCL/heparin fiber surfaces, and limit its diffusion and endocytosis. Successful outcomes will guide fabrication of three-dimensional engineered tissues suitable for testing in vivo with spatially discrete zones for ligament formation and bony integration.

477 - 3d Cell-scaffolding Approach Trough Extrahepatic Bile-duct Decellularization

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***Purpose/Objectives:** There are different injuries that affect the bile ducts. Therefore, it is necessary to produce adequate treatment that could preserve the anatomy and physiology of this region. One tissue engineered approach that could replace the bile duct with biological scaffolds is the decellularized tissues. At this time, the use of swine's extrahepatic-bile-duct acellular scaffolds has not been reported in the literature as a probable guide of bile duct regeneration. Therefore, in this study, we developed a decellularization protocol to generate a swine-derived acellular scaffold. We hypothesized that replacing the reagents before mentioned could lead to better preservation of the integrity of native ECM and promote cell viability and proliferation. Our approach is to decellularize swine's extrahepatic-bile-ducts to have a tissue-engineered scaffold with suitable biological and biochemical features to be repopulated by cells. This project was financially supported by PAPIIT-DGAPA grant N° TA-200218
***Methodology:** We obtain 9 bile ducts from pigs (25Kg-35Kg). The decellularization protocol was conducted by perfusion of all the solutions through the lumen of the bile duct using a peristaltic pump, a closed circuit was formed with the bile duct embedded in these solutions at a flow rate of 46.0 ml / min and room temperature. The selection of decellularization agents was proposed based on modifications of protocols previously used by Giraldo et al and Cheng et al. The protocol includes the use of SDS and DNase-I as decellularization agents. DNA quantification and immunohistochemical analysis labeling MHC-I histological analyses were performed in order to confirm the decellularization. To ensure the quality of the ECM histological analysis and SEM analysis were performed
***Results:** We observed a significant decrease in DNA content in the decellularized matrices, furthermore, immunostaining of the MHC-I in the acellular biliary duct scaffold confirmed no residual presence of Major Histocompatibility Complex (MHC-I) in the decellularized samples.
In native bile ducts, the ECM is composed primarily of collagen and other proteins. After decellularization process the major components of the ECM was retained, the main structural protein as collagen remained after the decellularization process. In order to follow the microarchitectural changes in the decellularized matrices these were observed by SEM the micro photos showed the apical surfaces of the cholangiocytes with numerous microvilli, as well as pores of the extracellular matrix with cells. In the decellularized samples there is an absence of cells, microvilli, and cilia. The pores of the extracellular matrix and the collagen fibers in the decellularized samples are conserved with the absence of cellular material inside them
***Conclusion/Significance:** The decellularization process proposed by our group allows the removal of cellular material and preservation of the tissue architecture. The possibility of regenerating tissues such as the bile duct with organ-specific decellularized scaffolds is extremely attractive and promising because it allows not only to preserve structural features but also biochemical signals that can induce an adequate migration and normal development of cells through the cellular scaffolding generated by this technique.

478 - Effect Of Bioactive Ceramics In Viability Of Fibroblasts, Keratinocytes And Stem Cells

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***Purpose/Objectives:** Bioactive ceramics are synthetic biomaterials, which show highly positive interactions with hard and soft tissue. Recently, these materials have been used for wound healing. In this study, the bioactive ceramics composed of P_2O_5-CaO-SiO_2-Na_2O (BC) were synthesized and tested regarding their influence on skin cells (fibroblasts, keratinocytes and stem cells).
***Methodology:** Fibroblasts (MRC-5), keratinocytes (HaCat) and stem cells isolated from human teeth (SC) were used. The cells (2,500) were seeded in 96-well tissue culture plates, and treated with the BC in the following concentrations: 0 (G1, used as the control group); 100 (G2); 250 (G3); 500 (G4); 1,000 (G5) or 1,500 µg/mL (G6). Cell viability was evaluated by 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide (MTT), after two and seven days of cultivation.
***Results:** The BC did not significantly affect the MRC-5 viability, with values of absorbance mean ± mean standard error after two days and seven days, respectively, of 0.089 ± 0.009 and 0.113 ± 0.022 for the G1; 0.078 ± 0.008 and 0.101 ± 0.018 for the G2; 0.101 ± 0.018 and 0.075 ± 0.010 for the G3; 0.091 ± 0.011 and 0.081 ± 0.010 for the G4; 0.111 ± 0.015 and 0.098 ± 0.011 for the G5; 0.111 ± 0.012 and 0.119 ± 0.007 for the G6. In HaCat, a statistical difference was found between 0 and 1,500 µg/mL of BC at day 2 and 7. The values for HaCat after 2 days and 7 days were 0.044 ± 0.002 and 0.137 ± 0.003 for the G1; 0.043 ± 0.003 and 0.137 ± 0.004 for the G2; 0.056 ± 0.003 and 0.157 ± 0.005 for the G3; 0.053 ± 0.004 and 0.154 ± 0.003 for the G4; 0.060 ± 0.006 and 0.133 ± 0.006 for the G5; 0.084 ± 0.009 ($p<0.01$) and 0.054 ± 0.011 ($p<0.01$) for the G6, respectively. The stem cell results demonstrated a significant cell increase in several doses tested with values after two and seven days, respectively, of 0.030 ± 0.001 and 0.051 ± 0.003 for the G1; 0.032 ± 0.001 and 0.054 ± 0.003 for the G2; 0.037 ± 0.003 and 0.087 ± 0.005 ($p<0.01$) for the G3; 0.044 ± 0.002 ($p<0.05$) and 0.084 ± 0.008 ($p<0.01$) for the G4; 0.066 ± 0.005 ($p<0.01$) and 0.099 ± 0.004 ($p<0.01$) for the G5 and 0.087 ± 0.004 and 0.097 ± 0.007 ($p<0.01$) for the G6.
***Conclusion/Significance:** The absorbance values obtained related to viability indicated a proliferation between two and seven days; however, when excessive proliferation occurred, cell viability decreased. The results are interesting because during the repair it is important to have the presence of SC, but no increase in fibroblasts, which can trigger the exacerbated growth of hypertrophic and keloid scar tissue production, preventing the restoration of the morphofunctional properties of the native tissue. Considering that the bioceramic did not affect the fibroblast viability and increased SC, this material is interesting for the repair of cutaneous lesions. Financial support: MCTI, FINEP, CNPq, CAPES, FAPESP, FAPERGS, UFRGS and Instituto de Pesquisa com Células-tronco (IPCT).

479 - Hybrid Hydrogel/3D-printed Scaffold Composites In Complex Geometries With Tunable Small Molecule Release

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Temporomandibular joint (TMJ) degeneration affects over 10 million Americans. Tissue engineered TMJ reconstruction will require scaffolds that provide mechanical support and biological factors to induce tissue regeneration[1]. As a first-pass effort to produce scaffolds that address both of these needs. The goal of this study was to control release of a model small heparin-binding molecule, crystal violet (CV), through a tunable PEGDA/heparin hydrogel layered within a porous 3D-printed load-bearing scaffold. Hydrogel solutions included PEG diacrylate (PEGDA) and heparin (15 wt.%), dithiothreitol (DTT, hydrolytic degradation reagent), and radical initiator/catalyst ammonium

persulfate/tetramethylethlylenediamine (18 mM each). Heparin derivatives of varying sulfation levels were synthesized by solvoyltic desulfation. Laser-sintered porous PCL scaffolds were placed in custom molds and injected with hydrogel solution prior to 20-minute crosslinking at 25°C. Phase-contrast microscopy demonstrated hydrogel penetration throughout void spaces of the 3D-printed porous scaffold in two distinct layers representing cartilage and bone compartments. Stress-relaxation compression tests demonstrated viscoelastic properties for composites (~23% decrease in force after 5-minute constant strain). Our goal of 4-week hydrolytic degradation was achieved in hydrogels with 20 mol.% DTT (Fold swelling (FS) ~14.2± 1.4) and greater sulfate groups (Hep and Hep^{-N}) (FS ~16.5± 0.1). Zero-order release of CV was observed over 7 days. Cumulative release decreased by ~24% when heparin wt.% was increased from 10 to 15 due to greater electrostatic interactions with the positively-charged CV. These results suggest that further development of our composite system may lead to an alternative to current treatments for anatomically complex osteochondral defects.
1.TMJ. *NIDCR,* 2017.

480 - Strontium Carrier Matrix Based On Oxidized Bacterial Cellulose And Apatite

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Bacterial cellulose (BC) is a polymer produced by fermentation with several interesting properties. However, for specific uses this biomaterial presents some limitations due its non-degradability "in vivo". This polymer has aroused interest in the area of bone repair when combined with apatite (Ap) favoring interactions with bone cells. The additional incorporation of strontium (Sr) ions in this hybrid matrix could improve bone repair, The objective of this work was to improve BC biodegradability through periodate oxidation and to produce an hybrid strontium carrier matrix based on oxidized BC and apatite for future applications as a strontium release system or other drugs. BC membranes were produced after cultivation of *Komagataeibacter xylinus*. To obtain the hybrid matrix, the BC membranes were previously oxidized with sodium periodate and then mineralized. The hybrid matrix was characterized by the degree of swelling, electronic microscopy, X-ray diffraction, *in vitro* strontium release and *in vivo* biocompatibility tests. It was possible to identify that the hybrid matrix achieves strontium release more rapidly when the oxidized BCis used as a polymer matrix component than when unmodified BC was used. The biocompatibility results showed that the hybrid matrix was able to degrade *in vivo* with low acute inflammatory responseand without causing rejection by the mice organism, indicating that this is a biocompatible material. In this study it was shown successful production of a strontium carrier matrix by the synthesis route proposed and that its degradation was related to the chemical oxidation treatment in which the bacterial cellulose component was submitted.

482 - Synthesis And Characterization Of A Polymer Scaffolding By Electrospinning For The Formation Of Ligament

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Ligament rupture is the most common musculoskeletal problems. Nowadays, two types of graph transplants are used to replace the ligament deformation, however, they have disadvantages: autograft, lose their mechanical properties when being cultivated, while allograft could be rejected by the patient (Pereira, et al., 2014). The present study focuses on the synthesis and characterization of polycaprolactone (PCL)/chitosan (QS) nanofibers with hydroxyapatite (HA) and nanowhiskers of cellulose (CNC) by electrospinning not only to mimic the mechanical properties of an Anterior Cruciate Ligament (ACL) but also to grow fibroblast cells in it. The methodology for obtaining blend nanofibers of PCL/QS with HA and CNC is stirred the four of them with acid acetic at 70% and acid formic at 30% solvent, then, made the electrospinning process. After that, we characterize the nanofibers at stress test and proliferation cell test. The results at the mechanical test show that the nanofiber's ultimate stress is 19.4 MPa. According to Santos, et al., 2014 the parameters of ACL's ultimate stress is between 13-46 MPa. Therefore, the nanofibers could mimic an ACL's mechanical properties. For the proliferation test, we cultured 18000 cells of fibroblast (ATCC PCS-201-012) on the nanofibers with nanoparticles. We left the cells to grow it for 17 days. And the results demonstrated a growing of 62000 cells on it. Hence, we demonstrated the growth of 244 % of the original culture.

483 - Slow Release Of Sitagliptin Promotes Osseointegration At The Bone-implant Interface Via Mediation Of M2 Macrophage Polarization Under Diabetic Conditions

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Macrophages, the key players in immunoregulation, are important for bone repair and regeneration process. As a chronic inflammatory disease, (diabetes mellitus) DM creates a pro-inflammatory microenvironment of implants, which resulting in a high rate of implant loosening or failure. Methods to locally control the polarization of macrophages is of great interest for biomedical implants and tissue engineering. In this study, we use silk protein as a carrier to modulate the release of sitagliptin and control macrophage responses to create a pro-healing microenvironment in the DM condition. The results indicated sitagliptin significantly induced polarization of macrophages into the M2 phenotypes in a manner of dose-dependence and obviously alleviated impaired osteoblast behavior on Ti under diabetic conditions in vitro. Further, the in vivo studies using silk scaffolds loaded sitagliptin indicated that implantation of this scaffold around titanium implants markedly induced the recruitment of healing-associated M2 polarization to the implanted sites, which in turn had promotive effect on osteointegration compared with oral sitagliptin administration under diabetic conditions. Taken together, our findings suggested that slow release of sitagliptin from the SF scaffolds resulted in an immune-regulating effect and enhanced bone regeneration in support of the success of implants in diabetic patients.

484 - Characterization Of Uniaxially Aligned PNIPAM Nanofibers As Thermoresponsive Scaffolds

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Thermoresponsive polymers, such as poly(N-isopropyl acrylamide) (PNIPAM), are particularly applicable for use as cell culture substrates due to their temperature dependent physico-chemical properties.

PNIPAM's physiologically relevant lower critical solution temperature (32°C) allows for temperature-induced mechanical harvesting of cells, which conveniently circumvents the use of traditional biochemical enzymes that compromise important cell-cell and cell-matrix connections. In this study, the process of electrospinning was used to fabricate and characterize thermoresponsive, aligned PNIPAM nanofibers capable of supporting cell adhesion and proliferation. A factorial design of experiments (DOE) approach was employed to systematically determine the effects of different electrospinning process parameters on PNIPAM nanofiber diameter and alignment. Results show that high molecular weight PNIPAM can be successfully electrospun into both random and uniaxially aligned nanofiber mats with similar fiber diameters as a function of the speed of the rotating mandrel collector. Dynamic mechanical analysis was used to characterize the mechanical properties of the scaffolds, which revealed an order of magnitude difference in storage modulus (MPa) between cured and uncured samples. Thermoresponsive properties were confirmed by measuring the attachment and release of L-929 fibroblasts on cured electrospun scaffolds. Cell proliferation was also assessed and the rate of cell growth was found to be statistically similar on PNIPAM nanofibers compared to controls. In conclusion, uniaxially aligned PNIPAM nanofiber scaffolds were determined to be stable in aqueous culture, biocompatible, and thermoresponsive, enabling their use as tissue engineered scaffolds where uniaxial alignment is ideal, such as in vascular, bone, or neural tissue.

485 - Rat Liver Scaffolds In Health And Fibrosis: Proteomics Analysis

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While the cellular mechanisms of liver regeneration have been thoroughly studied, the role of extracellular matrix (ECM) in this process is still poorly understood. Scaffolds obtained by decellularization are based on native ECM and represent efficient tools to understand mechanisms underlying the pathogenesis of liver fibrosis. Here, we propose a proteomic based approach to identify the hepatic ECM composition after carbon tetrachloride (CCl_4) treatment. For that, female Wistar rats were submitted to fibrosis induction through CCl_4(50% in olive oil) intraperitonially, 1 mL/kg 3x per week in association with 5% of alcohol in drinking water while controls received only olive oil. After three months, their livers were removed and decellularized through portal vein perfusion with water (2h), 1% Triton X-100 (2h), 1 % SDS (24h-48h) and water (48h). The scaffolds were freeze-dried, minced and the proteins were extracted with a solution containing 7 M urea, 2 M tiourea, 4% SDC, 100 mM DTT and 100 mM ammonium bicarbonate (AB) for 72 hours after sonication. The remaining pellet was deglycosylated with ABC chondroitinase and solubilized with DMSO, following alkylation, digestion with trypsin (18h) and purification with an *in-house*spin-column. The dried peptides were ressolubilized in 10 mM AB and injected in a nanoLC-LTQ Orbitrap Velos mass spectrometer. We identified notable increases in ECM structural components (fibronectin, collagens I, II, III, V and XI) and non-structural components (lumican, elastin and laminins), showing that proteomic approaches following decellularization are adequate tools for enriched analysis of ECM proteins.

486 - Tri-layer Placental Allograft Membranes: Enhanced Handling Properties And In Vitro Bioactivity

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***Purpose/Objectives:** The growing demand for cell and tissue-based products, such as placental allografts rich in growth factors, cytokines, and extracellular matrix, stems from their ability to accelerate the wound healing process, especially in chronic wounds. However, the handling properties of placental tissues are typically poor and processing of these placental tissues may damage the inherent factors contained in these membranes. The aim of this study is to assess the enhanced handling properties as well as the *in vitro* bioactivity of a novel, dehydrated tri-layer placental allograft membrane (TPAM), consisting of amnion, chorion, and amnion.
***Methodology:** Handling properties of the membranes were assessed by performing mechanical testing as well as a conformability assay. For *in vitro* bioactivity assays, TPAM was homogenized and extracted in media at 37^{o}C for 72 hours. Chemotactic migration was assessed with human dermal fibroblast cells in a trans-well plate setup. Proliferation of fibroblasts as well as secretion of collagen I ECM in the presence of TPAM extracts were also assessed. An inflammation assay was performed with lipopolysaccharide activated peripheral blood mononuclear cells (PBMCs) to determine whether TPAM could reduce the expression of pro-inflammatory cytokines. To elucidate TPAM's ability to promote regenerative macrophage phenotypes, macrophage polarization in the presence of TPAM was evaluated through analysis of a diverse panel of secreted proteins.
***Results:** TPAM membranes have a higher allowable maximum load to break than single or double layer placental membranes (n=4 per group; $p<0.05$). In a conformability assay, TPAM had a higher percentage of conformation to an obscurely shaped object, representing a complex wound bed, than a double layered membrane. TPAM promoted enhanced adult fibroblast migration, which was significantly higher than controls (n=4 per group; $p<0.05$). A very similar trend was observed with fibroblast proliferation. There was a significantly higher amount of proliferation in the presence of TPAM extract than a negative control (n=4 per group; $p<0.05$). Furthermore, fibroblasts cultured for 7 days in the presence of TPAM extract had more collagen I secretion than basal media alone. The addition of TPAM extracts to PBMCs significantly reduced the expression of pro-inflammatory mediators by more than 90% for TNF-alpha, IL-1beta, and IL-6, and by 40% for IL-8 (n=4 per group; $p<0.05$). Finally, macrophage polarization evaluation indicated that TPAM favorably modulates the macrophage cytokine secretion profile, including lower levels of IFN-gamma and RANTES as well as higher levels of CCL22 and CCL18 compared to non-stimulated M0 controls, to maximally enable tissue regeneration.
***Conclusion/Significance:** The culmination of this work suggests that TPAM not only has enhanced handling properties, but may also improve the chronic wound milieu by providing essential cytokines, and growth factors to create a beneficial environment for wound healing.

487 - A Circadian Clock Gene, Neuronal Pas Domain 2 (npas2) Regulates Dermal Wound Healing

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***Purpose/Objectives:** Circadian rhythms that maintain cellular homeostasis during a 24-hour cycle have been shown to regulate a wide range of peripheral tissues. We have hypothesized that dermal wound healing is also under the regulation of circadian gene. In this study, we investigated the role of one of the circadian genes, Neuronal PAS domain 2 (*Npas2*) in the homeostasis of dermal structure using

in vivo and in vivo wound healing models.
***Methodology:** Primary fibroblasts were isolated from homozygous knock out (KO) (*Npas2**-/-*), heterozygous KO (*Npas2**+/-*) and normal C57Bl6J (WT) mice. The expression of core clock genes was determined by quantitative RT-PCR (qPCR). Fibroblast behaviors were characterized in terms of cell proliferation (WST-1 test), cell migration (Scratch test), and cell contraction (floating collagen gel culture). In addition, gene expression of alpha-smooth muscle actin (alpha-SMA) and extracellular matrix collagens (type I, III, XII, and XIV) was determined by qPCR and *in vitro* collagen accumulation was evaluatedby Picrosirius red staining. The time-course healing of full-thickness punched-out wounds was monitored in WT and *Npas2* KO mice. Moreover, we screened 1,120 FDA-approved compounds for*Npas2* expression and fibroblast migration. One candidate compound exhibited the increased fibroblast migration *in vitro*and the accelerated full-thickness dorsal skin punched-out wound healing *in vivo*
***Results:** There was no effect on the core clock gene expression by *Npas2* KO mutation. The KO fibroblasts showed higher cell proliferation, migration and contraction capabilities. While alpha-SMA expression was not affected, FACIT collagen XII and XIV gene expression was significantly increased in *Npas2* KO fibroblasts. Picrosirus red staining was strongly positive in *Npas2* KO fibroblasts. *Npas2**-/-* mice demonstrated faster dermal wound closure than the other groups ($p<0.01$). Furthermore, candidate compound-treated dermal wounds suggested accelerated wound healing.
***Conclusion/Significance:** Our study demonstrated that *Npas2* suppression in dermal fibroblasts modified cell behaviors demonstrated by accelerated cell proliferation, cell migration and cell contraction force *in vitro*. Moreover, *Npas2* suppression resulted in accelerated dermal wound healing. This study suggests that *Npas2*may be a novel therapeutic target for dermal homeostasis and wound healing.

488 - Soft Tissue Approximation And Repair Using Laser-activated Biomaterials

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***Purpose/Objectives:** Sutures, staples and tissue glues remain the primary means of tissue approximation and vessel ligation. Despite widespread use, conventional sutures do not immediately seal approximated tissue, resulting in bacterial leakage and infection. Non-absorbable sutures and staples are especially traumatic, requiring subsequent removal. Use of cyanoacrylate glues has been limited due to brittleness and toxicity.
***Methodology:** Laser-activated tissue sealing is an alternative approach which conventionally employs light-absorbing chromophores and nanoparticles for converting near-infrared (NIR) laser to heat, resulting in tissue sealing. Here, we demonstrate three novel technologies for light-activated soft tissue sealing and repair.
***Results:** In the first, we describe generation and characterization of laser-activated nanosealants (LANS) in which, gold nanorods (GNRs) are embedded within a biopolymer matrix (collagen, silk or elastin-like polypeptides). Laser-activated sealing of porcine tissue resulted in a 7-fold increase in burst pressure compared to sutured intestine. Laser sealing also enhanced the recovery of skin tensile

strength in a mouse model of dermal closure following spine surgeries. LANS demonstrated significantly higher efficacies compared to sutures or cyanoacrylate skin glue. Secondly. we describe novel laser-activated tissue-integrating sutures (LATIS) that synergize the benefits of conventional suturing with laser repair, resulting in accelerated wound closure and healing. The mid-dorsal surgical incision model was used and closed with commercial sutures (PGA, silk), and LATIS with our without laser irradiation. Laser irradiation resulted in greater recovery of skin biomechanical properties compared to commercial sutures. Three days post-surgery, the incision site was connected with immature fibrous tissue in all cases. LATIS and LATIS+laser showed no additional inflammation. LATIS+laser closed mice skin incisions showed the highest increase in skin tensile strength after 3 days, which corresponds to ~50% recovery of intact skin tensile strength. The third technology describes the use of a chromophore or nanoparticle-free approach for tissue sealing by employing non-ionizing mid-infrared (midIR) laser light. Biomaterials, based on their ability to absorb midIR light, demonstrate a local photothermal response which results in rapid tissue sealing and approximation. Biomaterials (silk and chitosan) irradiated with midIR laser light were employed to seal surgical incisions in ex vivo porcine intestine and in skin in live mice. Tissues sealed with midIR irradiated biomaterials demonstrated enhanced recovery of biomechanical properties with no adverse inflammation. Cytotoxicity and biocompatibility of midIR laser were also determined using MTT assay indicating that midIR light can be used for rapid sealing and repair of tissues using conventional biomaterials.
***Conclusion/Significance:** Taken together, the conversion of non-ionizing near and mid-infrared light to local heat can be used for accelerated tissue sealing and repair resulting in improved outcomes in trauma, surgical repair, and wound healing.

489 - Photothermal And Immunomodulatory Nanomaterials For Tissue Repair

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***Purpose/Objectives:** Sutures, staples and tissue glues remain the primary means of tissue approximation and vessel ligation. Despite widespread use, conventional sutures do not immediately seal approximated tissue, resulting in bacterial leakage and infection.
***Methodology:** Laser-activated tissue sealing is an alternative approach which conventionally employs light-absorbing chromophores and nanoparticles for converting near-infrared (NIR) laser to heat, resulting in tissue sealing.
***Results:** In the first, we describe generation and characterization of laser-activated nanosealants (LANS) in which, gold nanorods (GNRs) are embedded within a biopolymer matrix (collagen, silk or elastin-like polypeptides). Secondly. we describe novel laser-activated tissue-integrating sutures (LATIS) that synergize the benefits of conventional suturing with laser repair, resulting in accelerated wound closure and healing. The mid-dorsal surgical incision model was used and closed with commercial sutures (PGA, silk), and LATIS with our without laser irradiation. Laser irradiation resulted in greater recovery of skin biomechanical properties compared to commercial sutures. The third technology describes the use of a chromophore or nanoparticle-free approach for tissue sealing by employing non-

ionizing mid infrared (midIR) laser light. Biomaterials, based on their ability to absorb midIR light, demonstrate a local photothermal response which results in rapid tissue sealing and approximation. Biomaterials (silk and chitosan) irradiated with midIR laser light were employed to seal surgical incisions in ex vivo porcine intestine and in skin in live mice. We have also used this technology for accelerating slow healing diabetic wounds and acute wounds. 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. Significant reduction in the 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 and 11 days in diabetic wounds. 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.
***Conclusion/Significance:** Taken together, the conversion of non-ionizing near and mid-infrared light to local heat can be used for accelerated tissue sealing and repair resulting in improved outcomes in trauma, surgical repair and wound healing.

492 - Accelerated Full Thickness Wound Healing In Streptozotocin Induced Diabetic Rat Using Honey Incorporated Silk Fibroin Substrates

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***Purpose/Objectives:** Diabetes mellitus is one of the causes of impaired wound healing, these wounds are characterized by chronic inflammation, and decreased granulation tissue formation and vascularization, and it is a great challenge for researchers to promote chronic wound healing. In this study, we tried to develop an improved dressing of honey(2% v/v) loaded silk fibroin(8wt%) for diabetic wound healing.
***Methodology:** Fabrication of honey loaded silk fibroin micropillared substrates by soft-lithography using lotus-leaf as a master template. The fabricated membranes were physicochemically characterized and tested for *in vitro* biocompatibility. For *in vivo* wound healing study, male Wistar rats were divided into two group models- acute and streptozotocin-induced diabetic model. Full-thickness wound of diameter 10mm was created at the dorsolateral plane of animals and studied wound healing rate. Non-invasive optical coherence tomography (OCT), histological and immunohistological studies were performed on reconstituted skin after 21 days of implantation. Ultrastructure and nanomechanical property of reconstituted skin tissue also have been studied.
***Results:** The fabricated honey loaded silk fibroin substrates showed Young's modulus of 175±0.84 MPa and maintained its shape upon water immersion. The swelling ratio was 6.5±0.4 and after 21 days of

incubation in phosphate buffer saline (1M, pH-7.4, 37°C), the degradation rate was 7.79±1.1%. Compared to honey free fibroin substrate, the blended substrate enhanced the attachment and proliferation of 3T3 fibroblasts. In vivo testing of fabricated substrates on acute wounds on Wistar male rats indicated the fast wound closure and healing in compared to the sham group. In streptozotocin-induced diabetic rats, the wounds treated with honey-silk fibroin substrates were smaller by day 7 after wounding, compared to the untreated and honey free substrate indicated accelerated wound healing. OCT showed the complete re-epithelization (epithelial thickness ~ 101 ± 3.5 µm) and dermal construction of repaired diabetic wound skin which is equivalent to the normal rat skin (epithelial thickness ~108 ± 1.2 µm) and corroborated with histological findings which showed fibroblast distribution, collagen fiber organization, and formation of hair appendages to be almost similar to the normal homeostasis. The collagen I and III expressions and, the ultrastructure of collagen fibers and fiber thickness of acute and diabetic wounds tissues are comparable to the normal skin (without any wound). The nanomechanical parameters of diabetic tissue were Young's Modulus ~ 7.89 ± 4.79 kPa, deformation~82.8 ± 23.6 nm, and adhesion~3.2±0.14pN were also found to be comparable to the normal skin tissue.
***Conclusion/Significance:** This study thus, suggests that such accelerated wound healing along with attaining the normal homeostasis under the influence of micropillared fibroin-honey substrates may find its potentiality in treating diabetic non-healing skin ulcers and other chronic wounds.

494 - Engineering An Anti-inflammatory Islet Transplant Microenvironment Through Il-10 Cell-surface Immobilization

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***Purpose/Objectives:** Clinical islet transplantation (CIT) is an emerging curative treatment option for labile type 1 diabetes patients to provide physiological blood glucose regulation. CIT's widespread clinical application, however, is limited by the drastic, inflammation-mediated early graft loss. Macrophages, an innate phagocytic immune cell, play a seminal role in the orchestration and resolution of inflammation. Due to their phenotype plasticity, macrophages can exhibit an anti-inflammatory (M1) or anti-inflammatory (M2) profile depending on the stimuli present; therefore, macrophage polarization is an attractive target for modulation of an anti-inflammatory, pro-engraftment islet transplant microenvironment. In this study, we sought to engineer a platform in which Interleukin-10 (IL-10), a potent anti-inflammatory cytokine, could be immobilized onto the islet surface to locally promote an M2 macrophage polarization upon transplantation.
***Methodology:** In order to immobilize IL-10, a Michael's addition conjugation strategy (maleimide-thiol) was employed. This was accomplished by using a cell-surface binding heterofunctional poly(ethylene glycol) linker (NHS-PEG-maleimide) and thiol-functionalized IL-10 (IL-10-SH). To first validate the feasibility and specificity of this conjugation scheme for islet-surface engineering, collagen coated dextran beads (150-212 µm) were used as an analogue. The surface of these idealized beads was first conjugated with a fluorophore-functionalized polyethylene glycol (NHS-PEG-FITC). Next, a FITC functionalized thiol group (SH-FITC) was used as a fluorescent reporter to confirm conjugation. The density of the NHS-PEG-maleimide-SH-FITC coating was examined at varying incubation times to identify optimal experimental conditions. Next, the bioactivity and M2 macrophage polarizing capacity of IL-10-SH was determined. This was done by incubating lipopolysaccharide (LPS)-stimulated bone marrow-

derived macrophages with soluble IL-10-SH for 6 and 24 hours and measuring TNFα response.
***Results:** To verify the IL-10 conjugation strategy (NHS-PEG-mal-SH-FITC), results were compared to beads conjugated with methylated polyethylene glycol (NHS-mPEG-SH-FITC) and with the thiolated fluorophore (SH-FITC) alone. Image analysis confirmed a 3 to 4-fold increase in FITC labeling, when NHS-PEG-maleimide-SH-FITC groups were compared to control groups. Additionally, 30 minutes proved a sufficient incubation time to tether SH-FITC onto the bead surface. For the bioactivity studies, when LPS-stimulated bone marrow-derived macrophages were incubated with unmodified soluble IL-10 and soluble IL-10-SH, the soluble IL-10-SH was found to significantly lower the TNFα response in a concentration dependent manner, comparable to that of the unmodified soluble IL-10 at both 6 and 24 hours.
***Conclusion/Significance:** Image analysis of the idealized bead islet analogues revealed the feasibility and specificity of using Michael's addition to immobilize IL-10 onto the islet surface. Additionally, thiol functionalization of IL-10 did not hinder the anti-inflammatory capacity of IL-10. Future work will explore the impact on viability and functionality of immobilized IL-10 on the islet surface, as well as further characterization of the macrophage phenotype derived from immobilized IL-10 modulation *in vitro* and *in vivo.*

495 - Engagement Of Cadherin-11 Promotes Cell Growth In Cooperation With Platelet Derived Growth Factor Receptor (pdgfr) Via Akt Pathway

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***Purpose/Objectives:** This research is focused on understanding the fundamental biomechanics of cadherin-11 engagement as well as the hetero-interaction with platelet derived growth factor receptor in the regulation of cell growth during wound healing process
***Methodology:** Cadherin-11 deficiency mouse (*cdh11*$^{-/-}$) and littermate (WT) were employed as *in vivo* experiment. Both *cdh11*$^{-/-}$ and *WT* mouse were subjected to a subcutaneous wound healing experiment for 2 weeks. Wound healing process were monitored and skin tissues were isolated at the end for further histology examination. *In vitro,* both mouse and human fibroblast were isolated and subjected to study the function of CDH11. Engineered surface coated with CDH11-11 Fc were used to mimic the homotypical interaction of CDH11. Co-immunoprecipitation were employed to prove the hetero-interaction between cadherin-11 and PDGFR. Various mutations of CDH11 structure were constructed and expressed in L cell to verify the functional binding domain between CDH11 and PDGFR.
***Results:** Surprisingly, *cdh11*$^{-/-}$ mouse has a significant thinner dermis layer of skin which is featured with less number of fibroblasts compared with WT. Consistently, *in vitro*, fibroblast isolated from *cdh11*$^{-/-}$ grow much slower characterized with diminished PDGFR expression. Similar observation was captured by knocking down CDH11 expression in human fibroblast. In contrast, plating cells on CDH11-Fc surface, CDH11-CDH11 engagement promotes cell growth by activation of AKT pathway in a dose depend manner within 30 minutes. Furthermore, by blocking other growth factor receptors with corresponding chemical inhibitors, we verified CDH11 engagement activates AKT pathway through PDGFR. Specifically, CDH11 physically binds to PDGFR and increases PDGFR sensitivity to its ligands by 10-100 times. Deficiency of CDH11 in mouse tissues dramatically slow down the natural wound healing process due to lack response to the growth factor stimulus.
***Conclusion/Significance:** Overall, our research indicates a novel function of CDH11 in mediating dermal

fibroblasts proliferation via AKT pathway activation, specifically as a result of CDH11 binding and modulating the PDGF receptor sensitivity to its ligands. This novel discovery may help comprehensive understanding of cell survival and proliferation even under nutrient deprived settings and have implications in both tissue regeneration and tissue engineering.

496 - Bacterial Cellulose: Matrix For Production Of Bioactive Wound Dressing

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The high cost of wound care and the impact on patient quality of life has motivated the medical market to obtain innovative materials for the healing process. Bacterial cellulose (BC) is a successful membrane in the healing process and aiming to improve the therapeutic action of the dressing, biomolecules can be immobilized. Therefore, the aim of this work was to evaluate physical-chemical and biologic properties of BC bioactive dressings obtained through immobilization of papain. BC was produced by static fermentation of *K. hansenii* in Hestrin-Schramm medium for 5 days. Membranes were purified with K_2CO_3 and oxidized with $NaIO_4$. The papain immobilization was performed by immersion of membrane oxidized BC. The results showed that the BC bioactive dressings were non-cytotoxic to human fibroblasts and keratinocytes (cell viability of 89.8 % and 86.5 %, respectively) and non-haemolytic when in contact with blood. Moreover, the moisture vapor transmission rate for the curative (2678 ± 181 g/m^2. 24h) was like commercial curative highly permeable (300-3000 g/m^2. 24h/Opsite Post-op® and Hydrocoll®). The fluid absorption capacity was above 100% of its weight, being considered a high absorbent material. Microbiological assays showed that the material presented bacteriostatic activity against gram-negative bacteria. The *in vitro* drug release was conducted using Franz diffusion cells and showed a liberation profile of papain around 78.2% in 24h. These properties indicate that this BC bioactive dressing could acts in the preventing infections, maintaining ideal moisture, promoting gas exchange, as well as acting by removing necrotic tissue, accelerating tissue reconstitution

497 - Electrospun Phbhv Fibers Loaded With Anti-inflammatory And Antioxidant Molecules For Wound Repair

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Objectives: Polyhydroxyalkonates (PHAs) are a class of biodegradable and biocompatible bio-based polyesters produced by bacterial fermentation. In this study, we electrospun Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBHV) meshes incorporating drug models and evaluated their cytocompatibility. Dexamethasone (Dex) was loaded inside the fibers as an anti-inflammatory drug and olive leaf extract (OLE) as an antioxidant compound. Cell metabolic activity and viability were investigated. **Methodology:** PHBHV was dissolved in chloroform/methanol (9:1 w/w) mixture and electrospun into fibers using 15 (w%) polymer solution, 30 kV voltage and flow rate of 0.001 ml/min and 58% relative humidity. Fiber morphology was analyzed via scanning electron microscopy (SEM). OLE was characterized via HPLC.

Total polyphenols (TP) in OLE were determined using Folin-Ciocalteau method. Dex was added at 10% w/w, while OLE at 3% w/w with respect to PHBHV. The sample were analysed via Fourier-transform infrared spectroscopy (FTIR) and sterilized via UV irradiation. Human fibroblast-like cells were cultured on the scaffolds for 9 days. AlamarBlue and LIVE/DEAD® assay were used to evaluate cell viability. **Results:** The TP content ranged in 14.99-27.83 mg Gallic Acid Equivalent (GAE)/g due to seasonal differences, with Oleuropein being the main component. Electrospinning process produced drug loaded and unloaded fibers with similar size and morphology. FTIR showed the presence of DEX and OLE inside the fibers. The preliminary cell culture results demonstrated that human fibroblasts were able to adhere and grow on PHBHV scaffolds in presence of Dex or OLE. **Conclusion/Significance:** Electrospun PHBHV can be used to produce bioactive wound dressings. **Acknowledgements:** BBI-JU H2020, POLYBIOSKIN (G.A. 745839)

498 - A Role For Fibroblast Bioenergetics In Mediating Distinct Fibrotic Responses To Injury

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Energy metabolism governs wound healing and fibrosis, with fibroblasts being main arbiters of scarring. It is unknown why different people scar differently from similar injuries, and whether fibroblast energy metabolism underlies this observation. We hypothesize that differences in intrinsic fibroblast energy metabolism regulates scarring, which can be attenuated by metabolic-optimization using engineered biomaterials. Fibroblasts were isolated from ‘uninjured’ normal-skin and scars of patients who are "low(LS)" or "high(HS)" scarrers. Oxidative phosphorylation(OCR), glycolysis(ECAR) and ATP at resting state and under cellular stress/hypoxia(seahorse assays), mitochondrial membrane potential(ΔΨm;JC-1) and ROS(MitoSOX) were measured.Fibroblasts were cultured on gelatin methacrylate crosslinked with thiolated lignosulfonate(GelMA-TLS) and the expression of α-SMA/Col1/3 were assessed(qRT-PCR). p-values by ANOVA;(n=3-4patients/group). HS fibroblasts had higher basal OCR and ECAR, indicative of being more energetic, as compared to LS phenotype($p<0.01$). ATP production was similar in HS and LS normal-skin fibroblasts. HS fibroblasts responded better to biochemical stress, evidenced by a greater increase in spare respiratory and glycolytic reserve capacity upon FCCP/Oligomycin treatments as compared to LS($p<0.01$). Under hypoxic conditions, HS fibroblasts showed a significant increase in glycolysis. ΔΨm was higher in normal-skin fibroblasts from both HS and LS as compared to scar fibroblasts($p<0.001$), despite HS normal-skin fibroblasts staining for more depolarized mitochondria compared to LS($p<0.01$). Consequently, mitochondrial-ROS was higher in HS normal-skin fibroblasts($p<0.05$). GelMA-TLS composites scavenged free radicals and attenuated the upregulated fibrotic markers, α-SMA and Col-1a in HS fibroblasts($p<0.05$). Differences in fibroblast bioenergetics may govern fibrotic responses, which can be regulated by engineered biomaterials, representing new frontiers in improving fibrosis.

499 - Transcriptome Dynamics Of Long Non-coding RNAs And Transcription Factors Demarcate Human Neonatal, Adult, And MSC-derived Engineered Cartilage

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***Purpose/Objectives:** *The engineering of a native-like articular cartilage is a long-standing objective that could serve the clinical needs of millions of patients suffering from osteoarthritis and cartilage injury. An incomplete understanding of the developmental stages of articular cartilage (AC) has contributed to limited success in this endeavor.*

***Methodology:** *Using next generation RNA sequencing we have transcriptionally characterized two critical stages of AC development in humans - i.e., immature neonatal and mature adult, as well as tissue-engineered cartilage derived from culture expanded human mesenchymal stem cells (MSCs).*
***Results:** *We identified key transcription factors (TFs) and long non-coding RNAs (lncRNAs) as candidate drivers of the distinct phenotypes of these tissues. AGTR2, SCGB3A1, TFCP2L1, RORC, and TBX4 stand out as key transcription factors whose expression may be capable of reprogramming engineered cartilage into a more expandable and neonatal-like cartilage primed for maturation into biomechanically competent cartilage. We also identified that the transcriptional profiles of many annotated but poorly studied lncRNAs were dramatically different between these cartilages, indicating that lncRNAs may be also playing significant roles in cartilage biology. Key neonatal-specific lncRNAs identified include AC092818.1, AC099560.1, and KC877982.*

***Conclusion/Significance:** *Collectively, our results suggest that tissue-engineered cartilage can be optimized for future clinical applications by the specific expression of TFs and lncRNAs.*

500 - Therapeutic Effect Of Cell Therapy On Improvement Of Erectile Function In A Rat Neurovascular Injury Model

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***Purpose/Objectives:** Neurovascular injury induced erectile dysfunction (NVED) is one of the most common complications in complex pelvic trauma in men. Despite advances in stem cell therapeutic approaches, the long-term impact of cell therapy of the ED is unknown. The goal of this study is to determine the long-term therapeutic effect of mesenchymal stem cells on erectile function recovery in a rodent model of NVED.
***Methodology:** NVED model was established in athymic rats by crushing bilaterally the cavernous nerves and ligations of bilaterally the internal pudendal bundles. Three different types of human cell populations used included endothelial cells (EC), adipose derived stem cells (ASC) and amniotic fluid derived stem cells (AFSC). Aged-matched animals served as a positive control and defect induced animals receiving normal saline (NS) injections served as a negative control. Cell populations ($2.5x10^6$ cells in 0.2 ml) were injected intracavernously into the penile tissue. Erectile function and histomorphological analyses of penile tissue were assessed 12 weeks after defect creation and cell injection.
***Results:** The ratio of intracavernous pressure and mean artery pressure (functional indicator) significantly increased in the stem cell and endothelial cell therapy groups when compared to the NS

injection group. Immunofluorescence staining showed that more cells expressing biomarkers of endothelial, smooth muscle, and nerve cells within the corpora cavernosa was seen in the cell therapy groups when compared to the NS injection group.
***Conclusion/Significance:** Stem cell therapy enhanced erectile function and ameliorated the histological changes 12 weeks after pelvic neurovascular injury in vivo, indicating that cell therapy may improve the long-term outcomes in vascular, myogenic and neurogenic tissue regeneration in the treatment of NVED.

501 - Quality Control Of Human IPSC-derived Motorneurons

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***Purpose/Objectives:** Motorneurons (MNs) are central nervous system (CNS) neurons that control essential voluntary muscle activity such as speaking, walking, breathing and swallowing. Disruption of MN function has been involved in diseases such as spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS). To tackle these diseases an increasing number of in vitro models have been developed to reproduce human MNs and MN-associated physiological systems, mostly neuromuscular systems (NMJs), by utilizing patient derived stem cells. Human induced pluripotent stem cells (hiPSCs) have been an increasingly important cell source for these models due to their ability for almost unlimited expansion, pluripotency for differentiation, and capability for recapturing disease-relevant pathologies in the differentiated cells.However, a significant problem concerning iPSCs and the differentiated cells generated from iPSCs is the variability, observed between different iPSC sources/lines and between different cultures
***Methodology:** We have established a protocol for differentiating MNs from iPSCs, and have differentiated MNs from multiple ALS patient-derived iPSC lines, which demonstrate representative pathological phenotypes. These MNs are utilized for subsequent disease models by integration into our established in vitro NMJ system to develop patient-specific NMJ models for ALS. However, the variations in the MN differentiation efficiency and the qualities of generated MNs have caused difficulty in these projects. To reduce the variations and increase the consistency of related experiments, we have established a series of quality control steps for these iPSC-MNs
***Results:** For each batch after differentiation and before being utilized in experiments, the morphology and identity of the MNs were analyzed by phase microscopy and immunocytochemistry with the MN markers HB9, Islet1 and SMI32. The percentage of MNs in the culture was quantified by flow cytometry. The functionality of MNs was also examined by patch clamp electrophysiology. Finally, the capability of these MNs for forming functional NMJs was then confirmed in functional NMJ systems
***Conclusion/Significance:** The application of these quality control mechanisms has significantly increased the consistency and efficiency of the studies that utilize these MNs

502 - Double-induction: Osteogenesis And Angiogenesis In MMSC Spheroids

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***Purpose/Objectives:** The problem of proper vascularization is still one of the most actual challenging questions of regenerative medicine limits the formation of full-thickness artificial tissue, like bone tissue. The general approach to generate vascularized tissue involves co-cultivation of osteogenic multipotent mesenchymal stromal cell (MMSC) and endothelial cells (EC). Moreover, the appearance of endothelial cells stimulates an early activation of the factors that regulate osteogenesis. On the other hand, in the last decade, it was shown that 3D cultivation of MMSC as spheroids also enhances osteogenesis. However, these approaches have never considered according to endothelial differentiation and the crosstalk between osteogenesis and angiogenesis. Thus, the objective of this research was the analysis of cell behavior after double-induction of both osteogenic and endothelial differentiation in MMSC spheroids.
***Methodology:** The study was conducted using human adipose-derived multipotent mesenchymal stromal cells (MMSC) isolated from an adipose stromal-vascular fraction of lipoaspirate cultured in the standard condition of incubation. On the 4^{th} passage, these cells were placed in agarose plates with microwells (Microtissue, USA), the cell suspension concentration was 3.3×10^6 cell/ml. Experimental groups included 4 types: 1. Control group - intact spheroids; 2. Osteo-group - the group with osteogenic induction; 3. Angio-group - the group with endothelial induction; 4. Double-group - the group with double-induction. Spheroids were characterized using scanning electron microscopy (SEM), immunocytochemical (ICC) staining and real-time PCR at 1, 7, 14 and 21 days of 3D cultivation.
***Results:** SEM images confirm that spheroids had the standard morphology with surface epithelial-like cells and central stromal cells surrounded by extracellular matrix. ICC showed expression of early osteogenic marker Osteopontin (OstP) and endothelial marker Flk-1 at day 7 of 3D cultivation of the all experimental groups including Control group. These results show the opportunity spontaneous osteogenesis and angiogenesis in adipose-derived MMSC spheroids. But ICC demonstrated that endothelial induction and double-induction significantly enhanced expression OstP and Flk-1 at days 7 and 14 for both groups. Moreover, PCR confirms that double-induction stimulated early stages of osteogenesis including significant increasing of Osterix expression.
***Conclusion/Significance:** To sum up, the current study showed that in non-adherent culture adipose-derived MMSC are able to spontaneous osteogenesis and angiogenesis. However, was shown that double-induction stimulates and enhances osteogenesis in MMSC spheroids, also providing their angiogenesis. These results open new approaches to generation of bioequivalents of *in vitro* vascularized bone tissue fragments for the rapid and efficient repair of large bone defects of the axial skeleton or face.
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503 - Enhancement Of Lymphangiogenesis By Human Mesenchymal Stem Cell-secreted Extracellular Matrix

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***Purpose/Objectives:** Injury to the lymphatic vasculature disrupts tissue homeostasis and impedes tissue fluid balance, resulting in lymphedema. Tissue swelling due to lymphedema brings inconvenience and severely affects patients' life quality. Moreover, the accumulation of cell metabolized waste and immune cells in tissue fluid might cause chronic inflammation and immune system disorder. Hence, it is critical to develop novel strategies to reconstruct lymphatic vasculature and recover lymphatic circulation. Injection of mesenchymal stem cell (MSC) suspension has previously been used in both human and animal lymphedema, and has shown beneficial effects on ameliorating lymphedema swelling due to the paracrine impact of MSCs. However, the cell suspension delivery damages the cell-cell and cell-extracellular matrix (ECM) connections, which significantly lowers cell retention and engraftment. Herein, the impact of the hMSC-secreted ECM on lymphangiogenesis was investigated by *in vitro* by co-culturing hMSC sheet with lymphatic endothelial cells (LECs).
***Methodology:** hMSCs were cultured for 9 days under physiological hypoxia (2% O_2) to create hMSC sheets. LECs were co-cultured on the top of hMSC sheets for 12 days (hMSC sheet group). As a control, LECs were cultured with single hMSCs trypsinized from hMSC sheets (hMSC suspension group). Lymphatic vasculature was observed and quantified by immunofluorescent staining using LEC marker CD31. lymphangiogenic growth factors and basement membrane proteins (collagen IV and laminin) were examined using Enzyme linked immunosorbent assay (ELISA) and western blot, respectively. Matrixmetalloproteinase-2 (MMP-2) was examined using MMP zymography.
***Results:** The hMSC-secreted ECM served as a growth factor reservoir that contained 1.2-2.4 ($p < 0.05$) folds of hepatocyte growth factor (HGF) and 2.2-3.6 ($p < 0.01$) folds of basic fibroblast growth factor (bFGF) in hMSC sheet samples compared with hMSC suspension samples after co-culturing with LECs for 12 days. The ECM in the hMSC sheet also significantly stimulated hMSCs to release more HGF and promoted the remodeling of ECM. Moreover, the ECM increased MMP-2 release and activation while elevating the synthesis of basement membrane constituents collagen IV (1.41-1.51 folds, $p < 0.05$) and laminin (1.38-1.67 folds, $p < 0.01$). The lymphatic network formed on hMSC sheet showed incomplete basement membrane structure surrounding LECs, similar to the native lymphatic capillaries, which benefit the extra interstitial fluid adsorption.
***Conclusion/Significance:** The hMSC sheets, which combine hMSCs with ECM, promoted LECs to form lymphatic networks by storing growth factors and promoting crosstalk between hMSCs and LECs. The performance of hMSC sheet in stimulating lymphangiogenesis makes it promising in treating lymphedema. **Acknowledgements:** This study was supported by the National Institutes of Health (1R15CA202656 and 1R15HL145654) and the National Science Foundation (1703570) to FZ. It was also supported by the Portage Health Foundation to both FZ and WJ.

504 - Human Placental Derived Stem Cells Promote Erectile Function Recovery In A Rodent Pelvic Neurovascular Injury Model

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***Purpose/Objectives:** Erectile dysfunction is one of the complications associated with pelvic injury. Pelvic injury can involve damage to nerves as well as the vascular system. This type of injury remains

difficult to treat although advances in pharmacotherapy has been achieved. The aim of this pilot study was to determine the possibility of improving erectile dysfunction using cell therapy comprised of human placental-derived stem cells (PSCs) in the treatment of a neurovascular injury in a rat model.
***Methodology:** Neurovascular injury induced erectile dysfunction (NVED) was established in a nude rat model. Human PSCs ($2.5x10^6$ cells/0.2 ml) were injected intravenously into the penile tissue of the injured animlas. Aged match animals were used as a positive control. Defect induced animals injected with normal saline (NS) injection served as a negative vehicle control. Erectile function and histological analysis of penile tissue was assessed 1, 6 and 12 weeks after defect creation and stem cell injection.
***Results:** The ratio of intracavernous pressure to mean arterial pressure was significantly improved in the defect induced animals 6 and 12 weeks and post-treatment with PSCs compared to the PBS treatment group. No difference was seen between the one-week PSCs treated group and the PBS-treated group. Histological analysis of the penile corpora tissue showed more cells expressing nerve, smooth muscle and endothelial cell markers was present in the PSCs treated group than in the NS treated group.
***Conclusion/Significance:** Intracavernous injection of human PSCs improved the erectile function as well as the cellular composition of the penile corpora in a double-pelvic neurovascular injury at 6 weeks post-injury and treatment and was maintained out to 12 weeks post treatment. The data suggest that cell therapy using PSCs provides an alternative approach in the treatment of ED following pelvic injury.

505 - Development Of Consistent Xeno-Free Human Umbilical Cord MSC (hUC-MSC) Cell Populations For Tissue Engineering And Regenerative Medicine

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***Purpose/Objectives:** Many tissue engineering and regenerative medicine (TERM) approaches include cells as a critical intermediate material. Mesenchymal stem/stromal cells (MSCs) are a prominent translation-friendly cell in the field with over 900 registered clinical trials. A critical bottleneck in TERM applications involving MSCs, however, is generating the large number of cells required for clinical trials and commercial therapies. This consideration is critical during development studies preparing for translation. While a tissue engineering research animal model might require only 1 million cells, translation to clinically-relevant human tissue engineered constructs instead require 100's of millions to billions of cells. Furthermore, for consistency of the TERM approach, it is necessary that the state of the cells be consistent, requiring a defined range in population doubling level (PDL) for each use. Thus, well-characterized, reproducible, and scalable sources of MSCs are needed to enable the success of TERM approaches during development of commercial therapies. These studies focus on a cell bank model that allows for repeated manufacture of human umbilical MSCs (hUC-MSCs) at a consistent range of PDL and functional quality attributes.
***Methodology:** hUC-MSCs were isolated from the perivascular region of human umbilical cords from nine donors. Using a xeno-free culture system (medium, reagents and materials), cells were cryopreserved to create a Working Cell Bank (WCB). The designed paradigm is for a vial or vials of WCB cells to then be thawed and expanded for use (without further banking), thus limiting the variability of the hUC-MSCs across uses.
***Results:** Cells at the PDL of use were then assessed for critical quality attributes: final PDL, typical MSC surface marker expression (positive for CD90 and CD166, negative for CD34 and CD45), trilineage

differentiation potential (osteogenesis, adipogenesis, and chondrogenesis), and functional properties including IDO secretion and angiogenic cytokine secretion (VEGF, IL-8, bFGF, HGF, TIMP1 and TIMP2). Multiple vials from the same cell bank will be evaluated for these critical quality attributes to demonstrate the consistency of cell supply in final tissue quality.
***Conclusion/Significance:** This study establishes a cell banking model to reproducibly generate populations of hUC-MSCs that have consistent properties. Repeatedly generating cell populations with similar attributes is critical for well-designed studies during the development of tissue engineering and regenerative medicine approaches and as these approaches move towards clinical translation.

506 - Modeling Molecular Responses And Stem Cell Therapies To Treat Traumatic Brain Injury

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***Purpose/Objectives:** Meta-analysis of large data sets and stem cell engineering technology have opened new avenues to study human pathophysiology, in particular modeling disease progression in vitro using living cells. Traumatic brain injury (TBI) is one neurological pathology that occurs frequently and routinely due to causes like falls and car accidents. Despite the potential for severe quality of life impairment for patients with TBI, objective molecular criteria to diagnose the severity of a brain injury remain unclear.
***Methodology:** Our objective was to identify potential biomarkers of TBI severity using meta-analysis of big data. We developed custom code to scan large biomedical literature databases (millions of articles) against standardized text analysis (approximately 100,000 keywords). We performed data analysis to predict possible biomarkers for the severity of TBI. The responses of these genes were measured using in vitro cell culture models of chemical and mechanical damage to neural stem cells.
***Results:** This systematic meta-analysis included more than 10,000 TBI-related PubMed-indexed articles. We identified the primary cell types and model organisms used to study TBI, as well as the cell types and molecules of greatest relevance to the disease. We next correlated these genes with studies of mild, moderate, and severe TBI to identify potential biomolecules whose state varies with the severity of brain injury. These data produced a panel of predicted biomarkers, each supported by cumulative prior experimental evidence. We have deployed established in vitro injury methods to mechanically or chemically mimic the physical shearing or inflammatory related processes associated with traumatic brain injury. Ongoing studies are testing how neural stem cells and their derivatives respond to TBI-like injuries using the panel of predicted biomarkers for brain injury severity.
***Conclusion/Significance:** Injury severity is a dominant factor in determining the motor and cognitive consequences of a patient's brain injury. Furthermore, neural stem cells may contribute to long term increased risk for neurodegenerative diseases associated with repeated mild TBI. This systematic meta-analysis of TBI subtype biomarkers and related stem cell therapies for brain injury may help advance efforts to more precisely diagnosis TBI and to improve design of cell therapies to treat common but challenging brain injuries.

507 - Simplified MSC-derived Extracellular Vesicle Production In A Scalable Bioreactor System

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***Purpose/Objectives:** There have been over 800 registered clinical trials using mesenchymal stem/stromal cells (MSCs) for various therapeutic applications, showing MSCs as a well-tolerated, safe therapy. Additionally, MSC-derived extracellular vesicles (MSC-EVs) are being increasingly investigated as a clinical therapy for a broad range of indications due to their similar therapeutic effects to MSCs and their potential as a key bioactive agent in regenerative medicine applications. With growing numbers of MSC-EV clinical applications, there is a critical need for a scalable MSC-EV production paradigm that can generate the EV numbers necessary for clinical doses. The objective of this project is to establish a simple streamlined process for generating MSC-EVs in a microcarrier-based bioreactor system.
***Methodology:** In recent studies, human Bone Marrow-Derived MSCs (hBM-MSC) were cultured on polystyrene-based microcarriers in a xeno-free (XF) fed-batch suspension culture using a 0.1L PBS-MINI bioreactor system. Initially cells were seeded onto microcarriers using a prescribed seeding protocol. After 3 days of culture, a bioreactor feed was added followed by 1-2 additional days of culture.
***Results:** This process has successfully been scaled-up to the development scale (3L) and pilot scale (15L), where cell concentrations of >0.5M cells/ml were achieved within 4-5 days. After a switch to a low-particle EV collection medium, EVs were collected for 3 days. Particle concentration (in the size range of EVs) was $2x10^9$/ml in the bioreactor samples, which was >2X the productivity in 2D culture. Additional optimization is still needed to explore microcarrier types with other distinct cell-binding strategies. Particles will be further analyzed for EV characteristics, including expression of CD9, CD63, CD81, protein and RNA content, and wound-healing capability in an in vitro migration assay.
***Conclusion/Significance:** Leveraging this observed increased productivity in bioreactor cultures is pivotal for all groups interested in generating MSC-derived EVs in clinically relevant numbers. Establishing expertise in microcarrier-based bioreactor cultures, however, can be an obstacle for many. To simplify this process, this project will now focus on developing a means to cryopreserve hBM-MSCs on microcarriers to facilitate a thaw-acclimate-harvest approach to generating EVs in a suspension bioreactor system. By optimizing the cryopreserved conjugates for culture in the PBS bioreactor product family, which includes systems suitable for 0.1L to 500L scale, this approach will provide a scalable manufacturing process of EVs that can support yields appropriate for laboratory benchtop research, translational development studies, and commercial clinical therapy production.

508 - StemRegenin 1 Promotes The Expansion Of High Proliferative Potential Endothelial Colony Forming Cells

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***Purpose/Objectives:** Endothelial colony-forming cells (ECFCs) are rare in peripheral blood and their numbers decline with age and become dysfunctional with disease. In search of an alternative source of functional ECFC for use as a cell therapy to treat patients with vascular disease, we have developed a novel human induced pluripotent stem cell (hPSC) differentiation protocol that produces umbilical cord blood (CB) ECFC-like cells at a clinically relevant number. Since the aryl hydrocarbon receptor (AHR) antagonist StemRegenin 1 (SR1) has proven beneficial to expand hematopoietic stem cells and CD34+ hematopoietic progenitor cells derived from human embryonic stem cells in vitro, we tested that ability

of SR1 to expand hPSC-derived ECFC.
***Methodology:** Human PSCs and ECFCs were obtained and expanded by our published methods. Culturing of CB-ECFCs and hPSC-ECFCs were performed in EGM-2 supplemented with 10 % FBS on collagen-coated dish. The medium was changed every other day thereafter until the appearance of endothelial cell colonies within 14 days.
***Results:** The high proliferative potential colonies (HPP) were picked, replated, and cultured until the formation of a confluent monolayer of HPP-ECFCs. We compared SR1 treatment (over a range of doses) compared to vehicle control. We found that the SR1 treatment group was significantly enriched in HPP-ECFCs (dose dependent) compared to the control group.
***Conclusion/Significance:** This is the first report of a molecular approach to enhance HPP-ECFC expansion in vitro. HPP-ECFC represent a therapeutic cell source for revascularization of ischemic tissues and tissue engineering.

509 - Regulation Of The Hair-inductive Capacity Of Human Dermal Papilla Spheres By Alkaline Phosphatase

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Recent studies showed that sphere formation enhances the ability of cultured dermal papilla (DP) cells to induce new hair follicles. Alkaline phosphatase (ALP) activity is known to be correlated with the hair-inducing capacity (trichogenicity) of DP cells and expression of ALPL (ALP, liver/bone/kidney) transcript is restored in DP spheres. To investigate whether restoration of ALPL expression by sphere formation plays a critical role in hair-inducing capacity of DP spheres, we employed *ALPL* siRNA-mediated gene knockdown and expression vector-mediated *ALPL* overexpression in combination with a hair reconstitution assay. Knockdown of ALPL impaired the trichogenicity of human DP spheres and overexpression of ALPL augmented the trichogenicity of DP spheres. Knockdown of ALPL in DP spheres reduced nuclear β-catenin levels, pTopflash activity, and the expression of target genes in the Wnt/β-catenin pathway and DP signature genes. Overexpression of *ALPL* in DP spheres dramatically increased nuclear β-catenin levels, pTopflash activity, and the expression of target genes in Wnt/β-catenin pathway and DP signature genes. These data show that ALPL plays a critical role in the hair-inductive capacity of human DP spheres by regulating Wnt/β-catenin signalling and maintaining the characteristics of the DP. Since competent dermal cells are necessary for the cell-based treatment of hair loss, our finding of ALPL involvement in hair-inductive capacity of human DP spheres will provide a rationale for a new strategy for preparing competent DP cells by augmentation of ALPL expression in DP spheres.

510 - Effects Of Stem Cell Culture Environments On The Cellular Characteristics Of Bone Marrow Cells In 4 Day Old Chicken

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This study was conducted to evaluate the effects of stem cell culture environment on the cellular characteristics of chicken neonatal bone marrow cells (nBMCs). Four-day-old white leghorn chicks were employed as the donor of bone marrow cells and the cells isolated from the femurs were subsequently cultured in different media, primordial germ cell medium (PGC medium), low glucose DMEM (low) or DMEM/F12. To evaluate colony forming ability and proliferation of nBMCs in various media, colony forming unit-fibroblast analysis and total number of counted cells are cumulative in each passages were conducted. PLOC-GLM in SAS program was employed for statistical analysis. Higher ($p=0.0016$) colony number and Better ($p<0.0001$) proliferation were detected in the group used PGC medium than the other group. To assess gene expression and specific protein of pluripotency- and osteogenic or chondrogenic-related marker, the nBMCs collected at the end of passage 0, 3 and 4 were provided. RT-PCR analysis showed that pluripotent related marker and osteogenic and chondrogenic related genes were expressed in regardless of culture media. Immunofluorescence and western blot analysis of collagen type II (Col II) at passage 0 and 3 showed that Col II-positive cells only were expressed on cultured in DMEM/F12 and low. There were no media effects on the osteogenic differentiations but adipogenic differentiation ($p=0.0003$) were significantly induced in the PGC compared with low. In conclusion, stem cell culture environments improved culture efficiency of nBMCs and was feasibility of using nBMCs for different applications.

511 - Enrichment Of Retinal Ganglion Cells Isolated From Mouse Embryonic Stem Cells For Cell Therapy

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Methods aimed at improving the regeneration of RGCs in order to restore vision have been promising. Transplantation of purified cells into host tissue to repair damaged cells is a novel concept. A key component of cell therapy is the direct and controlled separation of a population of target cells with high purity and viablity. The purpose of this study was to optimize enrichment and isolation methods that yield high purity and viability of RGCs from mESCs. Cells stained for the phenotype $Thy1.2^{+}$ $L1\text{-}CAM^{+}$ $CD\text{-}73^{neg}$ were isolated using the Miltenyi Tyto cell sorter, and then characterized with MACSQuant cell analyzer. Enriching the RGC population from mES were successful, yielding ~95% purity and ~90% viability. The experiment examined two phenotypes $Thy1.2^{+}L1CAM^{+}CD73^{-}$and $Thy1.2^{+}L1CAM^{+}CD11b^{-}$- and we found the former to be better at isolating the most enriched RGC population. Of the six million cells obtained from cell culture, 50,000 out of 6,000,000 (~4.1%) were identified as RGCs post sort. This population showed a ~95% purity of cells with the phenotype, $Thy1.2^{+}L1CAM^{+}CD73^{-}$ and 90% viability. The cells were characterized with antibodies: Brn3a, Arrestin, KI67, Thy1.1, CRX, NeuN, Thy1.2, Recoverin, Oct3. Under 5% of cells expressed Arrestin, CRX, and Recoverin, and around 3% expressed Oct4 indicating that the purity of sorted population. High percentages of Brn3A, Thy1.1, Thy1.2, and NeuN expression confirms a high purity of RGCs. This study verifies a new phenotype, $Thy1.2^{+}L1CAM^{+}CD73^{-}$, that yields enriched RGCs. The next steps include transplantation studies and the replication of the results in larger animal models and human cells