2017 McGowan Retreat
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Exploration of inflammatory biomarkers in three depots of adipose tissue in women with endometrial cancer

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Background: Obesity is characterized by a state of chronic, low-grade inflammation and is recognized as a primary risk factor in the development of endometrial cancer (EC). Increased adipose tissue mass may promote carcinogenesis through the increased expression of pro-inflammatory biomarkers. We explored the inflammatory environment in the three adipose tissue depots (omental, retroperitoneal, subcutaneous) in women diagnosed with EC by evaluating biomarkers secreted by adipose-derived stem cells (ASC). ASC have been investigated in inflammation, aging, and obesity, but not explored in the context of EC, the most common gynecologic malignancy.

Methods: Omental, retroperitoneal, and subcutaneous adipose tissue samples were collected from 22 women, aged 35 -83 years, undergoing hysterectomy for EC. Conditioned media was generated from the growth of ASC from the three depots. Angiopoietin-2, EGF, IL-8, leptin, VEGFA, VEGFC, and VEFGD levels in the conditioned media were analyzed by Luminex bead-based xMAP immunoassays. One-way ANOVA was used to compare the mean levels of biomarkers between the three depots.

Results: The 22 women had a mean age of 64.30 (12.35) years and a mean BMI of 37.48 (7.81) kg/m2. Angiopoietin-2, EGF, leptin, VEGFD and approximately 20% of VEGFC levels were below detection limits in the three depots. There was a significant difference between the three depots for IL-8, with the highest levels of IL-8 in the omental depot and the lowest levels in the retroperitoneal depot (p-value <0.0001). VEGFA levels were highest in the retroperitoneal depot and lowest in the subcutaneous depot; however, these differences were not statistically significant.

Conclusions: This is one of the first studies to compare inflammatory biomarker expression in ASC conditioned media from three depots of adipose tissue. Adipose tissue is a potentially attractive depot to evaluate biomarkers in relation to cancer risk and more research needs to be done.

Efficient reduction of vertebrate Cytoglobins by the Cytochrome b5 reducing system

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Cytoglobin (Cygb) is a heme-containing protein ubiquitous in mammalian tissues. Unlike the evolutionarily related proteins hemoglobin and myoglobin, Cygb shows a six-coordinated heme binding, with the heme-iron coordinated by two histidine side chains. Cygb is involved in cytoprotective and regenerative pathways through yet undefined mechanisms, and it has recently been demonstrated that cytoglobin has redox signaling properties via NO and nitrite metabolism.The reduced ferrous molecule can bind oxygen and will react with NO in a dioxygenation reaction to form nitrate, which dampens NO signaling. When deoxygenated cytoglobin can bind nirite and reduce it to NO. This oxido-reductase activity could be catalytic if an effective reduction system exists. The nature of the physiological Cygb reducing system is unknown although it has been proposed that ascorbate and cytochrome b5 could fulfill this role. Here we describe that physiological concentraitons of cytochrome b5/cytochrome b5 reductase system can reduce human and fish Cygbs at rates up to 250-fold higher than their known physiological substrates, hemoglobin and myoglobin and many times faster, as well as more thoroughly, than even high levels of ascorbate. These data suggest that the cytochrome b5/cytochrome b5 reductase system is a viable reductant for Cygb in vivo, allowing for catalytic oxido-reductase activity.

Adjuvant Statin Therapy Efficacy is dictated by Tumor Dormancy and Statin Lipophilicity in ex vivo and in vivo Models of Metastatic Breast Cancer

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Metastasis in breast cancer patients heralds mortality, as disseminated disease is generally chemoresistant. After tumor cells reach the ectopic tissue, they undergo an epithelial reversion to enter a period of quiescence, termed dormancy, which may last for decades before outgrowing again as mesenchymal/dedifferentiated masses. Thus, long-term, relatively non-toxic interventions that prevent metastatic outgrowth are needed to treat this mortal stage of tumor progression.

Epidemiological analyses have suggested that statin usage, for cardiovascular indications, is correlated with a reduction in metastatic emergent (though not in incidence of primary) breast cancer. The goal of this study is to demonstrate this is due to statins suppressing breast cancer cell proliferation, a hallmark of emergent outgrowth.

We have found that atorvastatin and simvastatin limit the growth of some cancer cell lines, but not others. The sensitive lines were marked by lacking surface E-cadherin, the hallmark of the mesenchymal phenotype. Furthermore, this is a direct effect, as we now have shown that hydrophilic statins are relatively ineffective compared to the membrane permeant lipophilic statins.

To determine whether the statins target emergent metastatic tumor cells, we are using an all human microphysiological system of the most common site for metastases, the liver. Briefly, a micro-hepatic tissue is established by seeding primary human liver cells in a porous scaffold subject to a physiological flow. RFP-labeled breast cancer cells are seeded into these microtissues and examined weeks later. Initial studies suggest that statins suppress the emergence of dormant tumor cells when challenged by stressors that lead to outgrowth. Statins were further found in vivo to influence breast cancer metastasis and metastatic growth using a mouse model of spontaneous liver metastases. As 26% of adults currently take a statin for other medical conditions, these studies may suggest the best statin to use in the context of maintaining breast cancer dormancy long-term and delaying or avoiding the morbid emergence.

Computer Aided Design and Evaluation of a Novel Stem Cell Therapy for Small Abdominal Aortic Aneurysms

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Exsanguination from abdominal aortic aneurysm (AAA) rupture is frequently fatal and is currently the 13th leading cause of death in the United States of America. AAAs can take years to progress to the point where surgical intervention is recommended (> 5.5 cm diameter). Our goal is to develop a novel stem cell therapy for small AAAs. Approximately 90% of patients with AAA do not meet the size criterion for intervention and could benefit from this alternative therapy.

Our proposed strategy is delivery of adipose-derived mesenchymal stem cells (ADMSCs) to the external surface of the AAA. In this way autologous cells can be isolated from a patient, culture-expanded if necessary, mixed in a hydrogel, and injected around that same patient's aorta in a minimally invasive procedure.

Aspects of this treatment paradigm were evaluated in-silico, in-vitro, and in-vivo. We found that allowing for elastin production reduces the aneurysm enlargement rate using in-silico models of aneurysm growth and remodelling. We also found that adult smooth muscle cells produced a mature elastin network when co-cultured with ADMSCs in-vivo. Lastly, we found that delayed, periadventitial delivery of ADMSCs halted two aspects of aneurysm progression – expansion of the aortic diameter and fragmentation of the elastic lamella – in an elastase perfusion mouse model of AAA.

The results of these studies show promise for using ADMSCs as a possible AAA therapy by stimulating new elastin production by adult SMCs.

Different miroRNA expression in MSC-derived exosomes: IPF patients and age-matched normal individuals

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Purpose: We aimed to observe the difference between exosomes isolated from mesenchymal stem cells (MSCs) from both healthy subjects and patients with Idiopathic Pulmonary Fibrosis(IPF).

Methods: Bone-marrow MSCs from vertebral bodies were isolated from IPF and age-match normal subjects. When the cells grow to 80-90% confluence, cell culture medium was replaced with MEM media without fetal bovine serum, differential centrifugation and 18 hours density gradient ultracentrifugation was used to isolate and purify the exosomes. The morphological features of exosomes were examined by Transmission Electron Microscope (TEM). MSCs derived-exosome RNAs were extracted by SeraMir Exosome RNA kit. MicroRNAarray were used to analyse different expression of microRNAs in exosomes from IPF patient's MSCs and normal subject's MSCs.

Results: The density of the gradient after 18 hours of ultracentrifuge is 1.020, 1.030, 1.042, 1.053, 1.066, 1.088, 1.104, 1.121, 1.182, 1.212, 1.251g/ml. Detected by their HSP70 expression, microvesicles were enriched in the Fraction 3, 4, 5, 6, 7 and exosomes were enriched in Fraction 3, 4, 5, 6. The corresponding floating density of exosomes is from 1.042g/ml to 1.088g/ml. TEM showed the exosomes were cup-shaped and the diameters were around 30-100nm. Bioanalyzer showed that MSCs have intact 18S and 28S ribosomal RNA but MSCs derived-exosomes contain little or no ribosomal RNA, instead of a lot of microRNA and mRNA. ExosomesRNA from IPF patient's MSCs express different microRNAs compared with normal subject's MSCs.

Conclusion: IPF patient's MSCs-derived exosomes differ both morphologically and in miroRNA expressions from exosomes present in normal subject's MSCs. Further studies need to be completed to elaborate functional differences between IPF and normal subject's MSCs.

Modified mesenchymal stem cells using miRNA transduction modify lung fibrosis progression

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Background/Objectives: .Although different preclinical models have demonstrated a favorable role of bone marrow derived-mesenchymal stem cells (B-MSCs) in preventing fibrosis, this protective effect is not observed with late administration of B-MSCs, when fibrotic changes are consolidated. The possibility of modify B-MSCs to prevent deleterious effects or even enhancing their therapeuthic properties could be relevant in their future potential therapeuthic use. We sought out to investigate if the modification of B-MSCs using miRNAs let-7d (antifibrotic) and miR-154 (profibrotic) could modify their ability to alter lung fibrosis in a murine bleomycin model

Methods: Concentrated let-7d, miR-154 miRNAs or a control sequence lentiviral vector were transduced into human B-MSCs. These modified B-MSCs were intravenously administered to mice at day 7 day after bleomycin instillation. Mice were sacrificed at day 14. Different epithetial/mesenchymal markers in B-MSCs were assesed. In addition, the effect of modiefied B-MSCs in physical signs and diferent lung fibrosis markers on mice were also evaluated.

Results: B-MSCs were successfully modified and overexpressed let-7d and miR-154 after transfection. Although no reverse in lung fibrosis was observed, bleomycin-injured animals that were treated with let7d- B-MSCs were found to have an improvement in weight and a reduction in collagen mRNA levels in lung tissue, what suggests a decrease in activity of lung fibrosis progression. This positive effect was probably associated with the changes of B-MSCs epithelial/mesenchymal properties after miRNA modification. Treatment with miR154 modified-BMSCs not only did not have a beneficial effect but miR-154 group had the worst survival.

Conclusions: Our results establish the use of modified B-MSCs with mi-RNAs as a potential future treatment in lung fibrosis.

Biodistribution of [F-18] FDG-labeled adult bone marrow-derived stem cells with PET/CT scan in an ARDS sheep model

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Acute respiratory distress syndrome (ARDS) is an acute, inflammatory lung injury with mortality rates around 40-60% in critically ill patients. To date, no specific treatment strategy exists. Human stem cells have emerged as a promising therapeutic strategy. Early results from our group and others have shown the efficacy and safety of the use of human stem cells in LPS-induced ARDS in sheep when administered by endo-bronchial or intravascular route. As part of the understanding of their molecular mechanism by which they are therapeutically beneficial, the evaluation of biodistribution, migration and homing in vivo of stem cells are needed. Noninvasive tracking method using Positron Emission Tomography (PET) has been successfully used for in vivo tracking of [18F]-FDG labeled cells. Here we aimed to compare in vivo human stem cell trafficking, their tissue tropism and their engraftment to the targeted organ lung, using bone marrow derived multipotent adult progenitor cells (MAPC®), comparing two methods of administration: endo-bronchial (EB) and intravenous (IV), in a sheep model of LPS-induced lung injury.

Sheep were kept under anesthesia with 100% oxygen. A dose of 5 µg/Kg E. coli endotoxin was infused over 21 minutes to induce lung injury. Arterial blood gases were measured before endotoxin, 1 hr, 2 hr, 4 hr, and 6 hr after endotoxin infusion to determine the onset of the acute phase of hypoxemia. MAPC cells were labeled with [18F]-FDG for an hour and delivered EB at a dose of 1 mill./Kg or IV at 10 mill./Kg one hour after the infusion of endotoxin. Computed Tomography (CT) and PET scans were acquired to evaluate injury and biodistribution of the cells. The free [18F]-FDG was also evaluated in both routes. Plasma samples were collected to evaluate toxicity.

Arterial blood gases demonstrated a decrease in oxygen levels as a consequence of injury induced by LPS and complete recovery of pO2 to normal levels after administration of MAPC cells with no significant differences observed between the two routes used to administer the cells. Neither of the two groups showed a deleterious effect in kidney and liver function. The [18F]-FDG labeled MAPC administered by EB route were found mainly in the compartment in which the cells were administered with a minimal fraction remaining in the upper airway and no changes in the distribution over 5 hours were observed. After IV injection of [18F]-FDG labeled MAPC, the main organ of cell uptake was the lung with re-distribution after 5 hours. Bio-distribution of [18F]-FDG labeled MAPC and free [18F]-FDG MAPC was administered IV in no-LPS and LPS-lung injury model, at 1 and 6 hours after LPS Injection. Administration of [18F]-FDG labeled MSCs resulted in significantly higher accumulation of radioactivity in the lungs compared with free [18F]-FDG. A high amount of activity was detected in the kidneys and brain.

The endo-bronchial and intravenous routes of administration of MAPC cells are equally effective for treatment of the ARDS sheep model. After endo-bronchial administration the MAPC cells do not migrate through the alveolar epithelium and remain entrapped within the bronchiole after six hours, suggesting that their effect was not mediated by integration into the lung tissue or systemic bio-distribution. The bio-distribution of MAPC cells after intravascular instillation is similar to previous reports. However, the bio-distribution was heterogeneous among the lobes and it was further aggravated by the variation in the lung volumes, structural alterations (hyperdensities).

Deficiencies in Mesenchymal Stem Cells from Idiopathic Pulmonary Fibrosis Patients Result in Lower Capacity to Protect the Lung from Injury

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RATIONALE: Aging is a natural process characterized by progressive functional impairment and reduced capacity to respond to environmental stimuli. The incidence of idiopathic pulmonary fibrosis (IPF) increases with age. It is plausible, therefore, that the abnormal regulation of the mechanisms of lung repair that characterizes aging contributes to the pathobiology of IPF.

We aimed to determine some of the differences in the biological and functional characteristics of bone marrow derived mesenchymal stem cells (B-MSC) of normal individuals and IPF patients in the same age range.

METHODS: B-MSC were obtained from vertebral bodies, procured from normal controls and IPF patients. Mitochondria were examined under transmission electron microscopy and their length and area were quantified and their respiration and glycolytic rates were measured by Seahorse XF Analyzer. Mitochondrial import receptor subunit Tom20 expression was determined by western blot and Mitotracker Deep Red staining was quantified to assess mitochondrial mass. Senescence was assessed by β-galactosidase assay and quantification of p21 mRNA expression which was determined by qRT-PCR. Telomere length was measured by FISH assay. Cell number in the proliferation assays was quantified by DNA staining. These cells were used in a murine model of bleomycin-induced lung injury and their ability to protect against fibrosis was measured.

RESULTS: We demonstrate that cells obtained from IPF patients are functionally defective with changes in mitochondrial morphology and function. IPF B-MSC mitochondria are smaller in size, exhibit decreased oxygen consumption rate and are less glycolytic than the B-MSC from age-matched healthy individuals. These data correlate with reduced total ATP production and lower ROS generation per mitochondrion, albeit a slightly higher mitochondrial content. In addition, IPF B-MSC show signs of accelerated senescence by β-Gal staining, with shorter telomere length, increased expression of senescence marker p21 and have longer doubling times. In functional studies, IPF B-MSC and their age matched controls were less effective than B-MSC from young controls in preventing fibrotic changes observed after bleomycin-induced lung injury in mice.

CONCLUSION: B-MSC from IPF patients have important differences in mitochondrial morphology and function that result in defects in critical cell functions when compared to age-matched controls. IPF B-MSC show signs of accelerated senescence which could suggest a link between aging and the late onset of the disease. Impaired function of B-MSC may have a role in the pathobiology of IPF.

The use of GMP-produced B-MSC Cells in Combination of Extracorporeal Membrane Oxygenation or Hemolung (Extracorporeal CO2 Removal) in ARDS in Combat Casualties

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Transfer of injured service members from the Level 3 combat support hospital to level 4 and 5 medical facility increases their chance of survival from devastating injuries. Aeromedical evacuation of patients with Acute Respiratory Distress Syndrome (ARDS) is sometimes beyond possibility due to limitations in providing ventilator support in flight with a possible further deterioration in patient status. Cell based therapy with adult bone marrow-derived mesenchymal stromal cells (B-MSC) in experimental models of ARDS has been the focus of intense investigation. Data suggest that administered allogeneic B-MSCs can mitigate hypoxemia in ARDS and promote recovery. However, it is unknown how this new form of therapy can be used as an adjunct to current supportive measures for lung failure.

Our objective is to complete a series of preclinical studies in a large animal model using extracorporeal membrane oxygenation (ECMO) or minimal invasive extracorporeal CO2 removal (Hemolung), alone or in combination with B-MSC in sheep with LPS-induced ARDS.

We will use a model to assess the efficacy of ECMO/Hemolung with B-MSCs and B-MSCs alone: A sheep model of LPS-induced ARDS (short-term support). Human B-MSCs will be generated from a single healthy normal adult donor by Athersys Co. We will utilize up to 50 sheep for the proposed two-year study.

Mesenchymal Stem Cells Drive Cell Repopulation In An In Vivo Model Of Lung Regeneration

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Rationale: The use of bone marrow mesenchymal stem cells (B-MSCs) to promote the recruitment of endogenous cells to a decellularized scaffold provides a novel approach for the generation of a functional organ for clinical use. Lung transplantation remains the only accepted treatment for end-stage lung, diseases however, long wait list times and scarcity of acceptable donor organs result in nearly 400,000 deaths per year for patients awaiting transplant. These reasons underscore the need for novel approaches to increase the number of organs suitable for transplant.

Methods: Lungs from C57BL/6 wild type mice were decellularized in situ by perfusion of the pulmonary vasculature. In short, the pulmonary artery was cannulated through the right ventricle and the vasculature perfused with PBS, water and SDS. The matrix was then seeded with GFP B-MSCs and heterotopically transplanted into the dorsum of wild type mice for 1 month. Lungs containing DMEM were implanted on the opposite side of the dorsum of the same mouse to serve as an internal control. Alternatively, lungs were seeded with fibroblasts and also placed in the dorsum of mice as a positive control. Revascularization of implanted lungs was imaged using two-photon microscopy prior to tissue retrieval. To determine the cellular makeup of the recellularized tissue, histological and immunofluorescent staining, qPCR and flow cytometry were used.

Results: Lungs seeded with GFP B-MSCs and heterotopically placed in recipient mice exhibited macroscopic re-vascularization confirmed by two-photon microscopy compared to control lungs. Markers for CD45, CD4, CD8, CD19, GR1, CD11b, Cd73, CD44, CD106, Ter119, Cd31 and cytokeratin were used in both IF and flow cytometry to confirm the presence of endothelial, epithelial and smooth muscle as well as immune cells in lungs seeded with GFP B-MSCs compared to control lungs. A lack of co-localized GFP signal with cells indicates cells where recruited to the matrix from the recipient mouse, not differentiated GFP B-MSCs.

Conclusions: These results indicate that decellularized lung matrix seeded with B-MSCs, serves as a viable scaffold for the recruitment of specific types of cells that will generate a functional and viable organ for transplant. Lack of co-localization of the GFP signal with cell markers and flow cytometry data indicate that repopulation of the decellularized matrix is by mesenchymal stem cell mediated recruitment of endogenous cells. Further studies are needed to interrogate the signaling pathways involved in this process.

A Phase 1/2 Study to Assess the Safety and Efficacy of MultiStem® Therapy in Subjects with Acute Respiratory Distress Syndrome

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ARDS is a common clinical condition and a major cause of morbidity and mortality in the critical care setting. Historically, ARDS has been associated with mortality ranging from 25% to 45%, with worse outcomes in the elderly population. According to the ARDS Foundation, the annual incidence of ARDS is 190,000. No drug treatment exists for ARDS and recovery from ARDS is a slow process. Only 34% of ARDS survivors are well enough to be discharged directly home which means extended rehabilitation in skilled nursing facilities. The treatment of moderate to severe ARDS therefore represents an unmet medical need for effective therapies. The primary objective of this trial is to evaluate the acute safety and tolerability of MultiStem therapy as a treatment for subjects with ARDS. A single dose of MultiStem Therapy (300 or 900 million cells) will be administered by intravenous (i.v) infusion. and cohort 3, control, is the vehicle in which MultiStem therapy is administered. Our primary objective of this trial is to evaluate the acute safety and tolerability of MultiStem therapy as a treatment for subjects with ARDS. The secondary objectives of this trial are to evaluate in a longer term: safety, tolerability, efficacy, pulmonary function and mortality of MultiStem therapy as a treatment for subjects with ARDS.

Cell-spray autografting of deep partial-thickness burns: A critical review of 44 patient applications

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Cell-spray autografting is an innovative treatment for the early treatment of deep- partial thickness burn wounds using isolated, non-cultured, adult, and, epidermis/ dermis-derived stem cells. This technique offers an improvement over non-operative management, particularly with larger wounds, while avoiding the need for large donor sites (donor-site to burn-wound surface ratio of 1:100) known for mesh grafting to achieve rapid wound reepithelialization. Our clinical routine includes donor area harvesting, cell isolation from the donor tissue, and spray grafting of single cells, including adult stem cells from the epidermis and dermis. We present data on 47 cell isolation procedures in 44 patients with deep partial-thickness burns performed over the last five years. The patients treated with cell-spray autografting presented with various etiologies and a wide range of TBSA. The average hospital length of stay following treatment was seven days. The focus of this review is to provide an analysis of technical problems, pitfalls, and solutions specific to the cell isolation procedure. We hope that presenting our data here may help to plan future clinical studies.

PCR-Electrospray Ionization Mass Spectrometry in Identifying Microbial Infections in Burn Wounds

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Background: Infection remains the major complication associated with burn injury and remains a cause of death. Managing burn wound infection is challenging and would benefit from early detection of microbes so as to initiate appropriate therapy.

Hypothesis: We hypothesized that molecular examination of the microbial DNA contents of burn wounds may be able to better and more speedily identify burn wound bacteria/pathogens versus routine culture-based methods.

Methods: Tissue samples from burn wounds were obtained from 141 patients that underwent first time surgical debridement at more than one body site (n=316). Tissues were analyzed by standard microbiological culture and compared to a novel culture-independent PCR/electrospray-ionization-mass spectrometric (PCR/ESI-MS) assay after genomic DNA isolation. Demographics, complications, and outcome data were prospectively collected during recruitment and also from the electronic medical records. Parametric and non-parametric analyses were used along with logistic regression. All tests were two sided with $\alpha=0.05$.

Results: PCR/ESI-MS analyses identified far greater numbers of microbial organisms resident in burn wounds compared to standard culture methods. Of the 316 patient samples analyzed, 80 derived from sites with clinical evidence of burn wound infection, of which 10 showed microbiological concordance between PCR/ESI-MS and standard culture methods, approaching statistical significance ($p=0.07$). The result of multivariate logistic regression showed that a model with six independent variables was statistically significant $P<0.0001$. The strongest predictor of burn wound infection was PCR/ESI-MS, OR=8.6, $p=0.007$, followed by concordance in microbiological identification between PCR/ESI-MS and culture. Wound culture alone is also a statistically significant predictor, along with degree of burn and high age-adjusted Charlson Comorbidity Index.

Conclusions: Our results indicate that using PCR/ESI-MS in identifying microbial pathogens could be a test of merit to complement standard microbiological wound culture that can be developed to identify patients that are at high risk for burn wound infection.

Small molecule-mediated inhibitions of transcriptional cofactor MKL and its downstream target profilin impedes endothelial cell migration and angiogenesis

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Angiogenesis is a fundamental mechanism of neovascularization that when dysregulated contributes to progression of many diseases including cancer. Current anti-angiogenic therapies often have limited success in clinical settings due to functional compensation by alternative pro-angiogenic pathways after selective blocking of one pathway and/or toxicity effects. Therefore, there is a critical need to discover new anti-angiogenesis targets and translational strategies. De novo synthesis of cytoskeleton-regulatory proteins triggered by the MKL (megakaryoblastic leukemia)/SRF (serum response factor) transcriptional system in response to pro-angiogenic growth factors lies at the heart of endothelial cell (EC) migration (a critical element of angiogenesis) and physiological/pathological neovascularization. In this study, we demonstrated that a small molecule inhibitor of MKL inhibits migration and angiogenic ability of microvascular EC in vitro and developmental angiogenesis in vivo. Our next goal was to identify key cell motility- and angiogenesis-promoting genes regulated by MKL pathway. To this end, we discovered that profilin (Pfn) family of actin-binding protein (an essential regulator of actin dynamics) is a major target of MKL, but surprisingly MKL-dependent regulation of Pfn does not require its traditional SRF-related activity. Through loss-of-function studies utilizing RNAi and conditional EC-specific gene deletion, we further demonstrated that Pfn is an essential molecular player of angiogenesis. Finally, we performed structure-based virtual screening followed by biochemical assays to identify novel first-generation inhibitors of Pfn that are able to inhibit migration and angiogenic ability of EC mimicking the genetic loss-of-function phenotypes. In conclusion, these findings provide a conceptual foundation for novel anti-angiogenic strategies that involve functional inhibition of a major transcription system and its downstream cytoskeletal target, setting a stage for further preclinical evaluation of these inhibitors in disease settings.

Omental Milky Spots as Sites for Ectopic Liver Development

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Liver transplantation, currently the only curative treatment for patients with end-stage liver diseases, is greatly limited by the shortage of available donors. Hepatocyte transplantation has been proposed to be an alternative approach to liver transplantation in treating end-stage liver diseases. However, most of the research has focused on cell engraftment in the diseased liver where hepatocyte survival and/or proliferation is limited due to fibrosis and cirrhosis. Therefore, hepatocyte transplantation at ectopic locations could facilitate the development of functional liver tissue.

We previously demonstrated that transplantation of hepatocytes in a lymph node generate an ectopic liver that rescue mice with lethal metabolic disease. Here we show that, besides lymph nodes, hepatocytes can engraft in omental milky spots as well, where they proliferated and formed multiple ectopic liver nodules that rescued the mice with lethal metabolic disease. How hepatocytes engineer a functional ectopic liver in vivo at lymphatic sites, and what is the cellular and molecular mechanism responsible for this liver organogenesis, is unknown. We hypothesize that hepatocytes borrow some of the molecular mechanisms lymphocytes use to interact with the lymphatic system.

As a first step to understand the cellular mechanism involved, we transplanted FRGN mice (Fah-/-/Rag2-/-/Il2rγ-/-). In FRGN mice, omental milky spots are lacking due to the absence of γc expression. Transplanted hepatocytes in FRGN mice still engrafted in the omentum but failed to expand to form ectopic livers. Next, milky spots were restored in FRGN mice by infusion of wild type bone marrow cells. Ectopic livers formed in the restored omental milky spots that prevented mice from dying of liver failure. This result indicates the importance of the hematopoietic system, directly or indirectly, influence the development of ectopic liver in milky spots.

As another step to understand the molecular mechanism responsible of ectopic liver growth, we used the alymphoplasia (aly) mice. These mutant mice have a point mutation in the NF-κB inducing kinase (NIK) that disrupts non-canonical NF-κB signaling pathway and lymph node development. We show that even though there are omental milky spots in aly mice, the engrafted hepatocytes had very limited expansion and were not able to form ectopic livers to rescue the mice from liver failure. This result suggested a role of the NIK pathway in ectopic liver development.

Collectively, omental milky spots are unique structures in the peritoneal cavity that facilitate liver development and we show that both a cellular mechanism with hematopoietic cells in FRGN mice, and the NIK pathway in aly mice are involved in ectopic liver organogenesis.

Modulating Macrophage Dynamics to Improve Autologous Fat Grafting Outcomes

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Fat grafting has shown great potential for soft tissue repair for tumor resection, trauma, burn, and congenital tissue loss. Published surveys show that 62% of US Plastic Surgeons use fat grafting techniques for breast reconstruction despite continued variability in patient and physician satisfaction. We propose that inflammatory biomarkers can be measured preoperatively to predict patient capacity for macrophage polarization and likelihood of long term fat graft success. We intend to test Calcitriol, a FDA-approved drug with known immune-modulatory capacity for improving long term fat graft outcomes. To design a culture system for adipose particles, we assessed the impact of three experimental parameters on upregulation of hypoxia-related genes and proteins to determine optimal experimental conditions for future drug screening studies. Fat particle size (300mg, 600mg, or 900mg), hypoxic oxygen concentration (1% or 2.5%), and culture period (24 or 48 hours) were assessed. Results showed that 600 mg and 900 mg particles depleted all the available free fatty acids by 48 hours and therefore significant differences between the experimental groups were not detectable. There was a significant difference in free fatty acid concentration of normoxic vs 1% hypoxic groups ($p < 0.05$, $n = 5$ per group) for 300mg particles, therefore future studies will be conducted using fat particles in this size range. Adipose tissue cultured in 1% oxygen has significantly increased CD68 macrophage gene expression and CA9 expression. Hypoxia-induced apoptosis is significantly higher in samples cultured in 1% oxygen compared with 2.5% or 8% oxygen ($p < 0.05$). Our data supports the hypothesis that adipose tissue has unique capacity to respond to hypoxic stress with variable apoptotic protective gene expression. We conclude that additional patients should be tested in our culture system with the experimental drug, Calcitriol.

Chloroquine, a pharmacological inhibitor of autophagy, attenuates hypoxia-induced pulmonary hypertension

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Autophagy is an important biologic process involved in maintaining cellular energy homeostasis. While its importance in diseases, such as cancer, has been well established, its role in other proliferative diseases, such as pulmonary arterial hypertension (PH), is just emerging. We hypothesized that autophagy contributes to the development of PH in a chronic hypoxia (CH) induced PH mouse model and that pharmacological inhibition of autophagy by chloroquine (CQ) will attenuate the disease. In this study, mice were exposed to 3-weeks of CH and treated daily with either chloroquine or saline. At the end of exposure, PH was assessed by measuring right ventricular pressure, obtaining right ventricular hypertrophy, and determining pulmonary vascular remodeling. In vitro experiments included cytotoxicity and proliferation studies in human pulmonary vascular cells in normoxia/hypoxia +/- CQ. Autophagy was assessed by Western blot analysis, LC3-staining and electron microscopy.

During the course of CH, the lungs of the hypoxic control mice showed an increase in autophagic flux. Daily CQ treatment of hypoxic mice, however, prevented the development of PH compared to mice receiving saline injections. The CQ treated mice displayed lower RV pressures, hypertrophy, and decreased pulmonary remodeling. We further determined that the increase in autophagy correlates with increased levels of the DAMP HMGB1 observed in hypoxic mice. In vitro experiments showed that the beneficial effects of chloroquine on hypoxia-induced PH involved the potent inhibition of hypoxia-induced cell proliferation with concurrent reduction of endothelin-1 release into cell media. In conclusion, we demonstrate here that autophagy, at least partially induced by HMGB1, contributes to the development of PH and that its pharmacologic inhibition prevents it creating a potential new therapeutic target.

Stat3 regulates hepatocyte and biliary epithelial cells proliferation and hepatocyte maturation in the zebrafish hepatic progenitor cell (HPC)-driven liver regeneration model

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The only definitive treatment for end-stage liver disease is liver transplantation. Unfortunately, the demand for liver transplants exceeds the availability of donor livers, underpinning the need for harnessing the liver's innate regenerative capacity. Following acute liver injury, regeneration manifests as either: (1) hepatocytes regenerating to restore the lost mass or (2) if hepatocyte proliferation is compromised, biliary cells (BECs) proliferating and contributing to the regenerating hepatocytes. To induce hepatic progenitor cell (HPC)-driven liver regeneration, our lab generated the zebrafish transgenic line, Tg(fabp10a:CFP-NTR), in which hepatocytes can be specifically and genetically ablated. Consequently, BECs de-differentiate into hepatoblast-like cells (HB-LCs), proliferate and then re-differentiate into newly generated hepatocytes, eventually proliferating to restore the liver mass. During this regenerative process, inflammatory pathways are considered important regulators of the HB-LC response as increased cytokine production is linked with HB-LC proliferation. One of the downstream mediators of inflammatory cytokine signaling is the evolutionarily conserved transcription factor, signal transducer and activator of transcription 3 (Stat3). The binding of certain cytokines, such as IL -6, to its respective receptor activates JAKs, which subsequently phosphorylate and activate Stat3; once active, Stat3 dimerize, translocate to the nucleus and activate downstream target genes. Using the zebrafish liver regeneration model, the purpose of this study is two-fold: (1) to elucidate the role of Stat3 and (2) its downstream targets in HPC-driven liver regeneration. In the regenerating liver, stat3 expression was upregulated. When Stat3 activation was blocked with a chemical inhibitor, JSI-124, HB-LC induction occurred normally. However, hepatocyte maturation was delayed and the number of mature BECs was significantly reduced in inhibitor-treated regenerating livers, manifesting in a reduction of liver size. The reduced liver size was due to diminished hepatocyte and BEC proliferation, as shown by EdU staining. Interestingly, JSI-124 treatment caused no significant difference in cell death. In addition, the defective intrahepatic biliary network in JSI-124-treated livers resulted in the loss of proper liver function. Stat3 homozygous mutants also displayed a lower number of BECs and aberrant hepatocyte maturation. For future studies, we will conduct qPCR analysis to elucidate downstream targets of Stat3 and examine mutants of socs3a, a Stat3 negative feedback regulator, in HPC-driven liver regeneration.

Retinal and optic nerve viability evaluated with optical coherence tomography, manganese-enhanced MRI and electroretinography after whole eye transplantation

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Purpose: The purpose of this study was to evaluate the viability and structural integrity of the visual system in a rodent whole eye transplantation (WET) model with in vivo optical coherence tomography (OCT), manganese-enhanced MRI (MEMRI) and electroretinography (ERG).

Methods: We performed syngeneic orthotopic WET in 9 Lewis rats. 7 rats underwent OCT at 1 week and MEMRI at 3 weeks after WET. ERG was performed at 7 weeks in 2 separate animals to evaluate retinal function.

Results: OCT revealed visualization of retinal layers in five of the seven transplanted eyes. Corneal opacities of the other two rats prevented visualization. Results from MEMRI showed significantly increased signal post-Mn injection at all the levels of visual pathway, from the left, untreated eye to the right visual brain centers compared to pre-Mn injection. Mn-enhancement was also observed in the right, transplanted donor eye and intraorbital ON when compared to the eye and visual system prior to Mn injections. No apparent signal difference was observed between naïve and transplanted intraorbital ON post-Mn injection. No apparent difference in Mn-enhancement was observed between pre- and post-Mn injection in the right recipient prechiasmatic ON, left lateral geniculate nucleus or left superior colliculus. ERG data revealed the presence of small a- and b-waves in response to stimulating the retina with light in the transplanted eyes.

Conclusions: Qualitative analysis of OCT revealed the existence of retinal layers after WET. The presence of anterograde Mn transport in the donor eye to the site of transection at approximately three weeks after WET was seen in MEMRI. ERG data suggested a small response to light stimuli in the photoreceptors of the transplanted eyes after WET. Viability of retina and ON found in transplants allows the potential for future therapeutic strategies for neuroprotection, optic nerve regeneration and restoration of vision.

Small Molecule Therapeutics Targeting Ubiquitin E3 Ligase

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Cellular protein abundance is higly regulated by the ubiquitin proteasome system that eliminates proteins by selective degradation either by the proteasome or via the lysosome. The ubiquitin conjugation to the target protein is orchestrated by an enzymatic cascade involving an E1 ubiquitin activating enzyme, an E2 ubiquitin conjugating enzyme, and an E3 ubiquitin ligase that generates an isopeptide bond between the C-terminus of ubiquitin and the substrate's ε-amino lysine. There are more than 1,000 E3 ligases classified into three families: Complex type E3 ligase, HECT E3 ligase and RING-finger E3 ligase. These E3 ligases are gaining attentions through their diverse roles in cancer, inflammation and metabolic disorders. Through unbiased screening, our laboratory identified a series of E3 ligases targeting substrates involving in cell cycle progression, inflammatory response, mitochondria function and metabolism. We then performed computational simulation based screening and developed a series of E3 inhibitors interrupting the association between E3 ligase and its substrate. We further tested the efficacy of these small molecule compounds in mice acute lung injury model, mice bleomycin induced idiopathic pulmonary fibrosis model and obese induced diabetic model. These small molecule inhibitor projects are currently under further development.

Extracellular Matrix and Spermatogonial Stem Cell Culture

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Successful human spermatogonial stem cell (SSC) culture could enable therapies restoring fertility in males subjected to chemotherapy or radiation therapy. Conditions for expanding rodent SSCs in culture are established and robust, but human SSC cultures are in early development and no human SSC culture system has yet been independently verified. Mammalian extracellular matrix (ECM) contains signaling molecules promoting mitogenesis, migration, and/or differentiation of various stem/progenitor cells which we hypothesize will improve human SSC cultures. Human testicular ECM (htECM) and porcine small intestinal submucosa ECM (SIS) were prepared and solubilized as a media supplement for culture experiments. SSCs were cultured on STO or C166 feeder cells, Matrigel, murine or human laminin, or human laminin with htECM or SIS. Cells were passaged at day 7 and stained for the SSC marker UTF-1 at 0, 7, and 14 days to identify the percentage of UTF1+ cells relative to day 0. By 7 days the wells with feeder cells, Matrigel, or murine laminin showed ~45% the number of starting UTF1+ cells, whereas wells with htECM or SIS showed 77% and 187%, respectively. At 14 days, wells with feeder cells, Matrigel, and murine or human laminin showed ~20% the number of starting UTF1+ cells, whereas wells with htECM or SIS showed 59% and 114%, respectively. ECM appears to improve SSC survival in culture and establishes a foundation for development of robust human SSC cultures that will be valuable for fundamental investigations and may have application for treating some cases of male infertility.

Establishing efficacy for a combination of physical and cell therapy for stroke

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Stroke is caused by the occlusion of a cerebral artery and results in death or severe, long-lasting sequel. Treatment options, however, are extremely limited, extensive, and cost-intensive without the guarantee to restore lost functions. Alternative approaches, such as stem-cell based therapies, are promising and can support tissue regeneration and functional recovery. Despite this, the restoration of sensory and motor deficits is still not complete and requires further enhancement. Of note, physical therapy (PT) is known to also improve behavioral functions. Thus, the aim of this study was to evaluate the efficacy of a combination therapy (CoT) consisting of human Neural Stem Cell (NSC) transplantation and PT for improved recovery in a model of stroke.

Adult male Sprague-Dawley rats underwent transient middle cerebral artery occlusion (MCAO). Success of MCAO was determined by T2-weighted magnetic resonance imaging (MRI) and animals were randomly assigned to groups (MCAO only, MCAO + NSC, MCAO + PT, MCAO + CoT; 4-5 rats/group). Sham-operated animals served as healthy control. Rats subjected to MCAO+NSC or MCAO+CoT groups received a peri-lesional NSC graft 2 weeks after MCAO. Experimental groups with PT underwent daily physical training. Functional deficits and improvements were followed over a time course of 12 weeks using a battery of behavioral tests, including maximal capacity test, bilateral asymmetry test, foot fault test, rotameter, and grip strength test. Serial MRI was conducted throughout the experiment, including diffusion tensor imaging (DTI), cerebral blood volume (CBV), and functional MRI (fMRI) based on electrical forepaw-stimulation in the concluding imaging session. After animal perfusion, brains and upper forelimb muscles were collected for histology. Behavioral assessment revealed functional improvements after PT, NSCs and CoT. CoT is a promising approach to further enhance the efficacy of NSCs and to improve the restoration of lost functions.

Stem Cell Resistance to Endoplasmic Reticulum Stress and its Implications for Glaucoma Treatment

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Glaucoma is an eye disease that can damage the optic nerve and result in vision loss. Elevated intraocular pressure is the major risk factor of glaucoma and it is associated with reduced cellularity of the trabecular Meshwork (TM). The mechanism by which cellularity is reduced in the TM is unknown. In glaucoma patients, mutant myocilin is not secreted and it accumulates within the endoplasmic reticulum (ER). The research aim was to develop an open angle glaucoma model demonstrating reduced cellularity of the TM due to ER stress with implications for stem cell therapies. It was hypothesized that TM stem cells (TMSCs) would be more resistant to ER stress than TM cells so that TMSCs would survive in a glaucomatous environment. TM cells and TMSCs were cultured and exposed to ER stress inducers (Thapsigargin, Tunicamycin, Brefeldin A) for 72 hours. The expression of ER stress markers in the cells were evaluated. Transmission Electron Microscopy revealed morphological changes in individual cells such as swollen and fragmented endoplasmic reticulum. Immonoflourescent staining revealed the increased presence of myocilin and GRP78 in ER stressed induced TM cells and TMSCs. Western blotting revealed the increased presence of the proteins BiP, GRP78 and PDI in ER stress induced TM cells and TMSCs. Quantitative analysis showed that the increase in TM cells was greater than in TMSCs. qPCR revealed a larger increase in the relative transcript levels of sXBP1 and Chop for ER stress induced TM cells than TMSCs. All of the data supports the idea that TMSCs are more resistant to ER stress than TM cells. The results from these in vitro experiments can be used to optimize the in vivo ER stressed induced glaucoma model in mice and indicate that stem cell-based therapy might be feasible for glaucoma treatment.

Lack of beta-catenin in hepatocytes impairs proliferation and promotes liver progenitor cell-mediated repair in response to the choline-deficient ethionine-supplemented diet

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Despite the liver's capacity for regeneration, liver disease is the 12th leading cause of death in the United States. Treatments for chronic liver disease are limited due to incomplete understanding of liver regeneration mechanisms. Typically, liver regeneration is mediated by proliferation of hepatocytes. When hepatocyte proliferation is impaired, liver progenitor cells (LPCs) are activated and are thought to mediate regeneration by differentiating into hepatocytes. However, the role and origin of LPCs remains controversial. The choline-deficient ethionine-supplemented (CDE) diet model of liver injury is known to induce proliferation of LPCs. However, since the CDE diet does not block hepatocyte proliferation, recent evidence has supported repair primarily driven by hepatocyte self-duplication in the CDE diet model. As a member of the WNT signaling pathway, beta-catenin plays an important role in liver regeneration by promoting hepatocyte proliferation. Therefore, we hypothesize that beta-catenin loss in hepatocytes would impair hepatocyte proliferation and lead to biliary-derived LPC-mediated hepatic repair in the CDE diet model. Mice that lack beta-catenin in both hepatocytes and biliary epithelial cells (BECs) display increased morbidity, mortality, and defective hepatocyte proliferation when compared to wild-type (WT) littermates after 2 weeks of CDE diet. To investigate whether liver regeneration is mediated by LPCs in this model, we performed genetic fate tracing in mice by utilizing adeno-associated virus serotype 8 carrying thyroid binding globulin-driven Cre (AAV8-TBG-Cre) to simultaneously delete beta-catenin and permanently label hepatocytes with EYFP (KO mice). Importantly, in this model BECs contain beta-catenin and do not express EYFP. When these mice were given two weeks of CDE diet they displayed increased liver injury and a lack of hepatocyte proliferation compared to beta-catenin WT littermates. Notably, in KO mice given two weeks of CDE diet followed by a two week recovery on normal diet we detected clusters of hepatocytes which expressed beta-catenin and did not express EYFP, indicating that they originated from the BEC compartment. We did not observe expansion of EYFP-negative hepatocytes in control mice where hepatocytes retained beta-catenin expression. Our results demonstrate that loss of beta-catenin in hepatocytes impairs hepatocyte proliferation after CDE diet-induced liver injury and supports the hypothesis that LPCs mediate liver regeneration when hepatocyte proliferation is blocked.

Platelet-Rich Plasma and Adipose Stem Cells Improve Burn Wound Healing in Yorkshire Pigs

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BACKGROUND: Full-thickness burns often result in hypertrophic scarring and contracture across joints. Adipose-derived stem cells (ASCs) and platelet-rich plasma (PRP) enhance neovascularization and epithelialization in excisional wounds, but data for burn wounds is limited. We sought to assess ASCs and PRP as treatment modalities for full-thickness burns.

METHODS: 40 burn wounds were created on the backs of two female Yorkshire pigs using an electric branding iron. Wounds were debrided 48 hours after injury and covered with STSG harvested from the flanks. Saline, autologous PRP (1.2*10^9 platelets/ml), low-passage allogeneic ASCs (48*10^6 cells/wound), or PRP + ASCs were injected into the superficial wound bed (n=8 wounds/group). Bolster dressings were applied for 7 days, followed by dressing changes 3 times a week. Wounds were assessed by surface area tracing and animals were sacrificed at 2 weeks and 4 weeks post-operative. At the time of sacrifice, wound biopsies were collected for histology and protein quantification via Western blot.

RESULTS: At 3 weeks post-grafting, wound contraction trended lower in ASC (14.8+/-9.8%; p=0.19) and PRP+ASC groups (19.1+/-4.1%; p=0.23) when compared to saline controls (27.7+/-9.9%) but did not reach statistical significance. When compared to saline control at 4 weeks, CD31 was significantly higher in groups receiving ASCs (10.1-fold increase, $p<0.01$) and trended higher in PRP and PRP+ASC groups (7.5-fold, p=0.08 and 11.6-fold, p=0.10 respectively). When compared to naïve, unwounded skin, CD31 content was significantly lower in the saline control group only (20-fold difference, p=0.05). CD31 content in groups treated with PRP, ASCs, and PRP+ASCs was much closer to unwounded controls, although it still trended lower by 2.7 (p=0.14), 2.0 (p=0.18), and 1.8-fold (p=0.26) respectively.

CONCLUSION: ASCs and PRP appear to reduce early wound contraction after skin grafting and elevate markers of neovascularization at 4 weeks in burn wounds. Longer studies are needed to fully evaluate wound contraction and scar remodeling.

Accumulation of Bone Marrow Derived Macrophages in the Aged Murine Liver

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Macrophages, members of the innate immune system, are heavily implicated in wound healing, response to infection, and cancer progression. Immunosenescence describes the dysregulation of immune cell function with aging, which preferentially effects tissue-resident macrophages compared to bone marrow derived macrophages. The liver contains 80% of the total mammalian tissue-resident macrophage population and therefore can serve as a reservoir to study macrophage aging. Recent findings suggest that liver macrophages can be divided into two F4/80+ subsets: CD68+ embryonically-derived Kupffer cells and CD11b+ bone marrow derived cells, which exhibit specific cell functions. It is currently unclear how macrophages residing in the liver are phenotypically and functionally impacted by aging, which may have implications for the onset and progression of inflammatory disease and liver pathology in elderly populations.

We characterized the liver macrophage compartment from young (8-10 week) and aged (18-20 month) C57BL/6 wild-type mice using immunohistochemistry and flow cytometry. First, we identified a significant increase in the total number of macrophages residing in aged livers. In addition, the aged macrophage population contained a greater proportion of CD11b+ cells, suggesting an increase in monocyte-derived macrophages residing in the liver. To confirm this, we examined the liver macrophage profile of aged CCR2 knock-out mice, which lack the receptor for monocyte chemoattractant protein-1 (MCP-1), an important signaling molecule for monocyte recruitment following injury. Interestingly, we found that mice deficient in CCR2 had a reversal in liver macrophage profile, from CD11b+ predominant to CD68+ predominant cell subsets, demonstrating that monocyte derived macrophages accumulate in the uninjured aged liver at least in part due to MCP-1. Furthermore, preliminary in vitro studies showed that aged macrophages were primed towards a higher phagocytic capacity, regardless of M1 or M2 stimuli, suggesting an inherent level of activation with aging that might exacerbate inflammatory disease in elderly populations. Future studies will further characterize the functions of the CD11b and CD68 liver-macrophages with aging.

Characterization of Tenascin-C signaling on Mesenchymal Stem Cells for Chronic Wound Healing Therapies

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Mesenchymal stem cells (MSCs) have already shown great promise in the wound healing process by their expression of paracrine factors as well as recruiting additional cell types to the wound bed. However, little is known about the effects of stem cell therapy on angiogenesis during skin wound healing. Angiogenesis is a crucial element during the tissue replacement/proliferation phase of wound healing. Vessels provide the wound bed with nutrients and oxygen needed to sustain high metabolic activity, the transportation of supporting cells such as peripheral blood monocytes, and the removal of debris. Our lab is examining how proximal signals from the extracellular matrix (ECM) could be used to influence MSC-mediated paracrine signaling during the wound healing process. In addition, we are exploring how the paracrine activity of the ECM-MSC interactions regulate the wound microenvironment in order to heal or prevent the onset of chronic skin wounds. We previously published that specific ECM components, such as the pro-survival and pro-motility stimulus TNC and the anti-survival and anti-migration factor Decorin, can act as physiological matrix switches during the wound healing process. Here we further characterize the ECM-MSC signaling dynamic by comparing the relative expression of genes involved in wound healing and angiogenesis. Preliminary data suggest that in addition to being a pro-survival and pro-migration stimulus, Tenascin-C also regulates key genes involved in the wound healing and angiogenesis processes such as Thrombospondin 2, PDGFA, SerpinE1, and MMP2. Whereas Decorin is seen to inversely regulate these same genes. Therefore, we hypothesis that different ECM-MSC conditions will act as important regulators in the process of angiogenesis and wound healing; and will hopefully provide insight into how to better combat chronic skin wounds using ECM-MSC cellular therapy approaches.

Novel calcineurin inhibitor strategies to prevent radiocontrast-induced organ injury

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Radiocontrast agents are used in a multitude of imaging procedures. However, the problem is that radiocontrast exposure can induce debilitating end organ injury. The goal of our studies is to devise effective preventatives for radiocontrast injury. In the current work, we focused on pancreatic inflammation, or pancreatitis, and then further extended the work to studying kidney injury, or nephropathy, with radiocontrast. A recent proof of concept discovery from our laboratory is that radiocontrast-induced pancreatitis, which occurs in 3-15% of patients undergoing a common GI procedure called an ERCP, results from signaling of the pancreatic epithelial cell calcium-activated phosphatase calcineurin. To further translate this finding, we first examined whether co-administration of calcineurin inhibitors with the ERCP radiocontrast could target pancreatic calcineurin. In a mouse model of post-ERCP pancreatitis (PEP), we found that a novel combination of the calcineurin inhibitor FK506 (1 uM) and the radiocontrast iohexol prevented post-ERCP pancreatitis by 61% ($P < 0.05$; $n=3$). We are now in the process of optimizing safety and efficacy of the novel FK506/radiocontrast formulation. Before completing an ERCP, a stent will often be placed into the pancreatic duct when there are concerns for the development of PEP. We are investigating whether coating pancreatic duct stents with calcineurin inhibitors will further reduce PEP. This proof of concept in the pancreas also led us to examine whether the use of calcineurin inhibitors alongside radiocontrast infusion will prevent radiocontrast-induced nephropathy. In tubular kidney cell lines, FK506 pretreatment prevented early inflammatory change associated with the radiocontrast. Overall, we believe that a strategy to target calcineurin will provide a novel and effective method for preventing the problem of radiocontrast-induced injury.

Pirfenidone Inhibits TGF-b1 stimulated Non-SMAD Signaling Pathways in Dupuytren's-derived fibroblasts

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Background: Dupuytren's disease (DD) is a complex fibro-proliferative disorder of the hand that is often progressive and eventually can cause contractures of the affected fingers. The increase in transforming growth factor (TGF-b1) expression has been implicated as a key stimulator of myofibroblast activity and palmar fascial contraction in DD. Pirfenidone (PFD), is an active small molecule with potential to inhibit TGF-b1-mediated action in fibrotic disorders. Our recent published findings show that PFD reduced TGF-b1 mediated cellular functions leading to Dupuytren's through SMAD signaling pathway. In the present study, the effect of PFD on TGF-b1 mediated non-SMAD signaling pathway was determined in both CT- and DD-derived fibroblasts.

Materials and Methods: Fibroblasts harvested from Dupuytren's disease (DD) and carpal tunnel (CT)-derived fibroblasts were treated with or without TGF-b1 (10 ng/ml) and/or PFD (800 µg/ml) and were subjected to Western blots analyses to determine the phosphorylation levels of phosphatidylinositol-3 kinase (PI3K/AKT), extracellular regulated kinases (ERK1/2) and Rho family related myosin light chain (MLC).

Results: Our results show that the basal phosphorylation levels of ERK1/2, Akt and MLC were increased in DD- in comparison to CT-derived fibroblasts. Treatment with PFD led to the inhibition of both basal and TGF-b1-induced phosphorylation of the above proteins in both CT-and DD-derived fibroblasts. PFD inhibits not only expression and activation of downstream p-SMAD2/3 effectors but also non-SMAD signaling targets.

Discussion: These in-vitro results indicate for the first time that PFD has potential to inhibit TGF-b1 induced non-SMAD signaling pathways. Furthermore, these in vitro results suggest PFD as a promising candidate to inhibit the TGF-b1 cellular signaling candidates that lead to fibrosis. Further, in vivo studies are required to determine the therapeutic efficacy of PFD in preventing the recurrence of DD.

Cardiac transcriptome profiling during regeneration in zebrafish

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Heart disease is one of the leading causes of mortality worldwide. While therapeutics to ameliorate these diseases exist, they are poor at completely reversing sustained damage. Myocardial infarction represents a disease that results in a large loss of cardiomyocytes (CMs) that results in the formation of scar tissue. In contrast to embryonic development, adult CMs are post-mitotic and do not proliferate. Understanding which genes that are involved during development that regulate CM proliferation will offer insights into stimulating cell division in the adult heart. Zebrafish provide an excellent system to explore these pathways in adult CM proliferation. Previous research has revealed that zebrafish possess a high capacity for cardiac regeneration, share considerable homology with mammalian genome, and can be manipulated easily for developmental/molecular studies. Current research has yielded a multitude of genes involved in cardiac regeneration within zebrafish, but their exact function is not completely understood. In this study, we performed RNA-seq on uninjured and injured (3dpa) zebrafish hearts. We confirmed their induction after injury via RT-PCR. Among these genes, cenpf, errfi1, and foxm1 show high levels of expression at 3dpa and we sought to further analyze their role in heart regeneration. cenpf encodes a kinetochore-binding protein critical for mitosis, is highly expressed in developing mammalian CMs, but rapidly down-regulated 6 days after birth. foxm1 mediates G1-S/G2-M transitions, binds murine cenpf promoter and upregulates it, and has limited expression in adult CMs. errfi1 is a negative regulator of neuregulin 1 (Nrg1) signaling, and Nrg1 administration was shown to induce re-entry of mammalian CMs into the cell cycle after injury. Currently, we are characterizing transcript localization in adult uninjured and 3dpa hearts via immunostaining with specific antibodies. Future studies will include analysis of zebrafish that harbor mutations in these genes to determine their role in CM proliferation and heart regeneration.

Controllability Analysis of Protein-Protein Interaction Networks for Antiviral Drug Development

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Interaction networks are a tool for analyzing collections of biomolecular data, particularly protein interactions (PPI networks) within a human host. The advantage of PPI networks lies in their ability to take a “whole-cell” approach in lieu of requiring detailed kinetic data to build a system of differential equations. In this method, large-scale protein interaction data is represented in a network and analyzed by its position within the network and its interacting partners. Performing a controllability analysis on a network can predict how the system can be driven to any desired state. In a cell undergoing viral attack, the goal of the virus is to seek control of the host machinery and carry out virus replication tasks, the “desired state” of infection. To better understand how the virus takes control of host functions, we completed a controllability analysis of the proteins of the human PPI network in combination with Influenza A viral protein-host protein interaction data. In the absence of proteins deemed “dispensable” by the analysis, it is easier for an external input (such as a virus) to influence and control total network behavior. We find that though 43.8% of proteins in the total human PPI network are dispensable, 61.5% of host proteins which directly interact with viral proteins are dispensable. This increased proportion suggests that viruses are more likely to attack proteins which will make it easier to take control of the network and ultimately hijack host cell function. Information about the types of proteins targeted by viruses can be used to identify protein candidates for further study and guide antiviral drug development.

High intensity focused ultrasound-sensitive perfluorocarbon nanoemulsions in targeted drug delivery

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Current cancer treatment advances focus on reducing systemic drug toxicity by targeting the tumor. This is accomplished by using the enhanced permeability and retention (EPR) effect, wherein widened endothelium gaps near the tumor result in elevated nanoparticle uptake. Stimulus-mediated drug delivery strategies take advantage of this by making use of light, pH, or other stimuli to cause drug release on demand (Rapoport 2007). Magnetic resonance imaging (MRI) -guided high intensity focused ultrasound (HIFU) has been used to induce drug delivery from nanoparticles (Zhang et al. 2016). Nanoemulsions (NEs) are suitable candidates for both (i) targeting via the EPR effect and (ii) undergoing stimulus-mediated drug delivery (Astafyeva et al. 2015; Rapoport et al. 2011). We have previously shown that perfluorocarbon (PFC) NEs are capable of selectively distributing into the tumor compared to normal tissue in a mouse tumor model (Balducci et al. 2013). Several studies demonstrate the utility of PFC NEs as participants in HIFU-mediated drug delivery (Zhang et al. 2016; Rapoport et al. 2011; Shiraishi et al. 2011) due to the 19F MRI capabilities of PFCs and acoustic cavitation resulting from PFC droplets converting to gaseous microbubbles in response to HIFU. Triggered drug delivery product development is limited by unstable excipients and hence short shelf life. Presented here, a careful selection of NE excipients is conducted and statistical modeling is performed to explore what excipient attributes are critical for balancing acceptable colloidal stability with favorable drug delivery properties in the NE. It is of interest to elucidate physicochemical properties that explain the differences in colloidal behavior of hydrocarbon oil-based nanoemulsions and perfluorocarbon oil-based nanoemulsions. Perfluoro-15-crown-5-ether (PCE) demonstrates the most promising HIFU responsiveness, but lacks drug loading capabilities. A triphasic NE, which combines a hydrocarbon oil phase with PFC phase, allows for simultaneous drug loading capacity and favorable drug release properties.

Predicting all cause mortality for LVAD patients using Bayesian Networks

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The two main treatment options for end stage heart failure are heart transplant or implant of a left ventricular assist device (LVAD). Heart transplant is the gold standard, but the availability of viable hearts is limited and many patients are cannot receive a transplant due to age or health status. LVADs allow patients to either bridge to transplant or can add years to the life of a patient not eligible for transplant. However, LVAD implantation is a major surgery with risks for bleeding, infection, and stroke and requires a significant change in lifestyle. The goal of this work is to use pre-implant information to calculate the risk of mortality in order to improve patient selection for LVAD implant.

Using data from Interagency Registry for Mechanically Assisted Circulatory Support (INTERMACS) we built predictive algorithms for all-cause mortality at six timepoints (1, 3, 6, 12, 24, 36mo) using Bayesian Networks in Weka and GeNie software packages. The number of patients used to construct the model for each timepoint ranged from N = 9568 to N = 361. Patients were excluded at each timepoint for explant, transplant, death in a previous period, and lack of adequate follow-up time. Models were validated using 10-fold cross validation.

Model performance ranged from c-statistic of 0.65 to 0.90 for 1mo to 36mo timepoints. Key variables that influenced outcomes were hemodynamics, kidney and liver status for early timepoints, and then shifted to patient medical history and quality of life for later timepoints.

This work will be used to build a personalized prognostic tool for both physicians and patients to improve decision making for LVAD implantation.

The Modified Mechanical Fragility Index: A New Tool for Clinical Measurement of Red Blood Cell Fragility

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Study: Red blood cell (RBC) susceptibility to mechanically induced hemolysis, or RBC mechanical fragility (MF), is an important parameter in the characterization of erythrocyte membrane health. The rocker bead test and associated calculated mechanical fragility index (MFI) has proven to be a simple and reliable method for RBC MF measurement. While we have recently made progress towards making the rocker bead test clinically applicable by reducing the blood volume requirements from 15.5 ml to 6.5 ml, the MFI produces hematocrit (Ht) dependent results, making direct measurement of blood obtained from patients impossible. In the interest of applying the reduced volume rocker bead test in the clinic, we have developed the modified mechanical fragility index (MMFI): a numerical estimation of MFI if performed at 'standard' Ht (40%). Methods: The reduced volume rocker bead test utilizes 3.0 ml plastic collection tubes containing 2.0 ml of whole blood each. Each of two experimental tubes contains three 1/8" stainless steel beads while one control tube does not, and all tubes are rocked using a platform mixer (18 cycles/min ±17° from horizontal) for one hour. MFI is calculated as a function of total hemoglobin and the difference in free hemoglobin between experimental and control tubes. 96 tests were performed across hematocrit ranges of 25-50% using blood from 4 human donors. Results: A strong non-linear relationship was observed between sample Ht and the resultant MFI. After linearization, multivariate linear regression analysis yielded a highly correlated fit (r=0.965). The proposed correction factor accurately predicted (R^2 =0.923) MFI at 40% Ht from native hematocrit. With the MMFI equation, the reduced volume rocker bead test may be used to compare blood samples between 25–50% Ht without normalizing for hematocrit.

Computational Assessment of Design Parameters for a Torsional Ventricular Assist Device (tVAD)

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Purpose
The goal of this study is to quantify changes in hemodynamic function with the application of apical torsion as a means of cardiac assist. These results will help identify optimum parameters for the design of a torsional ventricular assist device (tVAD).

Methods
Parametric computational simulations were performed using ContinuityPro software (Insilicomed, Inc., La Jolla, CA) and used to determine design parameters for a second-generation tVAD prototype. These simulations utilized beating heart models of both ventricles attached to a closed-loop circulatory system. Ventricular size, shape and dimensions were based on anatomic measurements of a dysynchronous heart failure patient with dilated cardiomyopathy. Biomechanic and circulatory model parameters were taken from literature values. Varying degrees of applied apical torsion were simulated by altering coverage areas (12.5%, 18.2% and 24%) and rotation angles (0, 45, 55, 65, and 75 degrees) to determine the effects on global cardiac function. Results were compared to baseline hemodynamic values of the clinical heart failure model (No VAD) before torsion was applied.

Results
Increasing the parameters of tVAD coverage areas and applied angles of rotation produced pronounced increases in ejection fraction (EF), peak systolic pressure (PSP), and stroke work (SW) while simultaneously lowering end-systolic volume (ESV) in both ventricles. The simulation representing the most aggressive level of tVAD assist, with a large tVAD coverage area (24%) and 75 degrees of applied rotation, produced left ventricular EF of 30.4%, PSP of 164 mmHg, ESV of 145 mL, and SW of 9145 mmHg*mL. When compared to our baseline No VAD hemodynamics - left ventricular EF of 20.3%, PSP of 123 mmHg, ESV of 200 mL, and SW of 5321 mmHg*mL - we observed increasing returns of 49%, 34%, 28% and 72% for EF, PSP, ESV, and SW, respectively. Large coverage and 75 degree rotation angle yielded right ventricular EF of 69.2%, PSP of 54.6 mmHg, ESV of 26.6 mL, and SW of 2673 mmHg*mL. When compared to the No VAD hemodynamics - right ventricular EF of 43.9%, PSP of 44.3 mmHg, ESV of 64.5 mL, and SW of 1793 mmHg*mL - we observed increasing returns of 58%, 23%, 58% and 49% for EF, PSP, ESV, and SW, respectively.

Conclusion
These initial results have helped us to identify the optimal working parameters for tVAD coverage area and rotation angle. Based on these findings, we believe that applied apical torsion has the potential to help restore cardiovascular hemodynamics toward healthier values for both ventricles.

Lowering the risk of injury in head immobilization devices by design

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Precise and firm cranium fixation is critical in craniotomy and neurosurgery, because small head motion could disrupt the operation, cause complications, and may even lead to fatalities, especially in high risk patients. Hence, head immobilization devices (HIDs) have become a staple instrument in neurosurgery. Despite their abundance over the past few decades, serious injuries are still being reported today for HIDs which motivates our detailed analysis of existing HID designs. This work is a design-based approach aimed at reducing the risk of injuries arising from the use of HIDs, especially in pediatric neurosurgery.

The Mayfield head clamp is one of the earliest designs of HID and is one of the most widely used instruments today. However, complications were reported in case studies published since 1984, including skull fractures, depressed skulls, and epidural hematoma. These findings were recently supported by an FDA safety communication issued in 2016, which reports more than 700 injuries in relation to skull clamp complications before or during surgeries in the period from 2009 to 2016. Although design modification to HID were proposed, such as combining the clamp with a headrest, the mechanics of pin-bone contact have not been systematically studied.

This work applies finite element modeling (FEM) techniques to analyze the mechanical loading of HID pins on the skull during clamping. Our findings reveal a detailed understanding of the indentation caused by pushing pins against skull bone, highlighting the shortcomings of current pin designs. We simulate skull failure by exploring the effect of pin material, geometry and loading on bone deformation and stresses. Using the developed FEM model, we show the tradeoff between maximizing slip-resistance and compressive stresses. We also propose and evaluate new pin geometries with optimized designs to reduce the risk of skull fracture, while adequately preventing slippage.

In vitro and in vivo hemocompatibility assessment of the VADovations novel pediatric left ventricle assist device (LVAD)

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VADovations is developing a platform of miniaturized continuous flow ventricular assist devices (VADs), which permit exchanging 2 hydraulic components during manufacture to meet the hemodynamic requirements of different patient populations. In this early stage effort, adult RVAD hydraulic designs were evaluated for use as pediatric LVADs in vitro and in sheep implants at the McGowan Institute Center for Preclinical Studies. Hemolysis was measured in a mock flow loop at a flow rate of 2.0 L/min against 45-75 mmHg. Two hydraulic designs were compared using the same pump housing and blood pool. The average blood parameters were hematocrit (29.7±0.6%), total plasma protein (7.15±0.05 g/dL), and pH (7.10±0.03). The Normalized Index of Hemolysis (NIH) values for the two rotors were 0.103±0.031 and 0.212±0.095 g/100L, respectively, acceptable for pediatric LVAD use.

In vivo, pumps with small variations of hydraulic designs were implanted through the apex of the left ventricle with a 10mm graft anastomosed to the ascending aorta. Pump speeds were adjusted from 17,500–24,000 RPM producing average flows of 0.8-3.8 L/min. Four studies reached or exceeded planned duration (1-3 months), two studies were electively ended early due to outflow graft kinking, two studies were terminated peri-operatively, and one study due to right ventricular thrombus from an indwelling i.v. line. Plasma free hemoglobin (pfHb), total hemoglobin/hematocrit, total plasma protein, and fibrinogen returned to baseline pre-op values by the end of the second week of the study. Post-operative hypertension produced intermittent retrograde flow in some studies, but renal infarcts were only observed in one of 10 chronic implants.

The results demonstrated acceptable hemocompatibility as a pediatric LVAD, but presented opportunities for improved hemodynamic and hemolysis performance. Based on these findings, three pediatric specific hydraulics have been developed, Infant 0.5-1.5 L/min, Toddler 1.0-3.5 L/min, and Child 3+ L/min, which will be undergoing testing in 2017.

Ultrasound Triggered-Release Embedded Anti-Rejection Therapy (TREAT™) for Targeted Immunomodulation in Vascularized Composite Allotransplantation

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Purpose

More than 30,000 people receive organ transplants every year in the US. Vascularized composite allotransplantation (VCA) is the newest realm of solid organ transplantation. The skin component of VCA is highly antigenic and mandates high doses of systemic immunosuppressive drugs. Oral dosing of immunosuppressive drugs such as tacrolimus (TAC), rapamycin (Rapa), and mycophenolic acid (MPA) leads to fluctuating, erratic, or unpredictable blood levels risking toxicity or lack of efficacy . We propose a drug delivery platform that can not only provide sustained drug release but also on-cue triggered drug release upon ultrasound stimulation (USS) in graft tissues with stable, low blood levels, minimizing overall drug exposure and facilitating long-term VCA survival with no systemic complications.

Method

An injectable, re-loadable, biocompatible drug eluting hydrogel was prepared. We characterized the in vitro release kinetics of the drugs from alginate gels in absence and /or presence of USS. We evaluated feasibility and efficacy of the system in vivo in absence of USS. Brown Norway to Lewis rats received fully mismatched Brown Norway rat hind limb VCA (4/group) and a single dose of gel subcutaneously injected into the allograft. The gel was loaded with either TAC (10mg), Rapa (10mg), or TAC+Rapa (10mg) each in 1 ml. Drug levels in blood and VCA tissues were analyzed by LC–MS/MS. Flow cytometry was performed to detect expression of regulatory marker, FOXP3. In addition to allograft survival, systemic toxicity was evaluated using percent change in body weight (BW) and creatinine clearance (CrCL).

Results

In vitro, TAC and Rapa exhibited a low baseline level (without fluctuation) of release from alginate gels in the absence of USS. Pulsatile USS triggered drug release, leading to increased drug levels after each pulse. Sustained drug release occurred from alginate gels in the absence of ultrasound with blood levels within the therapeutic range (5-10ng/ml). Drug concentration in allograft tissues was higher than in blood and contralateral limb ($P<0.05$). In the first 2 weeks post gel injection, there was a ≤15% change in BW which stabilized with time. BW gradually increased over time. No significant change in CrCL occurred post gel injection over time (>0.05). Rats receiving Rapa developed Banff grade 3 rejection on day 21, while rats receiving TAC or TAC+Rapa showed allograft survival (>100 days). Expression of the regulatory marker FOXP3 was observed which may indicate peripheral immunomodulation.

Conclusion

We successfully developed, for the first time, a smart hydrogel drug delivery system with sustained baseline and on-demand release of drugs upon USS for use in VCA. The TREAT ™ system provides stable, low drug levels in the blood with preferential drug concentration in VCA tissues facilitating long-term VCA survival/outcomes with no systemic adverse effects. Further efforts are being made to use USS to optimize the on-cue drug release. We developed dibenzocyclooctyne (DBCO)-immunosuppressant prodrugs that can be reloaded into the azido-modified alginate gels via Click chemistry, with the goal of refilling the alginate scaffolds with immunosuppressive drugs once the encapsulated drugs are running out and thus maintaining the immunosuppressive effect for a long term without replacing the gel scaffolds via surgery method.

High Efficiency Respiratory Dialysis at Ultra-Low Blood Flows

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Clinical adoption of new extracorporeal carbon dioxide removal (ECCO2R) systems for management of hypercapnic respiratory failure is hindered by invasive connections strategies and complex blood circuitry, limiting use to specialized medical centers. We have developed a high efficiency ECCO2R device capable of treatment analogous to renal hemodialysis to close the logistical gap between respiratory and renal dialysis.

The Ultra-Low-Flow ECCO2R Device (ULFED) uses an array of rotating impellers within a gas exchange bundle to generate an “active mixing” effect. Impeller mixing dramatically enhances gas transfer rates and fiber washing to ease anticoagulation requirements. Significant improvements in CO2 removal efficiency versus available systems enable operation at blood flow rates comparable to renal hemodialysis (250 mL/min), facilitating potential for adoption of common dialysis cannulation strategies and use with existing dialysis circuitry.

Three ULFED prototypes (fiber surface area = 0.42 m2) were fabricated with varying bundle aspect ratios (length versus width) and two impeller arrangements (impeller length effects) to evaluate the effects of bundle and impeller design in the active mixing system. CO2 removal rates up to 75 mL/min from blood were achieved during in vitro gas exchange testing (4000 impeller RPM, inlet blood pCO2 = 45 mmHg, blood flow 250 mL/min), matching performance of leading approved ECCO2R systems at half the necessary blood flow rate. Subsequent in vitro hemolysis testing in a clinical circuit (including pump and cannula) revealed with no significant difference versus a control circuit using time-of-therapy normalized rates of cell trauma (ULFED Therapeutic Index of Hemolysis (TIH) = 0.190 ± 0.041 g/100min versus Control TIH = 0.123 ± 0.013 g/100min; $p>0.05$).

Performance of the ULFED matches or exceeds the abilities of all available ECCO2R devices, but does so at half the blood flow rate of leading systems, and 30% lower fiber surface area. Under these operating conditions, the ULFED could be connected to patients using dramatically simplified methods directly adopted from routine renal hemodialysis, with potential to be integrated directly into existing dialysis circuitry.

An Extracorporeal Neutrophil Reprogramming Device for the Treatment of Acute Inflammatory Conditions

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Sepsis is a systemic inflammatory response due to an infection. This condition affects 900,000 Americans per year and its incidence is expected to increase over the next 10-20 years as the population ages. As one of the first responders to sites of inflammation, neutrophils play a critical role in innate immune response and sepsis. High quantities of neutrophils migrating to remote organs such as the lungs has been associated with increased morbidity and mortality by initiating multiple organ dysfunction or immune paralysis in septic patients. The migration and activation of neutrophils is regulated by CXCR-1 and CXCR-2, g-protein coupled receptors that bind to interleukin-8 (IL-8).

An extracorporeal device is being developed to attenuate neutrophil chemotactic response in inflammatory conditions such as sepsis. This device achieves cell phenotype reprogramming by exposing neutrophils to immobilized IL-8 within an artificial microcirculation. First IL-8 was covalently immobilized on the inner lumen of amine functionalized fibers [Baxter Intl.] using a bifunctional polyethylene glycol spacer. After perfusion of human whole blood through modules constructed from modified fibers, neutrophil surface receptor expression was quantified using flow cytometry. In scaled devices surface receptor expression of CXCR-1 and CXCR-2 decreased by 19% and 70% respectively when compared to baseline blood. When hAlbumin was immobilized in place of IL-8, downregulation closely resembled baseline blood, indicating IL-8 was responsible for receptor downregulation. IL-8 ELISAs (Thermo Fisher) were completed to determine the extent of nonspecifically bound IL-8 leaching from the modules. Tests determined IL-8 concentrations of 662 ± 31 pg/ml after 1 hour 5% BSA recirculation; potentially enough to induce partial downregulation. Future tests will evaluate chemotaxis of neutrophils treated with the extracorporeal device. Beyond sepsis, a similar device platform may be applied to alternative disease states such as ischemia reperfusion and solid organ rejection, which are regulated by cell-cell interactions.

Corrosion Characterization of Magensium Coated Via Micro-arc Oxidation

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INTRODUCTION: Devices used for orthopaedic applications have been made using inert metallic alloys such as titanium and stainless steel as well as bioresorbable polymeric materials like poly(lactic-co-glycolic acid) (PLGA). Recently, there has been a renewed interest in the use of magnesium (Mg). While Mg has been used experimentally for development of bone plates and screws, it has not been used for soft tissue applications such as healing of ligaments and tendons. In our research center, we have developed a Mg device to facilitate the healing of the anterior cruciate ligament (ACL). We have chosen this material because it has suitable mechanical properties, controllable degradation, and biocompatibility that makes it acceptable for ACL healing. Specifically, Mg is more ductile than resorbable polymeric materials and the rate of degradation of Mg can be controlled through surface coatings without compromising its mechanical properties. Hence, Mg can be engineered to degrade away over time being replaced by the native tissue. Thus, the goal of this study was to characterize the corrosion rate of Mg with a surface coating to identify the appropriate coating time for use in the intended application of ACL healing.

METHODS: Pure magnesium (99.9%) were machined into 10 mm diameter by 1 mm thick disks. The samples were then rinsed with acetone and polished with 1200 grit SiC abrasive paper. After being polished, the samples were rinsed in methanol and allowed to air dry. In order to apply an micro-arc oxidation (MAO)surface coating, the samples were immersed in an electrolytic solution (6 M Na3PO4 + 8 M Na2SiO3•5 H20 + 4 M KF). An adjustable DC pulse source was used to apply a constant potential of 300 V through titanium wire for 2 and 5 minutes. Uncoated samples were used as a control. The corrosion rate was determined by in vitro dorrosion testing which involves submerging the samples into Hanks' Balanced Salt solution and monitoring the change in mass, change in pH, and the release of Mg ions. Measurements were taken daily for 1 week and weekly for 4 weeks.

RESULTS: The uncoated samples degraded faster than the samples coated for 2 minutes and 5 minutes. After 1 week, the uncoated, 2 minute, and 5 minute samples had an increased in mass after which the change in mass decreased up to 1% at 4 weeks. In terms of the pH measurements, the Hanks' solution became more alkaline after immersion of the samples. For the uncoated samples, there was a sharper increase in the pH value at the initial stage and an increase in pH of 3 by 4 weeks. Lastly, after measurement of the Mg ions remaining in the Hanks', a similar trend was seen as with the change in pH. For the uncoated, 2 minute, and 5 minute samples, there was a faster increase in Mg concentration within the first week which slowed over the remaining 3 weeks with a 4 times increase by 4 weeks.

DISCUSSION: These results confirm that the Mg samples would begin to degrade when immersed in Hanks' Balanced Salt Solution within the 4 week time period. Also, the initial increase of change in mass can be attributed to the accumulation of corrosion products on the surface of the sample. However, as the sample continues to undergo corrosion, there was more mass loss. The trends of pH and Mg ion concentration indicate that the uncoated samples degraded faster than the 2 minute and 5 minute samples as expected because the MAO coating slows the corrosion at initial time points. However, there was no significant difference for the corrosion rate between the groups. Thus, the coating times of 2 minutes and 5 minutes would allow a device for ACL healing to have a surface favorable to cell interactions, initially resist degradation as the ligament heals, and ultimately degrade away with the healed ligament remaining. With these promising results, we have used MAO coated Mg in subsequent animal studies for ACL healing

The Effect of Inlet Pressure on Gas Embolism and Hemolysis in Continuous-Flow Blood Pumps

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Purpose: While there is an increased enthusiasm in the development of small, high speed, continuous-flow ventricular assist devices, in clinical use these pumps can encounter negative inlet pressures. To date, in-vitro tests have not quantified the effect of low preload on hemolysis.

Methods: Two preliminary in-vitro studies examined the effect of inlet pressure on hemolysis and gas embolism in a magnetically levitated, pediatric, centrifugal blood pump: (1) gaseous emboli exiting the pump in a saline filled mock loop were measured with the EDAC Quantifier at variable inlet pressures (-40-10 mmHg) and speeds (2500-4000 RPM); and (2) hemolysis testing was performed at 0.5 and 1.5 L/min using a mock loop for four hours with three inlet pressures (-40 -0 mmHg) while holding speed, flow and pressure drop constant.

Results: In saline, significant numbers of gas emboli (>50 μm) exited the pump at negative inlet pressures. Hemolysis testing revealed a strong correlation between the NIH and negative inlet pressure at both flow rates with foam generated at all negative pressures. On-going studies combining the two experiments will enable the quantification of the gaseous emboli size in blood and further elucidate the effect of inlet pressure on blood trauma in continuous-flow pumps.

Pittsburgh Pediatric Ambulatory Lung (P-PAL)

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Acute and chronic respiratory failure are a significant source of pediatric morbidity and mortality. Current means of respiratory support used to bridge these children to lung recovery or transplantation typically render the children bedridden, a condition that can worsen long-term patient outcomes. The Pittsburgh Pediatric Ambulatory Lung (P-PAL) is a wearable pediatric blood pump and oxygenator (0.3 sq meter membrane surface area) integrated into a single unit that is designed to provide long-term respiratory support while enabling patient ambulation. During the current study, a P-PAL prototype was fabricated and used to perform in vitro evaluation of pumping, gas transfer, and hemolytic performance. The P-PAL was shown able to provide adequate blood flows (1 - 2.5 L/min) against the large resistance anticipated for use with pediatric cannulas. An oxygenation rate as high as 109 mL/min was achieved at a blood flow rate of 2.5 L/min, thereby meeting our design target of providing 90% respiratory support to children up to 25 kg. Hemolytic evaluation of the P-PAL resulted in normalized index of hemolysis values of 0.022 - 0.035 g/100L for the intended blood flow rate range, indicating low device-induced hemolysis. Thus, in vitro results demonstrate that the current P-PAL design meets all specified performance targets and has significant potential to improve treatment for pediatric respiratory failure. Future work includes in vivo evaluation of the P-PAL in acute and chronic animal studies.

RegenMatrix: Collagen-mimetic Bioactive Hydrogels for Growth Factor Free Approach for Bone Regeneration

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Bone and teeth are two major hard tissues in human body. These hard tissues are formed by a natural process of mineral deposition called biomineralization. In this process, collagen acts as an organic template to guide mineral deposition bone extracellular matrix (ECM) and apatite minerals act as inorganic phase, both of which interact at molecular level forming hierarchical nanocomposites. However, most of the currently explored materials are just physical blends of organic and inorganic phases. In this study, we focused on re-creating biomimetic regenerative microenvironment for guiding biomineralization and bone formation by employing fibrous hydrogel scaffolds (RegenMatrix) with collagen-mimetic multi-scale hierarchy and mineral sequestration. We assessed their potential for biomineralization in vitro and in vivo.

Two different RegenMatrix were fabricated from pairs of oppositely charged natural polymers. In vitro mineralization in simulated body fluid was assessed by fourier transform infrared spectroscopy (FTIR) and x-ray diffraction (XRD), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In vivo mineralization potential of RegenMatrix was assessed in a critical sized mouse calvarial defect model. Regenerated bone was quantified as bone volume using micro computed tomography (μCT). Histology was employed to assess progenitor and other native cell infiltration in RegenMatrix and the injury site.

Both RegenMatrix showed biomimetic mineralization by promoting amorphous calcium phosphate inside the matrix and apatite minerals on the surface when tested in vitro in SBF. RegenMatrix was proven to be safe in mice calvarial defect model. A quantitative comparison of regenerated bone volume from μCT analysis showed significantly higher bone regeneration with one of the two RegenMatrix compared to empty defect group. It is to our knowledge, the first material that could promote osteogenesis without any growth factors and exogenous synthetic minerals. Moreover, μCT images show evidence of guided bone regeneration indicated by the directionality of the regenerated bone with one of the two RegenMatrix.

Traditional method small volume drug delivery method fails to provide a therapeutic dose in small infants with supraventricular tachycardia

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Study Objective: Adenosine administration using a traditional three-way stopcock mechanism is the recommended treatment for pediatric patients with acute supraventricular tachycardia. Recent reports suggest that many infants do not respond to the first dose of adenosine as recommended by the American Heart Association. We observed that a portion of the adenosine remains in the stopcock and is not delivered to the patient. Our aim was to determine whether administration of adenosine using a stopcock delivers lower than expected drug doses in patients weighing less than 10 kg.

Methods: We developed an in vitro model of adenosine delivery. Doses of adenosine corresponding to weights 2-25 kg were calculated using a dose of 0.1 mg/kg and administered through one port of a stopcock. Water was administered through the second port. The adenosine concentration of the output was measured using mass spectrometry and results were confirmed using spectrophotometry of Evans blue.

Results: The mean doses of adenosine delivered through the stopcock increased as weights increased. The mean doses delivered were 0.07±0.01, 0.09±0.01, and 0.1±0.01 mg/kg for weights 2-5, 6-9, and 10-25 kg, respectively ($p<0.05$ for 2-5 vs. 10-25 kg). The percentage of the intended adenosine doses delivered via the stopcock method were 67±10%, 87±8% and 98±15%, for weights 2-5, 6-9 and 10-25 kg, respectively. Similar results were obtained using spectrophotometry.

Conclusion: Administration of adenosine via a stopcock delivers doses lower than intended in infants, which may account for decreased responsiveness of infants to the first dose of adenosine. Engineering of a delivery system for small volume medications that mitigates a finite volume of "drug left behind" may improve the accuracy of the intended dose. Such a small volume drug delivery mechanism may then improve patient responsiveness to the initial dose of a medication.

Ventriculo Amniotic Shunt for Fetal Aqueductal Stenosis

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Neonatal hydrocephalus due to aqueductal stenosis is a debilitating disease characterized by an excessive accumulation of fluid within the brain. Current treatment consists of a postnatal shunt, but this does not prevent poor neurological outcomes. VASFAS, a prenatal shunt which can be placed during pregnancy to prevent the buildup of pressure from fluid in the brain, may drastically decrease the morbidity, mortality and cost of overall care of these children. Fetal aqueductal stenosis affects an estimated 25-60,000 infants annually on a global basis. The application of our device can be widened to treat conditions such as lower urinary tract obstruction and pressure hydrothorax, both of which are of similar incidence to aqueductal stenosis.

Application of novel ultrahigh ductility (UHD) magnesium stents for the treatment of airway obstruction

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Airway obstruction is rare, but could be extremely difficult and challenging to handle. Standard treatment is open surgery. However, these reconstructive surgical procedures are usually associated with serious complications. Stent based interventional pulmonology has raised great interest by providing immediate relief with reduced morbidity and risk. Due to the permanent nature of current non-degradable stents, no stent provides satisfactory long-term effectiveness. A biodegradable stent could potentially solve this problem. In this study, we have developed proprietary ultrahigh ductility (UHD) magnesium based absorbable stents and evaluated the biocompatibility and in vivo degradation in rat airway bypass model and rabbit tracheal model. First generation magnesium stents were made from commercially obtained pure Mg and AZ31 alloys and Mg-Y alloys with an outer diameter of 2.25mm, inner diameter of 1.25mm and length of 5mm. Donor female Lewis rats were sacrificed and the tracheas harvested to provide a bypass graft for stent evaluation. The stent was placed intra-luminally in the donor trachea, which was anastomosed to recipient rats in and end to side fashion. The animals were euthanized at 1, 8, 16, and 24 weeks for post evaluation by μCT and standard histologic analysis. Second generation stents were machined from ERC-P6 alloy based on the in vivo results of the first generation stents. The size of the stents was scaled up to fit the trachea size of rabbit. Stents were directly delivered inside the trachea of rabbits without a bypass. Animals were euthanized at 4 weeks for post evaluation by endoscopy and standard histological analysis. First generation stents showed the potential of Mg alloys for use as airway stents. A ciliated epithelium was maintained even after 24 weeks in vivo. The in vivo corrosion results showed that Mg-Y alloys appeared to loose configuration at 24 weeks. For the second generation stents, novel ERC-P6 alloys showed excellent cyto-compatibilities in vitro. Airway lumen was well maintained after 4 weeks of implantation as shown in the endoscopy image. Mg stents were covered by degradation product; on top of which healthy epithelium layers were observed. Results of these studies will be presented and discussed.

Magnesium Single Crystals for Medical Implant Applications

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Magnesium is a promising material for orthopedic implants due to its biocompatibility and the ability to resorb in body. Single crystal of Mg is expected to reveal absence of grain boundaries which will result in high strength, non-catastrophic failures, high purity and increased corrosion resistance. Mg single crystals were grown by Bridgman method. The single crystal structure was confirmed by Laue XRD, pole diffraction figures, X-Ray tomography, electron backscatter diffraction, metallography, and synchrotron characterization at the Argonne National Labs. Magnesium single crystals revealed mechanical properties close to those of the natural bone with an ultimate tensile strength of 60-70 MPa and a remarkable tendency for higher ductility of 50-60%. Thanks to the absence of grain boundaries and the applied proprietary nanometer thin coating, the corrosion of Mg single crystal was controlled and significantly suppressed in vitro and in vivo. Orthopedic devices were machined from Mg single crystals and tested in 3 animal models such as mouse, rabbit and goat. Implanted in a rabbit ulna fracture model, single crystal Mg plates & screws showed promising Mg resorption and bone overgrowth around the device. Anterior Cruciate Ligament (ACL) rings implanted in goats successfully repaired the damaged ligament and have fully degraded in 12 weeks. No abnormal inflammatory response was observed. The conducted experiments confirmed that Mg single crystal is a promising material for biodegradable implant applications. Current efforts are reported on fabricating stents from Mg single crystal by EDM machining and laser cutting.

Dental Pulp Derived Extracellular Matrix as a Scaffold for Pulp Regenerative Therapy

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In the theme of dental pulp regeneration, two main concepts have emerged: cell-based and cell-free approaches. These are the current models for pulp regenerative treatment. While attempts have been made using cell-based approach utilizing cells from dental populations alone or combined with a scaffold as a carrier. The cell-free approach offers minimal manipulation and eliminates the need for cells to be harvested from the host, since it depends on the scaffolds ability to support cellular infiltration and proliferation. As scaffolds gained attention in this field, biologically inspired and synthetic scaffolds were developed to mimic the natural environment of dental pulp. The extracellular matrix (ECM) represents a natural scaffold material resembling the native tissue chemical and mechanical components. In the past few years, ECM-based scaffolds showed promising results in terms of recruiting progenitor cells, promoting constructive remodeling and modulation of host response. The aforementioned properties make ECM-based scaffolds an ideal candidate for pulp regenerative therapy.

Development of strategies for clinically relevant tissue engineering using dental pulp extracellular matrix (DP-ECM) can provide an alternative to conventional root canal treatment. This alternative could serve our goal to provide a suitable environment for pulp regeneration. In this work, we successfully decellularized ECM derived from porcine dental pulp. The resulting scaffold was characterized using immunostaining for extracellular proteins. To determine the effect of the decellularization process on growth factors, we performed ELISA for TGF beta, VEGF and bFGF. Furthermore, the matrix was implanted orthotopically for 8 weeks in canine model. In-vivo, the ECM supported cellular infiltration and remodeling of the scaffold. The resulting tissue had higher expression of DSP and CD31 when compared to control. Herein, we validated the concept of using ECM based scaffolds for pulp regeneration. To fully investigate the ECM potential in endodontics, development of clinical protocols and further work is needed.

Arterial remodeling in autologous vein and cell-free, fast-degrading vascular grafts using a rat carotid artery interposition model

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Background
The clinical need for vascular grafts is increasing with the high incidence of cardiac and peripheral vascular diseases. Autologous grafts remain as a gold standard, but are not always feasible and need an additional surgery to obtain. We have developed cell-free, biodegradable vascular grafts, which have demonstrated accelerated cell infiltration and host remodeling in a rat carotid artery interposition model.

Methods
Study included 2 groups. Group 1 (n=21) consisted of vascular grafts and group 2 (n=18) consisted of vein grafts. The left common carotid artery of 2-month old male Sprague-Dawley rats (body weight = 225-250 g) was exposed, clamped, and transected. In group 1 bi-layered composite vascular grafts with a fast-degrading elastomer, polyglycerol sebacate (PGS) core and an electrospun polycaprolactone (PCL) outer sheath grafts were used. In group 2 left external jugular vein was harvested (10mm long). Both synthetic and naïve vein grafts (10 mm long) were interpositioned by end-to-end anastomosis with 10-0 suture. Graft patency was checked with flow Doppler at 0, 5, 10, 30 min after anastomosis and ultrasound scanning was done at 2, 4, 8, 12-week post-implantation. Grafts were explanted at 2-week (n=7 for synthetic, n=6 for vein), 4-week (n=7 for synthetic, n=6 for vein) and 12-week (n=6 for both) post-implantation. Histology and immunofluorescence staining was done.

Results
Overall patency of composite and vein grafts were 90.5 and 100%, respectively. Ultrasound scanning confirmed unobstructed lumen and blood flow in both grafts without narrowing and dilation. Histology and immunofluorescence staining demonstrated artery-like structures: endothelial monolayer in lumen and organized smooth muscle cells in medial layer.

Conclusion
Our results showed successful neoartery remodeling in the cell-free, fast-degrading vascular grafts following rat carotid artery interposition. Our vascular graft can be ideally suited for small-diameter arterial regeneration.

Surface Modification of Electrospun Gelatin/Fibrinogen Scaffolds to Encourage Endothelial Cell Function

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Surface modification of biomaterials is a popular method in tissue engineering for increasing the biocompatibility of a polymer intended for graft design. In the case of tissue engineered vascular grafts (TEVG), they need to have the ability to promote the formation of a healthy endothelial cell monolayer in the inner lumen of the graft. In this study, gelatin/fibrinogen and gelatin/fibrinogen/PCL scaffolds were electrospun to create flat scaffolds. In order to increase the biocompatibility of these scaffolds with endothelial cells, the surface of each sample was modified using a thermoforming process and then coated with a blend of collagen IV and fibronectin. Human umbilical cord blood derived endothelial cells (hCB-ECs) were seeded onto the electrospun grafts and the important characteristics of a healthy endothelium were evaluated under static conditions. According to our findings, electrospun scaffolds composed of 50% PCL, 40% gelatin, and 10% fibrinogen, that were surface modified by thermoforming and coating with a mixture of collagen IV and fibronectin, are the most suitable for endothelial cell growth. hCB-ECs growing on these constructs have the ability to proliferate and form a stable monolayer on the surface of the planar scaffold, produce eNOS, respond to the addition of IL -1β by the upregulation of VCAM-1 and ICAM-1, and reduce platelet deposition and activation rate.

Measuring Changes in Force Generation in Cell Monolayers via Intranuclear Kinetics

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Intracellular force generation is critical to a cell's function particularly in areas such as wound healing, and embryonic development. We have developed a technique, which uses the nucleus as a force sensor within cells to determine changes in intracellular force propagation within cell monolayers. This technique is termed Sensors from IntraNuclear Kinetics (SINK), in which fluorescent, chromatin bound, proteins within the nucleus are tracked over time and mean square displacement of these particles is calculated. Upon fitting these displacements to a power law equation, we show that the exponent serves as a relative measure of intracellular force. Here we demonstrate this by decreasing cellular force generation, with the chemical Y-27632, and by physical decoupling of the nucleus from the cytoskeleton. In both cases we see a reduction in intranuclear motion, compared to control cells. Next, we investigate the changes in force propagation through cells in a monolayer, compared to isolated cells, which have different cytoskeletal structures. Then, we investigate the extent that monolayer cells respond to the stiffness of their underlying substrate, and the extent that this propagates to the cell nucleus. Finally, we investigate the role that a mechanically compromised cell in a monolayer, has on its surrounding cells. This demonstrates the power of the SINK technique for investigating force propagation through cell monolayers as it relates to diseases, which often stem from point defects in a monolayer.

Modeling Metastasis from Invasion to Colonization on a Human Physiomimetic Chip

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Melanoma progression from curable primary tumors to distant metastases heralds morbidity and mortality. The transitory state involves invasion from the epidermis through the dermis and dissemination to metastatic sites, where generally incurable micrometastases form. The newly seeded tumor clusters hone to canonical sites, namely liver (48% of cases, median survival [ms] = 4 mo.), brain (29%, ms = 4 mo.), and bone (23%), with an overall year fatality rate of 85%, [ms <12 months mo.]. Metastatic melanoma (MM) is extremely aggressive due to clinical resistance to a spectrum of chemotherapeutic, small molecule, and antibody targeted therapeutics. Effective treatments for MM remain elusive due to a lack of vital information regarding early seeding of MM, dormancy, and re-emergence at the site of metastasis. Several studies have partially elucidated mechanisms for MM progression using 2D models and xenograft animal models. Despite these advances, these models are limited in recapitulating the microenvironments in primary and distant sites, and in variations among species. We use an all-human organotypic skin organ culture system (SOCs) and human liver microphysiological system (MPS-LiverChip) to model early MM and key mechanisms for MM driven remodeling at distant sites. Previous data has implicated Tenascin-C (TNC), an extracellular matrix protein present in development and wound healing, as a key driver of MM invasion. TNC limits cell death through pro-survival signaling from epidermal-like growth factor (EGF-like) repeats present in the N-terminal domain. Accordingly, aberrant re-expression of TNC in MM is associated with poor clinical outcome. Preliminary data indicates that MM is capable of integrating with hepatocytes and non-parenchymal cells in the LiverChip, in both 2D and 3D culture. It is hypothesized that MM-TNC both “protects” MM in newly established metastatic sites and reciprocal interactions between MM-TNC and innate immune cells alters stroma/endothelial cell function to establish new tumor microenvironments

Accelerating Wound Healing with a Collagen VI - Heparin Sulfate Coated Matrix

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Treating burn related injuries remains challenging due to donor site morbidity, bacterial infection, and major scarring. Recent advances have led to the development of promising natural and synthetic polymer scaffolds, which aim to regenerate skin using a “top-down” approach in which the top layers of the skin populated by fibroblasts and keratinocytes are regenerated first. An often overlooked component of the integumentary system is the subcutaneous adipose tissue, which has been implicated in a variety of activities, including wound healing. We have created a novel coating composed of collagen VI (col VI) and heparin sulfate (HS) that promotes adipocyte attachment and adipogenesis at a rate significantly faster at both early and late timepoints (5 and 23 days) compared to a non-coated control. Additionally, Vicryl®, a biodegradable surgical implant mesh, was coated with the optimized col VI-HS ratio. Coated Vicryl® had an increased number of attached adipocytes with more pronounced growth when compared to uncoated Vicryl ®. These findings suggest that the combination of optimized col VI-HS coating and the fibrous woven Vicryl® may have excellent potential in developing skin graft applications using a paradigm-shifting ‘bottom-up’ approach. The next stage of this research will move beyond synthetic woven mesh and combine natural scaffolding materials with Collagen-Heparin coating to create a bio-absorbable felt-like adipomesh that mimics the extracellular matrix of dermal tissue.

Measuring SMC Death and Adhesion Forces Via Tissue Engineered Models Simulating ECM Microarchitecture

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Introduction: Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation and is associated with aneurysmal disease which predisposes patients to aortic catastrophe. (1) Our prior studies have uncovered collagen remodeling in extracellular matrix (ECM) and decreased oxidative stress defense of the smooth muscle cells in aortic specimens from BAV patients compared to specimens from patients with the morphologically normal tricuspid aortic valve (TAV). (2, 3) These alternations in ECM and SMC biology contribute to cystic medial degeneration (CMD) in aneurysmal disease, which are highlighted by cellular death and impaired cellular adhesion. To measure these two key components, we employ two tissue-engineered models to elucidate the interplay between the ROS-induced SMC changes and ECM.

Methods: Ascending aortic SMCs were harvested using previously established protocols. SMC populations isolated from BAV (n=9) and TAV (n=10) patients were seeded at a density of 5.0x103/well in a 96 well plate on tissue culture polystyrene (TCP) in the presence or absence of type 1 collagen substrate. Once 80-100% confluent, each well was treated with 0-4800μM of tert-butyl hydroperoxide (tBHP). The cells were stained with propidium iodide and read on a plate reader for cellular death. TAV SMCs (n=3) were seeded at 35μL of a 1.0x104 cells/mL onto polystyrene nanofiber scaffolds. After cells are allowed to attach onto the fibers, cells are visualized unto 20x magnification. A 20-30 minute time lapse is taken of each cell that fit the criteria, with pictures taken at 1-minute intervals. Fiber deflection is measured using a custom MAT LAB program. (4)

Results: At 4800μM tBHP, there was 86.7% BAV SMC death compared to 77.7% TAV SMC, p=.048. When seeded on collagen coated plates, TAV SMCs have significantly increased survival compared to BAV SMCs when treated with tBHP at 1200, 2400 and 4800μM, (57.9% vs 79.2%, p=.036; 59.4% vs. 78.1%, p=.01; 67.5% vs 87.5%, p=.001, respectively) . The average adhesion forces to the STEP fibers of the SMCs were found to be 12.4±2.4, 14.5±1.4, and 12.9±0.8 nN. The average IO force for the three cell populations was 12.9±1.0 nN. The calculated IO force in cells exposed to hydrogen peroxide (500μM) was decreased by about one-half that of SMCs under normal culture conditions (5.6±0.7 nN vs. 12.9 ±1.0 nN, $p < 0.01$).

Conclusion: The results of this study suggests that oxidative affects SMC interactions with the substrate, likely via integrin mediators. With these models, we can modulate the orientation and anisotropy of the collagen substrates via processes like macromolecular crowding to simulate ECM of BAV. Also, nanofiber scaffolds can also reproduce the orientation seen in the ECM of BAV. These models will allow us to further investigate ROS effects on ECM-cell interactions in future studies.

References

1. Keane MG, Wiegers SE, Plappert T, Pochettino A, Bavaria JE, Sutton MG. Bicuspid aortic valves are associated with aortic dilatation out of proportion to coexistent valvular lesions. Circulation. 2000;102(19 Suppl 3):Iii35-9.
2. Phillippi JA, Klyachko EA, Kenny JPt, Eskay MA, Gorman RC, Gleason TG. Basal and oxidative stress-induced expression of metallothionein is decreased in ascending aortic aneurysms of bicuspid aortic valve patients. Circulation. 2009;119(18):2498-506.
3. Tsamis A, Phillippi JA, Koch RG, Chan PG, Krawiec JT, D'Amore A, et al. Extracellular matrix fiber microarchitecture is

Hydrogel Composition Regulates Chondrogenesis by Mesenchymal Stem Cells and Endochondral Ossification in Engineered Cartilaginous Interfacial Tissues

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Introduction: The physis is the cartilaginous interfacial tissue that drives appendicular skeletal growth in children. Physeal cartilage exhibits a stratified cellular architecture with chondrocytes in zones of distinct differentiation states, including polymorphic, proliferative, and hypertrophic zones [1]. To successfully regenerate the physis, a tissue engineering approach is needed that supports the physeal chondrocyte states and proper interzonal signaling.

Materials and Methods: We evaluated the effects of two hydrogel formulations on osteochondrogenesis of human mesenchymal stem cells (hMSCs) and on progression of chondrocytes through their differentiation states (chick cells isolated from the sternae) using a subcutaneous model in mice. We encapsulated the cells and transforming growth factor β3 in methacrylated gelatin hydrogels (GEL, 10% w/v) and a blend hydrogel (PGH, 10% w/v) composed of GEL, methacrylated polyethylene-glycol, and methacrylated heparin.

Results: Over 8 weeks, the PGH hydrogel promoted only chondrogenic differentiation of hMSCs and augmented maintenance of chick sternal chondrocytes in a glycosaminoglycan (GAG) producing state while promoting timely development of the hypertrophic phenotype. The GEL hydrogel favored osteogenesis of hMSCs and led to decreased GAG synthesis, accelerated hypertrophy, and ECM mineralization in chick chondrocytes.

Discussion and Conclusions: The PGH hydrogel is ideal for physeal engineering because it supports endochondral ossification while inhibiting direct osteogenesis by progenitor cells. Biomaterials that can control cell phenotype progression are essential for regenerating cartilaginous tissue interfaces throughout the skeleton.

Reference:
1. Chung, Recent research on the growth plate. J Mol Endocrinol 53,2014.

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The effects of cardiac extracellular matrix aging on macrophage polarization

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Historically, the mammalian heart was thought to be a post-mitotic organ that possessed little to no regenerative capacity following cardiomyocyte depletion[1]. However, investigators have recently demonstrated potent cardiac regeneration in neonates and limited cardiomyocyte proliferation in aged individuals following myocardial infarction[2,3]. Of particular note, the observed neonatal cardiac regeneration was not holistically dependent on non-terminally differentiated cardiomyocytes but was instead dependent on the macrophage populations present in the tissue[3]. Recently, a study conducted by Lavine et. al. demonstrated the ability to recapitulate this neonatal regenerative response in adult cardiac tissue through the selective recruitment of extra-embryonic yolk sac-derived macrophages to the infarct site[4]. However, the factors governing the maintenance and recruitment of these macrophages in aging individuals remains unclear. Cardiac extracellular matrix (ECM) has been shown to undergo compositional changes with increasing age that can impact cardiovascular functionality[5,6]. Additionally, aging extracellular matrix has been shown to differentially regulate macrophage phenotype and function[7]. Thus, this study sought to examine the potential role cardiac extracellular matrix aging plays in altering macrophage phenotype and functionality. A skeletal muscle decellularization protocol was adapted and optimized to remove cellular antigens from cardiac ECM. Histology and DNA quantification assays confirmed the decellularization protocol sufficiently removed antigenic materials from the cardiac ECM without significant removal of ECM proteins. The decellularized scaffold was then pepsin-digested and macrophages were exposed to the cardiac ECM degradation products. Following cardiac ECM and cytokine polarization, we found enhanced nitric oxide concentrations in macrophage populations exposed to aged ECM compared to macrophages exposed to young ECM degradation products. Thus, the preliminary results of this study demonstrate the ability of cardiac ECM to modulate macrophage functionality in vitro.

Works Cited:

[1]. Soonpaa MH, Field LJ. Survey of studies examining mammalian cardiomyocyte DNA synthesis. Circ Res. 1998 Jul 13;83(1):15-26.
[2]. Beltrami AP, Urbanek K, Kajstura J, et. al. Evidence that human cardiac mocytes divide after myocardial infarction. N Engl J Med. 2001 Jun 7;344(23):1750-7.
[3]. Aurora AB, Porrello ER, Tan W, et. al. Macrophages are required for neonatal heart regeneration. J Clin Invest. 2014 Mar;124(3):1382-92.
[4]. Lavine KJ, Epelman S, Uchida K, et. al. Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. Proc Natl Acad Sci USA. 2014 Nov 11;111(45):16029-34.
[5]. Spadaccio C, Mozetic P, Nappi F, et. al. Cells and extracellular matrix interplay in cardiac valve disease: because age matters. Basic Res Cardiol. 2016 Mar;111(2):16.
[6]. Dworatzek E, Baczko I, Kararigas G. Effects of aging on cardiac extracellular matrix in men and women. Proteomics Clin Appl. 2016 Jan;10(1):84-91.
[7]. Sicari BM, Johnson SA, Siu BF, et. al. The effect of source animal age upon the in vivo remodeling characteristics of an extracellular matrix scaffold. Biomaterials. 2012 Aug;33(22):5524-33.

Towards a "Same-Day" Autologous Tissue-Engineered Vascular Graft: Seeding and Implantation of an Elastomeric Scaffold with the Stromal Vascular Fraction

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Significance: Tissue engineered vascular grafts (TEVGs) containing mesenchymal stem cells offer a possible treatment solution for those suffering from cardiovascular disease, however the time needed to culture remains a large hurdle for TEVGs as a treatment option. An alternative TEVG treatment option bypasses the time needed for culture making use of cells taken directly from the stromal vascular fraction (SVF) and obtained directly after liposuction or panniculectomy procedures. However, the ability of an uncultured, "same day" SVF seeded TEVG to remain patent and initiate remodeling in vivo and cell fate after implantation remain unknown.

Objective: The purpose of this study was to determine if SVF seeded TEVGs would remain patent in vivo and remodel after a "same day" implantation.

Methods: SVF, obtained from adult adipose tissue, was seeded within 4 hours after acquisition from the patient onto poly (ester urethane)urea bilayered scaffolds using a customized rotational vacuum seeding device. Constructs, after a short time in culture to allow for cell incorporation into the scaffold, were then surgically implanted as abdominal aortic interposition grafts in Lewis rats for 8 weeks. Patency was documented using angiography and gross inspection, while vascular components, along with the presence of any remaining implanted cells, were detected using immunofluorescent chemistry.

Results and Discussion: Initial findings show patency through both angiography and gross inspection after a period (i.e. 8 weeks) in vivo. IFC analysis was positive for von Willebrand Factor indicating the presence of an endothelium, and smooth muscle alpha actin and calponin suggesting the presence of smooth muscle cells in the neotissue. Such findings demonstrate that a "same day" SVF-seeded TEVG remains patent after implantation in vivo, with remodeling and neo-tissue formation occurring by 8 weeks.

Conclusion: Establishing a "same-day" implementation of a cell based TEVG will advance the technology towards for clinical use and greatly enhance its appeal.

3D Bioprinting of Organ-Scale Type I Collagen Scaffolds

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Collagen has numerous applications in tissue engineering because it is the primary constituent of extracellular matrix in the human body. However, as a material for 3D bioprinting, collagen type I and similar hydrogels cannot be assembled layer-by-layer without extensive support because each fluid must be held in place during gelation. In order to 3D print such fluids, we developed an approach termed Freeform Reversible Embedding of Suspended Hydrogels (FRESH), wherein fluids are crosslinked in a supporting medium and released after gelation.(1) A 3D printed tissue requires dimensional accuracy, manifoldness, and porosity. We demonstrate the simultaneous creation of these by 3D printing intricate objects from collagen solutions using an open-source 3D printing platform and FRESH printing. A cube with a perfusable conduit (designed in CAD software), a mammary epithelial network (generated from Optical Tomography data), and a human heart model (generated from MRI data) were FRESH printed and characterized for fidelity.

Collagen FRESH prints displayed manifold surfaces and details consistent with the small diameter nozzles used. A parametric cuboid with an internal, horseshoe-shaped conduit was designed and the resulting print contained an intact lumen with well-defined entrance and exit. A mammary epithelial network was imaged and converted to a model, and the print consisted of a hollow, dendritic tree that was manifold with well-defined internal and external surfaces. An open-source human heart model created using MRI data, was printed, released and preserved in formaldehyde and displayed intact vasculature as well as pectinate muscle.

Assembling hydrogels with this level of detail represents a first step toward complete control of biopolymer scaffold architecture. Through this method, it is possible to fully render 3D models as hydrogel, regardless of geometric complexity.

Development of a Topical Ophthalmic Biomaterial for the Controlled Release of Cysteamine

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Nephropathic cystinosis is an autosomal recessive disease characterized by the mutation of the CTNS gene and its protein product cystinosin. Cystinosin functions to transport cystine out of cell lysosomes. Lack of cystinosin function causes intracellular accumulation of cystine which crystallizes and damages organ tissues including the kidney, pancreas, thyroid and eyes. Cystine accumulates in all the ocular tissues but is most easily evident as crystal desposits in the cornea that lead to debilitating photophobia, corneal erosion, and can lead to blindness. Corneal cystine crystals are treated by hourly administration of topical cysteamine eye drops. The eye drop formulation requires a high concentration of cysteamine per drop to account for its instability as it is easily oxidized to inactive cystamine. The strict dosing regimen and high concentration of drug per drop make this treatment inconvenient and painful for patients leading to almost universal non-compliance. We hypothesize that an appropriately designed controlled release formulation may decrease the number of eye drops and prolong the effect of treatment, while decreasing unwanted side effects. The purpose of this study is to explore development of a thermoresponsive gel-based eye drop that contains cysteamine-encapsulated microspheres. The thermoresponsive hydrogel matrix is administered as a liquid drop and is retained within the conjunctival cul-de-sac. The target release kinetics for cysteamine is zero-order for a minimum of one day to a maximum of one week, providing patients with an alternative treatment for corneal cystinosis.

Extracellular matrix bioscaffolds reduce corneal scaring after traumatic wounding

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Biologic scaffold materials composed of mammalian extracellular matrix (ECM) have been used for a variety surgical repair reconstruction. They also exhibit a regenerative function in damaged tissues. Here we examined whether ECM bioscaffolds can suppress of scarring in a manner similar to that of the mesenchymal stem cells isolated from human corneal stroma (CSSC) cells. We used pepsin-soluble urinary bladder matrix (UBM) as reported by Huleihel et al. Sci. Adv. 2016. Suppression of scarring was examined in a mouse model of corneal wound healing with UBM applied in fibrin gel at the time of wounding. Scar area 14 days after wounding was calculated in masked fashion from ex-vivo light microscope images. 14 days after wounding corneal scar area was significantly reduced compared to untreated wounds ($p<0.001$, $n=16$). Expression of genes producing fibrotic proteins (tenascin C, smooth muscle actin, collagen III, and SPARC) was significantly reduced in UBM treated wounds in comparison with that of wounded tissue. In conclusion, UBM exerts a high regenerative potential in healing corneal wounds in vivo. It reduced corneal scarring and reduced fibrotic gene expression. These results suggest that UBM may allow a non-cell based therapy for corneal scars in the future.

Wnt7b and Wnt10a, beta-catenin independent signaling regulates cholangiocyte proliferation and function during cholestasis

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Cells found in bile ducts are believed to be bipotential, capable of differentiating into both cholangiocytes and hepatocytes, making them resemble stem cells for both the liver and biliary tract. Wnt/beta-catenin signaling has been implicated as an important factor in hepatoblast atypical ductular reaction and in the liver stem cells of rats and in a variety of liver pathologies. We identified upregulation of specific Wnt proteins in the cholangiocytes during cholestatic liver injury and found that mice lacking Wnt secretion from hepatocytes and cholangiocytes (Alb-cre Wls KO) showed fewer proliferating cholangiocytes and high mortality in response to DDC diet. Therefore, we hypothesize that Wnt induces proliferation of cholangiocytes, which may alleviate some complications caused by cholangiopathies. We have have shown Wnt7B and Wnt10A, via vitro studies, to promote proliferation of cholangiocytes in an autocrine manner. These Wnts are specific for cholestasis, as wild-type (WT) mice show normal expression of Wnt7B and Wnt10A after both 6 and 14 days of choline deficient, ethionine-supplemented diet, which induces hepatocyte injury and ductal hyperplasia unrelated to cholestasis. Proliferation and cell viability were unimpaired in a small cholangiocyte (sm-cc) cell line after β-catenin suppression in vitro under normal growth conditions. To confirm these findings, sm-cc cells were transfected with either Wnt7B or Wnt10A, in combination with control or β-catenin siRNA. The absence of β-catenin did not inhibit Wnt 7B- and 10A-induced increase in cholangiocyte proliferation. Media from sm-cc cells transfected with Wnt7B or Wnt10A was analyzed for cytokine production. Compared to control cells, Wnt7B and Wnt10A induced a significant increase in the expression of cytokines and growth factors, while expression of anti-inflammatory IL-2 decreases in the transfected cells. Thus, β-catenin independent Wnt signaling promotes ductular reaction during cholestasis and may also alter the phenotypic response of proliferating cholangiocytes.

N-cadherin adhesion is coordinated with the actomyosin network to regulate adherens junction dynamics and mechanical coupling in cardiomyocytes

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The junctional complexes that couple cardiomyocytes must transmit mechanical force while maintaining adhesive homeostasis. How these complexes are assembled and regulated to withstand the mechanical forces of contraction is unknown. The adherens junction (AJ) links the actin networks of adjoining cardiomyocytes. The core of the AJ is the cadherin-catenin complex, and N-cadherin is the primary classical cadherin in cardiomyocytes. Cadherins dimerize through a trans interface – forming an intermediate X-dimer and mature strand-swap dimer – and interact through a cis interface. In epithelia, mechanical tension is thought to regulate cadherin trans interactions and stability at AJs. We investigated the adhesive properties of N-cadherin in contracting cardiomyocytes. We measured N-cadherin-EGFP mobility by fluorescence recovery after photobleaching (FRAP) and found that N-cadherin was stabilized at sites of myofibril integration along the membrane. The N-cadherin mobile fraction in cardiomyocytes was similar to E-cadherin in epithelia (26% vs. 23%), whereas the recovery rate was significantly slower (t1/2=154 sec vs. 32 sec), which could reflect the unique topology of cardiomyocyte junctions and/or differential regulation of cadherin engagement. We then tested how trans or cis interactions affected cadherin dynamics. Strand-swap dimerization and cis interactions regulated N-cadherin stability at AJs, whereas X-dimer formation was not required. Our data show that, in cardiomyocytes, N-cadherin is stabilized by the actomyosin network and requires both trans and cis interactions to form stable adhesion complexes. We postulate that N-cadherin adhesive properties are coordinated with actomyosin tension to promote AJ assembly and mechanical coupling in cardiomyocytes.

Evaluation of a Novel Method of Treatment of Tendinopathy with the Use of Extracellular Matrix Hydrogel

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Tendinopathy is an overuse type of injury that is an inflammatory and degenerative disease process affecting over 32 million patients in the U.S. alone [1], and its incidence is rising each year. Although the Achilles tendon is the largest and thickest tendon in the human body, it is one of the most frequently affected. Achilles tendinopathy can range from mild and acute to chronic and severe and requires physical rehabilitation. Achilles tendon rupture associated with tendinopathy is not uncommon. Surgical repair of Achilles tendon rupture associated with tendinopathy is typically required but is associated with high patient morbidity and even re-rupture of the tendon. Ultimately, there is no consensus on the best method of treatment of the Achilles tendon pathologies.

The present study aims to investigate the use of a hydrogel form of extracellular matrix (ECM) as a non-invasive approach to treat tendinopathy. The ECM provides essential cues to govern tissue-specific function by “dynamic reciprocity”: the bidirectional crosstalk between a cell and its surrounding matrix to dictate cell behavior [2]. Herein, the effectiveness of ECM hydrogels to promote tendon repair were evaluated in a rat model of collagenase-induced tendinopathy. Experimental groups include a control group of sterile saline, hydrogels derived from tendon ECM, urinary bladder matrix (UBM), and matrix bound nanovesicles (MBVs) [3] isolated from UBM. Animals were sacrificed at 1 and 6 week time points to evaluate histologic and mechanical remodeling. Histologic evaluation and uniaxial mechanical testing results show that ECM hydrogel treatment promoted restoration of tendon mechanical strength and evidence of site appropriate tissue remodeling via hematoxylin & eosin staining. Future work will investigate the respective abilities of (1) homologous versus heterologous sources of ECM (i.e. tendon vs non-tendon), (2) ECM concentration, and (3) dosing regimens to promote efficient tendinopathy repair.

References:
1. Omae, H., et al., Multilayer tendon slices seeded with bone marrow stromal cells: A novel composite for tendon engineering. Journal of Orthopaedic Research, 2009. 27(7): p. 937-942.
2. Bissell, M.J., H.G. Hall, and G. Parry, How does the extracellular matrix direct gene expression? Journal of theoretical biology, 1982.
3. Huleihel, L., G.S. Hussey, and J.D. Naranjo, Matrix-bound nanovesicles within ECM bioscaffolds. Science …, 2016.

Development of Optimized Photocrosslinked Gelatin/Hyaluronic Acid Scaffold for Repair of Osteochondral Defect

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Objectives: Osteoarthritis (OA) affects about 60% of men and 70% of women above 65 years of age, and represents a global growing health and economic problem. Our laboratory has recently developed a visible light-activated methacrylated gelain/hyaluronic acid (mGL/mHA) hydrogel, which is biocompatible and biodegradable, maintaining high cell viability and promoting the encapsulated human mesenchymal stem cells (hMSCs) to undergo chondral or osseous differentiation with high efficiency by chondrogenic or osteogenic stimulation. The applicability of this novel scaffold for osteochondral repair in vivo was further examined using a rabbit model.

Methodology: A cylindrical defect of 4 mm in diameter and 3 mm in depth were surgically created in the young (5-7 weeks) or aging (13-15 weeks) rabbit femoral condyle and a 3-dimensional (3D) predifferentiated hMSC-biomaterial construct was fabricated in situ to fully fill the lesion site. The efficacy of the in situ formed neo-cartilage to repair the lesion site was estimated at 12 weeks post implantation.

Results: Young rabbits showed strong self healing ability 12 weeks post-surgery. In all groups including defect without treatment, the osteochondral lesion appeared completely covered. Tissue regeneration variation was significant among aging rabbits. The application of mGL/mHA scaffold generally improved filling of the defect and osteochondral regeneration in rabbits. However, no apparent histological difference was observed between the cell-free and cell-encapsulated groups. Tracking of transplanted cells is ongoing.

Delivering Antibodies in Skin Transplant Hosts using IgG-Binding Injectable Coacervates

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We have designed and characterized peptidic composites formed by coassembly of EAK16-II (amino acid sequence: AEAEAKAKAEAEAKAK) with novel Fc-binding domains for concentrating IgG antibodies subcutaneously in vivo. Mixtures of EAK16-II with the domains in low salt buffers undergo sol-gel phase transition upon exposure to physiological ionic strength. The coassembly approach allows mutual optimization of the Fc-binding domain concentration and gelling propensity because the two are decoupled structurally. Antibodies injected subcutaneously in these systems remain stable locally in vivo for up to 12 days, compared to less than 24 h with IgG injected in saline (Biomaterials. 2014, 35:5196; Acta Biomater. 2014, 10:4759; Mol Pharm. 2013, 10:1035; J Control Release. 2016, 28:230:1)

In the current study we characterized a second generation design in which antibodies are displayed on Z34c, a 4.18 kDa mimetic of Staphylococcal protein A (Mw~42 kDa). Z34c is a cyclic peptide, analogous to domain B in the native protein A. Z34c is estimated to have low immunogenicity, based on frequency and relative binding affinities of predicted human MHC-II ligands. We have engineered a tryptophan-inserted Z34c fused with EAK16-II into a plasmid vector. The resultant fusion protein (Mw = 9.02 kDa), named wZ34c_EAK2, was expressed in E.coli and purified. We hypothesized that the coacervates formed by intermixing wZ34c_EAK2 and molar excess of EAK16-II can be used to display IgG at the interface of skin graft and host cutaneous tissue for interdicting leukocytes and cytokines. The wZ34c_EAK2-EAK16-II coacervates were shown to bind IgG in vitro. Retention of IgG conjugated with near infrared dye injected subcutaneously in C57BL/6 mice was extended by the coacervates. The coacervate-IgG composite was found to remain stable underneath skin grafts in vivo for at least 48 h. These data indicate that the materials system can be developed as local or topical antibody delivery modules for suppressing inflammation.

A Single Injection of Protein-loaded Coacervate-Gel Significantly Improves Cardiac Function Post Infarction

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After myocardial infarction (MI), the heart undergoes fibrotic pathological remodeling instead of repair and regeneration. With multiple pathologies developing after MI, treatment using several proteins is expected to address this range of pathologies more effectively than a single-agent therapy. A factorial design of experiments study guided us to combine three complementary factors in one injection: tissue inhibitor of metalloproteinases-3 (TIMP-3) was embedded in a fibrin gel for signaling in the initial phase of the treatment, while basic fibroblast growth factor (FGF-2) and stromal cell-derived factor 1-alpha (SDF-1α) were embedded in heparin-based coacervates for sustained release and distributed within the same fibrin gel to exert their effects over a longer period. The gel was then tested in a rat model of myocardial infarction. Contractility of rat hearts treated with the protein coacervate-gel composite stabilized and slightly improved after the first week while contractility continued to decrease in rats treated with free proteins or saline over the 8 week study period. Hearts receiving the protein coacervate-gel composite treatment also exhibited reduced ventricular dilation, inflammation, fibrosis, and extracellular matrix (ECM) degradation. Revascularization, cardiomyocyte preservation, stem cell homing, and increased myocardial strain likely all contributed to the repair. This study demonstrates the potential of a multifactorial therapeutic approach in MI, using three complementary proteins delivered sequentially for comprehensive healing. The study also shows the necessity of controlled delivery for growth factors and cytokines to be an effective treatment.

Effect of Age on Macrophage Response to Muscle Extracellular Matrix

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Extracellular matrix acts as the supporting structure of a tissue and dynamically changes in a reciprocal relationship with cells of that tissue. Skeletal muscle strength and healing potential are known to decrease with aging. Macrophage polarization, which is dysfunctional with age, has been shown to be necessary for appropriate skeletal muscle healing. This study uses decellularized skeletal muscle ECM from young and aged mice to determine these microenvironmental effects on macrophage phenotype. Skeletal muscle ECM was decellularized from 4 mo and 20 mo old C57BL/6 mice abdominal wall muscle. ECM scaffolds were characterized with H&E, DAPI, Masson's Trichrome, Picrosirius Red, Collagen I IHC, Advanced Glycation End-Products fluorescence and IHC, PicoGreen DNA quantification, DNA agarose gel electrophoresis, SDS-Page protein gel electrophoresis, hydroxyproline, sulfated GAG and fatty acid quantification. Bone marrow macrophages were harvested from 4 mo old C57BL/6 mice. ECM scaffolds were digested in pepsin HCl buffer for 48 hours and used to treat macrophages for 24 hours with or without subsequent 24 hour IFN-y/LPS or IL-4 stimulation. Macrophage phenotype was assessed with immunofluorescent staining for iNOS and arginase. Macrophage function was assessed using Greiss reagent system for nitric oxide production and Vybrant phagocytosis beads for phagocytosis activity. Increased ECM age resulted in a non-significant trend toward decreased sulfated GAG content and significant decreases in hydroxyproline content and fluorescent AGEs. Staining of native and decellularized ECM scaffolds showed a reduction in collagen staining with aged ECM. Treatment of bone marrow-derived macrophages with IFN-y/LPS showed expected increases in iNOS, nitric oxide production and phagocytosis. IL-4 stimulation of macrophages resulted in increases in arginase staining and decreases in phagocytosis. ECM from aged skeletal muscle promoted elevated levels of iNOS labeling with and without IFN-y/LPS post-stimulation. Aged skeletal muscle ECM also promoted a decrease in arginase staining with and without IL-4 post-stimulation. ECM from aged skeletal muscle ECM resulted in enhanced nitric oxide production and phagocytosis with and without IFN-y/LPS post-stimulation. Aged skeletal muscle ECM promotes an increase in macrophage pro-inflammatory phenotype and function which proves that age-related ECM changes are a contributor to altered macrophage responses.

Double component electrospun fibers deposition (DCD): heart valve fabrication with controlled mechanics, microstructure, and anatomy

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Valvular heart disease (VHD) is a major source of morbidity and mortality, requiring approximately 280,000 replacement surgeries worldwide each year [1]. For patients with VHD, current surgical replacement options include: (I) mechanical heart valves made of synthetic materials such as polymers and metals; (II) bioprosthetic heart valves derived from bovine or porcine tissue, or homograft valves. Mechanical heart valves are durable and can last for more than 20 years, but are limited by potential thromboembolic complications which require lifelong anticoagulation therapy. Bioprosthetic heart valves have reduced thromboembolic effects and do not require anticoagulation therapy, but durability is affected by calcification and leaflet failure. Tissue engineering has the potential to overcome major drawbacks such as the need for anticoagulation therapy and the risk of calcification [2, 3]. Electrospun processed woven meshes have shown the ability to fine-tune structure and mechanics. This work introduces a novel processing method for electrospun valves: double component electrospun fibers deposition (DCD). DCD principle is based on a combination of a conductive collecting electrode and a non-conductive shield, which allows for microfibers deposition onto complex geometries, while maintaining the ability to control fabrication thickness, mechanics, and microstructure. Results documented control over in-plane and out-of-plane mechanics as well as microarchitecture and anatomy. Static and dynamic valve function has been assessed for the tricuspid indication, showing performance comparable with commercially available bioprosthetic valves.

1. Pibarot, P. and J.G. Dumesnil, Prosthetic heart valves. Circulation, 2009. 119(7): p. 1034-1048.
2. Hoerstrup, S.P., et al., Functional living trileaflet heart valves grown in vitro. Circulation, 2000. 102(suppl 3): p. Iii-44-Iii -49.
3. Mol, A., et al., Autologous human tissue-engineered heart valves. Circulation, 2006. 114(1 suppl): p. I-152-I-158.

Composite Adipose Derived Delivery System for Adipose Restoration

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Musculoskeletal injuries inflicted by wars, congenital deformities, tumor resection, and general traumatic injuries often require soft tissue reconstruction. Damages to soft tissue can not only affect cosmetic appearance, but also hinder function and emotional well-being. Autologous adipose grafting using processed lipoaspirate is a safe, resourceful, and minimally invasive option gaining tremendous momentum in clinical practice due to potential applications in trauma and reconstructive surgery, especially for breast cancer reconstruction. However, results can be unpredictable due to graft resorption rates reaching as high as 90%. These limitations serve as motivation for the development of new therapies to regenerate adipose tissue. The goal of this study was to evaluate quality and efficacy of a composite adipose derived delivery system (CADDS) containing adipogenic factors to increase adipogenesis in human adipose-derived stem cells (ASC) culture before examining volume retention in an immunocompetent rat model. Abdominal fat was obtained from a non-diabetic female undergoing elective panniculectomy at the University of Pittsburgh Medical Center, approved by Institutional Review Board. Decellularization consisted of alcohol rinses, delipidization, and disinfection of adipose matrix. Adipose derived matrix was frozen using liquid nitrogen and lyophilized. Room temperature milling reduces the matrix to a powder for pepsin digest. The pH of adipose extracellular matrix-pepsin solution was balanced to 7.4 and raised to 37 C to gel. Poly(lactic-co-glycolic) Acid (PLGA) (50:50) was used as the base polymer to encapsulate the adipogenic factor, dexamethasone, into microspheres being the second component of the composite delivery system. The decellularization protocol, confirmed by Quant-IT PicoGreen, resulted in DNA content less then 20 ng/mg while preserving total protein content of 23.67 ug/mg and 6.5 ug/mg of sGAG content in the matrix. After 58 days in degradation buffer, remaining CADDS mass percentage was 5% in total. Images of Oil Red O stained lipids from the 14-day in vitro study of ASC differentiation demonstrated increased adipogenesis when comparing the control sample to the CADDS experimental condition. Preliminary animal studies will be conducted using an immunocompetent rat animal model to observe volume retention and tissue remodeling up to 6 weeks.

The Role of Vinculin in Cardiomyocyte Adhesion and Mechanical Continuity

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Cardiomyocytes are joined end-to-end by a complex adhesive structure known as the intercalated disc (ICD) to create a functional syncytium. The ICD is composed of mechanical and electrical junctions including the adherens junction (AJ), whose core is the cadherin/catenin complex. Myofibrils – contractile machinery – are coupled to the ICD; however, this mechanical linkage is poorly defined. The force produced by a contracting cardiomyocyte is greater than epithelia, raising the question of how AJs are capable of maintaining adhesion through cyclical contractions. In epithelia, tension applied to the AJ is sensed in part through alpha-catenin, which connects the AJ to actin. Mechanical force alters alpha-catenin to reveal a binding site for vinculin, thus strengthening the connection. We investigated the role of alpha-catenin and vinculin in linking the AJ to actin in cardiomyocytes. Using a FRET tension sensor in N-cadherin, we found that N-cadherin is under tension at cell-cell contacts. We then measured protein mobility of EGFP-N-cadherin by FRAP and found that N-cadherin dynamics were reduced at sites of myofibril integration, suggesting that myofibrils stabilize N-cadherin. To examine the organization of the myofibril-AJ interface, we used immunogold-labeling platinum replica EM and found both vinculin and alpha-catenin decorating actin filaments at cell-cell adhesions. To further delineate the individual and combined roles of alpha-catenin and vinculin in regulating cadherin stability, we built a series of fluorescently-tagged N-cadherin:alpha-catenin fusion constructs. A constitutively active alpha-catenin increased vinculin recruitment and decreased the mobile fraction of the N-cadherin fusion, suggesting that vinculin recruitment stabilizes the AJ. In contrast, blocking vinculin binding and restricting actin binding to alpha-catenin increased the dynamics of the N-cadherin fusion, suggesting that vinculin binding is required to stabilize the AJ in cardiomyocytes. Together, our data support a model in which alpha-catenin and vinculin cooperate to anchor myofibrils at the ICD to provide mechanical continuity between cardiomyocytes.

Fluid Dynamics Assessment of Microfibrillar, Elastomeric Heart Valve Scaffolds

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Approximately 90,000 heart valve replacement procedures are performed each year in the United States alone. The two most common clinically used solutions, mechanical and bioprosthetic valves, have shortcomings that limit quality of life of their recipients and require further treatment, often reoperation, to ensure proper device function. For mechanical valves, the major drawback is device-initiated thrombosis and the need for anticoagulation therapy. Bioprosthetic valves do not require anticoagulants, yet still may fail due to calcification or structural deterioration. No current therapy would reduce the drawbacks of both bioprosthetic and mechanical valves and improve long-term patient quality of life. Recent advancements in tissue engineering have revealed valve prostheses that promote tissue growth and remodeling. Thermoplastic, fibrillar elastomeric scaffolds stand out for their capacity to be manipulated into complex geometries. Specifically, polyurethanes are noted for superior durability, biocompatibility, and support of endogenous tissue growth. Electrospinning, a technique by which a positively charged polymer is directed through a metal spinneret to a grounded target mandrel, has been identified as a potential method of producing polymeric scaffolds useful for valve replacement. Manipulation of fabrication parameters enables production of polymer scaffolds with any specified degree of fiber alignment and mechanical anisotropy. Relevant parameters in valve function are the mechanical response of the valve due to physiological stress conditions and the fluid mechanics environment surrounding the valve. I aim to mechanically characterize microfibrillar elastomeric heart valve scaffolds via biaxial and flexural mechanical testing and analyze the resulting fluid mechanical environment via particle image velocimetry and valve leaflet strain mapping. The goal of this work is to demonstrate and document that electrospun valve prostheses do not share the same limitations of current clinically utilized valves.

An in vitro Chondro-Osteo-Vascular Triphasic Model of the Osteochondral Complex

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The development of veritable in vitro models of the osteochondral (OC) interface tissue is essential to understand the biology of cartilage/bone and to develop high throughput screenings for the study of degenerative joint diseases, such as osteoarthritis. Tissue engineering approaches have exploited the combination of biodegradable polymers, cells and bioactive molecules in a variety of composite structures; however, there are as yet no satisfactory in vitro models of the osteochondral complex, mainly because of the inability to produce a stable vasculature in the bone compartment. Here we report the development, within a microphysiological system bioreactor, of a novel triphasic model of vascularized osteochondral interface tissue. The chondral construct is based on photocrosslinkable methacrylated gelatin (gelMA) seeded with human bone marrow mesenchymal stem cells (MSCs); the vascularized bone construct is based on additively manufactured poly(epsilon-caprolactone) (PCL) or PCL/hydroxyapatite (HA) (PCL/HA) porous scaffold seeded with MSCs and endothelial cells (HUVECs). Histology (alcian blue/alizarin red/H&E staining) revealed MSCs chondrogenic/osteogenic differentiation on the gelMA/PCL-PCL/HA constructs, respectively. qRT-PCR analysis showed up-regulation of chondral and osseous gene expression in the gelMA and PCL-PCL/HA construct, respectively. Constitutive GFP-expressing HUVECs formed interconnected networks of capillary-like structures in the bone compartment clearly detectable by epifluorescence microscopy. Presence of the HUVECs enhanced MSCs osteogenesis and chondrogenesis in both PCL-PCL/HA scaffolds and gelMA phases compared to HUVEC-free controls, based on histology and gene expression. These results indicate that our construct mimics native OC tissue in terms of structure and gene expression, in particular tissue-tissue communication, suggested by the effect of subchondral HUVECs on MSCs of the upper chondral compartment. The triphasic microtissue model recapitulates three different tissue types of the OC unit, and represents a key step towards the development of an effective, in vitro analog of this composite tissue, potentially applicable as a biomimetic platform for drug screening.

Peripheral Nerve-Specific Extracellular Matrix Hydrogel Supports Repair after Peripheral Nerve Injury

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Peripheral nerve injury commonly results in loss of neuromuscular function, often resulting in significant impact upon both quality of life and cost of care for patients. In moderate and severe cases of peripheral nerve injury, such as those involving nerve transection, long-term outcomes are poor even with state-of-the-art microsurgical repair techniques and products. One promising target for improving patient outcomes is the use of a peripheral nerve specific extracellular matrix hydrogel (PNS-ECM). PNS-ECM is an injectable gel generated from the extracellular matrix of healthy porcine nerve tissue. PNS-ECM provides a tissue-specific microenvironment which is conducive to nerve repair, including: nerve specific growth factors that are chemotactic signals for Schwann cells, promote neurite outgrown, as well as factors that modulate the macrophage inflammatory response to injury. PNS-ECM was created by decellularizing porcine sciatic nerve by a previously described method. The PNS-ECM was validated for removal of immunogenic cellular components, retention of beneficial nerve specific proteins, and tested for bioactivity in a series of in vitro and in vivo experiments. We found that PNS-ECM significantly enhanced Schwann cell migration and axon extension in vitro and in vivo using a critical length nerve defect rodent model. We also confirmed that exposure to PNS-ECM generates a unique macrophage phenotype and a greater M2:M1 ratio than negative controls. Rodents treated with PNS-ECM were also seen to experience about 15% less muscle atrophy and similarly significant improvement in function locomotion return when compared to conduit alone. PNS-ECM is now being compared to the standard of care or autograft repair using the sciatic functional index in a critical length sciatic nerve gap injury model. As an easily injectable material which promotes recruitment of alternately activated, M2 macrophages, Schwann cell migration, and axon extension, we believe that PNS-ECM would significantly improve quality of life for affected patients.

Improved Elastogenesis for Pediatric Patients with Genetic Defects of the Aorta

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Aortic aneurysms are enlargements of the ascending thoracic or infrarenal abdominal aorta that occur in the aging population (primarily smokers), and within pediatric patients with inherited connective tissue genetic disorders. Mediated by inflammatory damage to elastic fibers, these aortic aneurysms undergo dilation, reduction in wall strength, and ultimately rupture.

A regenerative therapy, wherein functional elastic fibers are restored, could offer a non-surgical therapeutic option for both adults below the “critical dilation diameter” necessitating surgical intervention, and for children with genetic connective tissue disorders as an aortic maintenance combination therapy with beta blockers and ACE inhibitors.

Recent work from our group has shown that adipose-derived mesenchymal stem cell (MSC) delivery to a growing elastase-induced mouse aneurysm slows growth and preserves elastic lamellae. While multiple mechanisms could explain the MSC effect, in this study we tested the hypothesis that MSCs secrete factors that lead to elevated elastic fiber production by vascular smooth muscle cells (SMC). The central method for this study was 3D fibrin gel culture of healthy adult human SMCs, in the presence or absence of MSC-conditioned media (CM). Total elastin content was measured by biochemical assay and elastic fiber morphology was analyzed by indirect immunofluorescence. Aspects of fiber microarchitecture were quantified by a custom Matlab image analysis script.

After 3 weeks of incubation within 3D fibrin gels, while SMCs produced substantial amounts of insoluble elastin (1% total protein) without exogenous stimulation, CM induced a 3-fold elastin deposition increase. Imaging the elastic matrix revealed increased continuous, thick elastic fibers when CM was used. Image quantification revealed a higher intersection density with CM, potentially reflecting more intrafiber elastin crosslinks.

We conclude that secreted factors from MSC are capable of stimulating elastic fiber assembly by SMCs, perhaps mediated through the activity of elastin accessory proteins. We are currently planning to integrate explanted aortic SMCs from pediatric patients with connective tissue disorders to further assess this pro-elastogenic CM therapy.

Nitrite Regulates Mitochondrial Dynamics to Inhibit Vascular Smooth Muscle Cell Proliferation

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Nitrite, a dietary constituent and endogenous signaling molecule previously thought to be a biologically inert product of endogenous nitric oxide oxidation, has recently been shown to regulate a myriad of biological processes. For example, nitrite has been shown to inhibit smooth muscle proliferation and attenuate restenosis after vascular injury. However, the mechanism of nitrite-dependent inhibition of smooth muscle proliferation remains elusive. Nitrite is an established regulator of mitochondrial morphology and function, and mitochondrial dynamics have been previously shown to regulate cell cycle progression. Thus, we hypothesized that nitrite modulates mitochondrial dynamics and function to inhibit cell cycle progression and attenuate smooth muscle cell proliferation. Using rat aortic smooth muscle cells (RASMCs) we demonstrate that nitrite inhibits RASMC proliferation induced by platelet derived growth factor (PDGF) in a concentration dependent manner. This phenomenon is associated with mitochondrial fusion dependent on the upregulation of mitofusin -1 (Mfn1). Further, nitrite treatment upregulates the cyclin dependent kinase inhibitor p21, an effect that is abolished in Mfn1 deficient RASMCs. Ongoing studies are focused on determining the mechanism by which nitrite upregulates Mfn1 and stimulates mitochondrial fusion. These data have important implications for dietary and pharmacological modulation of vascular health and uncover a novel potential physiological mechanism for the regulation of smooth muscle cell number.

Tissue-specific Effects of Normal, Metaplastic, and Neoplastic Esophageal Extracellular Matrix Hydrogels

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Cancer biology tools to recapitulate the microenvironment have largely remained unchanged: collagen gels and Matrigel have been the gold standard for the past 50 and 33 years respectively. One stimulus for the present study was to develop a new cancer biology technology: disease-specific extracellular matrix (ECM) hydrogels.

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

The three types of ECM showed distinctive fiber networks by SEM and showed distinctive protein profiles by Silver Stain/SDS-PAGE and targeted mass spectroscopy. Metaplastic and neoplastic ECM activate macrophages (human THP1) to a dual ``pro-inflammatory" (TNFalpha+) and ``immunomodulatory" (IL1RN+) state, with increasing expression as ECM tumorigenicity increased by qPCR/ELISA and corroborated by immunolabeling. Neoplastic ECM modulates the macrophage secretome to increase migration of normal esophageal epithelial cells, characteristic of migratory tumor cells. Top differentially regulated MBV miRNAs were notably related to epithelial-mesenchymal transition, a known mechanism of EAC progression; gastrointestinal cancer; and macrophage activation by small RNA sequencing/ pathway analysis, suggesting the direct role of the ECM in dynamically instructing cell behavior.

In conclusion, a novel ECM hydrogel “progression series” was developed. A better understanding of diseased ECM MBV miRNA cargo and disease-specific macrophage activation will guide EAC regenerative medicine strategies.

Construction of a Muscle-Derived Extracellular Scaffold intended to Promote Muscle Function after Nerve Injury

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Nerve injury is estimated to affect 22 million individuals in the United States with annual medical spending in the tens of billions of dollars. A disproportionate number of young adults suffer from these conditions due to higher incidence of trauma and debilitating battlefield injuries. Additionally, nerve damage of moderate severity promotes atrophy to the near muscles. Despite interventions having the primary goal of reinnervating target tissues, the nerve has proven to be notoriously difficult to therapeutically reinnervate after trauma, and in turn perpetuates an atrophic, denervating environment for the local muscular structures. Numerous studies have investigated muscle-derived ECM, amongst others, to regenerate muscle power and function in volumetric muscle loss (VML) models; poor muscle function and its regeneration as they pertain to nerve injury however, is a relatively unexplored niche with prospective solutions well-translatable into the VML arena. Using LabVIEW, Arduino, and various 3D-printed components, a small-scale bioreactor was developed allowing a scaffold to be in constant fluidic free fall while being exposed to a constant source of fresh solution. Using this system, a decellularized muscular scaffold was generated using skeletal muscle from the hind limbs of Lewis rats. Decellularization was performed using exchanges of sodium chloride and a surfactant cocktail solution, being sodium dodecyl sulfate and Triton X-100 in ethanol; the decellularized scaffold was then disinfected using peracetic acid (2% w/v). The scaffold was freeze-dried and digested into a hydrogel using a pepsin solution such that flow through a 26G needle was achievable. The protein concentration was able to be modulated using various centrifugal forces, also effecting the dispersive properties of the gel. These dispersive properties will translate rheologically in situ, relating to how the gel can be interspersed within a native tissue construct. It is our intention to use this muscle-derived scaffold to regenerate atrophied muscle with a process and material able to be readily translated to the clinical setting.

Form and Mechanical Function of Germ Layer Architecture in the Developing Embryo

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To understand the connection between genetics, the environment, and birth defects we are particularly interested in physical and genetic factors that regulate tissue mechanics in the developing embryo. Tissues within embryos of the frog Xenopus laevis increase in modulus six-fold during early development; however, the origin of this increase is not well understood. This stiffening happens at a time where the tissue is changing shape to form complex architectures.

To investigate the contribution of tissue architecture to bulk tissue modulus, we microsurgically isolate dorsal embryonic tissues from early neural (stage 14) and late neurula (stage 21) embryos, gently dissociate the tissues, and reaggregate cells into a novel, 'scrambled' tissue that is similar in shape and cell density to the original. Fibronectin immunofluorescence reveals only minor loss of ECM as local cell domains remain surrounded by fibrillar fibronectin. However, fibrils reveal that the overall laminar germ layer structure is effectively disrupted. Remarkably, mechanical testing of stage-matched scrambled tissues showed they retain the moduli of natively structured dorsal tissues. This suggests that the source of stiffness is inherent to individual cell stiffness, rather than the laminar architecture in which the cells are arranged. [Note: the laminar architecture plays a role in morphogenesis but not in differentiation since scrambled tissues no longer converge and extend as native dorsal isolates but are able to differentiate to somites and remodel their laminin and fibrillin ECM comparable to later stage native tissues.] We next tested whether tissues scrambled at stage 14 had the capacity to stiffen to equivalent stage 21. We found scrambled tissues stiffened by significantly by 45% but were not able to match the stiffening (250%) observed in either native or tissues scrambled at stage 21. Taken together, this suggested to us that tissue architecture is not important for maintaining tissue modulus in early and late stage dorsal tissues; however native architecture is required for maturation of tissue mechanical properties.

To elucidate the developmental regulators of tissue mechanics we are now focusing on dynamic changes in the F-actin cytoskeleton. Studies are underway to quantify how F-actin assembly and stabilization contribute to stiffening of the dorsal axis. Understanding how tissue architecture and cytoskeletal regulation affect embryonic tissue mechanics is imperative to uncovering the programs that control development, regeneration, and disease.

Resorbable vascular grafts support early cell infiltration and endothelialization in a porcine vascular access model

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50% of prosthetic vascular grafts will require reintervention by 1 yr when used as dialysis access conduits. Only 15% of native vessels will. We are developing a synthetic dialysis graft that remodels into a living vessel in situ. Grafts were fabricated from a tubular core of electrospun poly(glycerol sebacate) (PGS), a wrap of electrospun polycaprolactone (PCL), and an adhesive/sealant layer of poly(L-lactide-co-caprolactone) (PLCL). 2-5 cm long grafts were implanted as carotid-jugular or femoral-femoral shunts in a pilot set of pigs for 14 or 28 d. The external PCL/PLCL reinforcement improved the elastic modulus of the graft from 130±40 kPa to 2500±800 kPa. The composite graft had a compliance of 1.6±0.4%/100 mmHg and a burst pressure of 1605±298 mmHg. The suture retention load was 287±86 gf. In vitro cannulation testing confirmed that composite grafts demonstrated more complete needle hole closure than standard ePTFE grafts. By day 14 in pigs, staining indicated cells had infiltrated 2/3 of the graft wall in spite of the nonporous ablumenal PLCL coating. New ECM had been deposited on the lumenal surface, and CD31+ cells lined the lumen. Grafts were successfully cannulated with a dialysis needle at 14 d in vivo. However, grafts maintained past 14 d occluded. Explants and histology suggested degradation of the compression-resistant PGS core had enabled collapse of the stiff wrap/coating layer. Future work will utilize uncoated grafts and further study infiltrating cell phenotypes, neotissue structure, and medium-term patency rates.

Novel Conduits with Wall-Encapsulated Cells Improve Peripheral Nerve Regeneration

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Cell incorporation within biomaterial nerve conduits has been shown to confer a myriad of beneficial effects thought to derive from neurotrophic factor secretion. However, the application of cells lacks control and is generally limited to injection into the conduit lumen or adsorption to the surfaces of the conduit after fabrication. In addition, the cell sources typically used are limited in both quantity and immediate availability. We report here the application of a readily available and expandable cell type, induced mesenchymal progenitor cells (iMPCs), for peripheral nerve regeneration that exhibits neurotrophic potential similar to bone marrow stem cells (BMSCs). In addition, a method to immediately encapsulate cells into the walls of a nanofiber conduit during fabrication has been developed, allowing for tight control of cell number and spatial distribution away from the lumen. The resulting nerve conduit demonstrates flexibility and mechanical strengths necessary for suture retention. Immunofluorescence staining shows that the encapsulated cells respond to nanofiber topography with concentric distribution of cells visualized within the conduit. Chick embryonic dorsal root ganglia cultured on constructs with encapsulated human BMSCs or iMPCs both demonstrate remarkably enhanced neurite extensions up to twice the length of controls. This novel strategy of conduit fabrication incorporating a new cell type presents a promising new approach to enhance and optimize the potential of cell application in peripheral nerve repair. (Support: NIH CATER and DoD W81XWH-10-2-0084)

Biomechanical evaluation of gelatin/fibrinogen electrospun cylindrical scaffolds seeded with 3T3 mouse fibroblasts and porcine smooth muscle cells

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Heart disease is the major cause of death in the United States, with an estimated 83.6 million Americans with one or more type of cardiovascular disease (CVD). Mortality data from 2010 shows that CVD is the underlying cause of death for 1 out of every 3 Americans. There is an increasing demand for affordable, compatible and more easily accessible coronary vascular grafts. Tissue-engineered vascular grafts (TEVGs) offer a possible solution. The purpose of this study was to better understand the mechanisms of biomechanical changes that non-synthetic TEVGs undergo when seeded with 3T3 mouse fibroblasts (3T3FB) and porcine smooth muscle cells (SMCs) cultured ex vivo in a custom bioreactor.

Gelatin/fibrinogen cylindrical constructs were fabricated by electrospinning a 10% (w/v) of gelatin/fibrinogen (80:20) in HFP onto a rotating translating mandrel and crosslinked with glutaraldehyde vapor. The constructs were seeded with a normalized concentration of either cultured 3T3MF or a mixture of 3T3MF and PSMCs by using a custom-built vacuum lumen seeding device and by pipetting cellularized media onto the constructs. The seeded constructs were then transferred into custom-built bioreactors for culturing.

The cell proliferation assay results also suggested that constructs that were seeded with only 3T3FB cells proliferated better than those of the mixed culture. Biaxial mechanical testing indicated that there were some experimental groups that became slightly more compliant than the control. In addition, many of the construct experimental groups were able to achieve higher axial strains than that of the controls, suggesting more mechanical integrity and higher deformability. These results support the idea that it may be possible to further modulate the mechanical properties of non-synthetic conduits, which would allow more custom development of durable and biocompatible TEVGs. Furthermore, the current ex vivo bioreactor study may provide information related to the in vivo durability and biocompatibility of these TEVGs.

Biomechanical analysis of cell behaviors during neural plate convergent extension

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The formation of the neural tube, the primordial structure of the central nervous system, is one of the earliest embryonic morphogenetic events. At the start of neurulation, the dorsal ectoderm epithelium of the embryo, comprised of the neural plate and non-neural tissue, undergoes convergent extension (CE). The lateral edges of the neural plate converge towards the dorsal midline while the tissue extends in the perpendicular anterior posterior (AP) direction. To achieve this anisotropic tissue deformation, dorsal ectodermal cells must coordinate and orient their behaviors. Increasing evidence suggests tissue tension may direct cell behaviors. We seek to elucidate whether the tissue mechanical environment regulates the cell behaviors that facilitate neural plate CE in Xenopus laevis embryos.

From high-resolution confocal time-lapse sequences we performed a kinematic analysis of the apical dorsal surface of Xenopus laevis embryonic ectoderm as the neural plate is shaped. We observed distinct topological and cell shaping patterns between the neural and non-neural ectoderm during tissue CE. Within the neural plate, cells undergo directed rearrangement into narrow AP elongated multicellular arrays. Cells at the lateral border of the neural plate alter their apical shape becoming AP elongated. To understand if these behavioral programs are cell autonomous, we performed grafting experiments to introduce cells to different external tissue environments. When the neural plate is grafted into isotropically spreading non-neural explants neural cells continue to rearrange. Alternatively, non-neural cells grafted into the center of the converging and extending neural plate stretch in the AP direction and mirror the tissue level deformation. Grafted cells had similar phenotypes to neural plate border cells. We hypothesized that border cells may passively deform due to anisotropic tissue tensions generated during CE. To test this, we used dorsal tissue explants that maintain their ability to converge and extend in isolation. Physically constrained explants whose AP extension was blocked exhibited altered the tissue strains. Rearrangement persists in the neural plate in both constrained and freely elongating control explants. Border cell elongation and orientation in constrained explants significantly differ from control free explants. These results give insights into the multiscale mechanics of neural plate convergent extension and suggest processes that link cell behaviors to tissue level deformations. Further exploration of this relationship may reveal how aberrant mechanics cause defective morphogenesis.

Stem cell derived Extracellular Matrix Enhancement of Autologous Chondrocytes Implantation (ACI) for Articular Cartilage Repair

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Objectives: Mesenchymal stem cell derived extracellular matrix (MSC-ECM), a natural material with good biocompatibility and bioactivity, have been used as scaffold for tissue engineering. In this study, we explored the applicability of MSC-ECM as the culture substrate for chondrocytes expansion, and tested the capacity of chondrocytes impregnated MSC-ECM, without cell detachment after expansion, in forming cartilage tissue. We hypothesized that MSC-ECM would significantly enhance chondrocyte proliferation, maintain chondrocytic phenotype and promote robust in vivo articular cartilage tissue formation.

Methodology: Human bone marrow derived MSCs (hBMSCs) and human chondrocytes were obtained with IRB approval. hBMSC-ECM was collected after 10 days of hBMSCs culture. Chondrocytes were cultured on hBMSC-ECM after MSCs were devitalized. Cell proliferation, gene expression and chondrogenic capacity were analyzed. In vivo cartilage formation was examined by implanting constructs subcutaneously into SCID mice, with chondrocyte cell sheets as the control.

Results: Comparing to those on tissue culture plastic (TCP), chondrocytes grown on hBMSC-ECM showed increased proliferation rate as well as less dedifferentiation when reaching same cell number. In micromass culture, higher expression of chondrogenic genes and cartilage matrix deposition were observed in ECM-expanded chondrocytes, which were further supported by higher pSMAD2/3 expression with transforming growth factor-β (TGF-β3) stimulation. After being implanted into mice, chondrocytes impregnated hBMSC-ECMs produced significant amount of cartilage matrix, revealed by safranin O staining. Similar phenomenon was however not observed in chondrocyte cell sheets group, which were slowly absorbed without obvious new tissue formation.

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Long Gap Median Nerve Regeneration Using Tissue Engineered Guides in a Non-Human Primate Model

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Long gap (>3cm) peripheral nerve injuries are common among military and civilian patients. The gold standard of care for these injuries is autografting. The disadvantages of this are long operative times, sensation loss, multiple incisions, and nerve damage. Autografting may not be possible when multiple injuries have occurred because of insufficient donor tissue. To remedy this, we are investigating a biodegradable poly(caprolactone) (PCL) conduit with glial cell line- derived neurotrophic factor (GDNF) to actively support nerve regeneration in non-human primates (NHPs).

A 5cm defect was created on the median nerve and repaired with an autograft, decellularized nerve allograft, empty PCL conduit, or PCL/GDNF conduit. NHPs were trained using a modified Klüver board to assess functional return out to 1-year postoperatively. Baseline and one-year postoperative electrophysiology was recorded to assess nerve conduction velocity (NCV), motor evoked potentials (MEPs), compound nerve action potentials (CNAPs), and sensory nerve action potentials (SNAPs). Histology was performed to evaluate Schwann cell density and neurofilament.

NHPs demonstrated 70-80% successful precision grip at baseline. At POD 60, baseline pinch retrieval was observed. At POD 100 baseline time retrieval was observed. PCL/GDNF group had an average functional return of 58% at 1-year post operatively which is comparable to autograft. PCL empty had a 27% return of function at 1-year. NCV and MEPs were observed at one year in PCL/GDNF and decellularized nerve groups meaning the nerves were able to regenerate and reach the target muscle. No significant difference was found in Schwann cell density between PCL/GDNF, decellularized, or autograft at one year. PCL/GDNF had less nerve fibers and differences in fascicular structure at 1-year. Data suggests PCL/GDNF and decellularized nerve groups are able to regenerate the nerve across a 5-cm gap and support reinnervation of the target muscle. Future work will focus on completing the study and clinical translation.