

2022 McGowan Retreat

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Design, Development and In Vitro Testing of Solid Lipid Nanoparticles for Treatment of Inflammation

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Curcumin is a natural anti-inflammatory product obtained from the roots of *Curcuma longa* with poor bioavailability and stability in vivo. Here we report novel solid lipid nanoparticle (SLN) formulations designed to improve curcumin bioavailability and anti-inflammatory efficacy. Curcumin encapsulated SLNs were formulated employing Solvent evaporation method using the chosen Lipid and Surfactant. SLNs were then evaluated in detail for their in vitro stability and performance efficacy. Quality control tests included measurements of particle size (PS), Polydispersity Index (PDI), Zeta potential, and exposing SLNs to varied stress tests. The Scanning Electron Microscopy (SEM) revealed SLNs have the spherical shape. SLNs were stable in vitro upon storage and when exposed to biological media at body temperature. We also tested how well SLNs can be reconstituted post lyophilization. We found that lyophilized SLNs also are stable under stress and biological media conditions. In conclusion, curcumin was successfully loaded into SLNs which were stable and can be produced by scalable manufacturing processes.

Investigating the effects of physiological shear and nanoparticle size on particle phagocytosis in monocytes

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Monocytes are highly phagocytic cells which play a significant role in atherosclerosis development and progression. The role of monocytes in the disease and phagocytic nature makes them an attractive target for nanotherapeutics. Monocyte phenotype is very important to the way in which the cells will interact with nanoparticles and is modulated by a variety of stimuli. In this research we studied two stimuli: shear stress on the monocytes a physical parameter, and nanoparticle size an extrinsic parameter. Atherosclerotic plaque formation impedes blood flow thereby increasing the shear stress applied to monocytes. The altered shear stress could contribute to the abnormal monocyte phenotype populations in atherosclerotic patients and a change in phagocytic activity, which could subsequently change the way the cells interact with different size nanotherapeutics. To improve our atherosclerotic model, we developed a cellular model which mimics aberrant angiotensin converting enzyme 2 (ACE2) expression (an important vasodilator). The cell model (THP-1-ACE2-) and wild type (THP-1wt) was exposed to physiological shear stresses (5 dyne/cm² and 40 dyne/cm²) and determined how this impacts monocyte activation and nanoparticle uptake of 5, 100, 200, and 500nm spherical polystyrene particles. Results showed shear stresses of 5 dyne/cm² or 40 dyne/cm² have differential response profiles in both THP-1-wt and THP-1-ACE2- monocytes. Nanoparticle uptake was measured using flow cytometry and determining the percent positive cells using subtraction histograms with a cell sample not exposed to particles as a reference. The sustained effect of shear was determined by subtracting the % positive cells immediately after shear from the % positive after a 4-hour rest period post shear. Static versions of all samples were run with samples taken on the same time scale for comparison. At 5 dyne/cm², THP-1wt cells only saw an improvement in particle uptake with the 20nm particles showing a significant sustained effect from the shear after 4 hours. Pre-stimulation with lipopolysaccharide (LPS) to stimulate inflammatory conditions resulted in a significant sustained shear effect across all sizes in THP-1wt cells. ACE2 knockout cells showed similar trends to the wild type cells at 5 dyne/cm² but 100nm also saw an increased uptake after rest without LPS stimulation. Increasing the shear to 40 dyne/cm² increased the uptake of all particle sizes without LPS in THP-1 ACE2- cells. These results elude to the possibility that disease conditions can change the optimal nanoparticle size to use for therapeutic development.

Outbred mice are a cost-effective model for mRNA lipid nanoparticle delivery

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Introduction: Lipid nanoparticles are the most clinically advanced delivery vehicle for messenger RNA (mRNA) therapeutics. The applications of mRNA therapeutics are diverse and include vaccination, gene editing, cancer immunotherapy, protein replacement therapy, etc. Preclinical studies for mRNA therapeutics typically include screening of delivery efficacy and immunogenicity in C57BL/6 or BALB/c mice. (1,2) These mouse strains each contain genetically identical (inbred) mice, and are some of the most common mouse strains used. Screening in these inbred mice is advantageous, as they tend to reduce within-group variance, and thus, the number of animals needed for a particular study. This is beneficial for preliminary studies, but inbred mice fail to model the effect of genetic diversity on delivery processes and outcomes. (3) Outbred mice can mimic the genetic diversity of humans, and some strains (e.g. CD-1) are more cost-effective than inbred mice for this purpose. (3)

Methods: A library of 20 ionizable lipidoids were synthesized, formulated into lipid nanoparticles, and then screened using luciferase mRNA. Lipid nanoparticles were delivered intravenously at 0.5 mg/kg to three different mouse strains: C57BL/6, BALB/c, and CD-1.

Results: Across all three mouse strains total efficacy trends remain on the same order of magnitude. Outbred CD-1 mice demonstrated consistently higher total protein expression levels than inbred strains C57BL/6 and BALB/c. Delivery efficacy on the organ level was also assessed. It was found that delivery to each organ also remains on the same order of magnitude. This indicates that lipid nanoparticle specificity does not change across mouse models.

Conclusion: Efficacy and specificity trends remained the same across all three mouse strains, thus, we concluded that inexpensive and genetically diverse CD-1 mice could potentially be used to screen mRNA lipid nanoparticles for translational applications. Future work will evaluate immunological differences between the three mouse strains.

Use of Bioluminescence to Study Macrophage Polarization

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Macrophages are innate immune cells whose polarization phenotype, play an important role in tumor initiation and progression. Tumor-associated-macrophages (TAMs) can directly impact a therapeutic's success. This study introduces a bioluminescent platform that can be used to study the effects of therapeutics on the temporal dynamics of TAM polarization. Macrophage polarization can be affected by both intrinsic (previous polarization) and extrinsic (cytokine gradients) factors within the tumor microenvironment. Bioluminescent imaging (BLI) is commonly used to study circadian rhythm and has shown advantages for long-term live cell imaging. To study macrophage polarization, we created cell lines with bioluminescently labelled transcription factors. THP-1 cells, an immortalized monocyte line, were used to develop these platforms. Cells with labelled NF- κ B, a traditionally pro-inflammatory family of transcription factors to correlate with the M1 (pro-inflammatory and anti-tumoral) macrophage phenotype were purchased and created. We also created THP-1 cell lines in which we added a constitutively active bioluminescent label on the CMV promoter to normalize one of our lines as well as monitor proliferation and metabolism. Cell lines were exposed to polarizing agents (TNF α and LPS for M1 polarization and tumor-conditioned media for TAM-like polarization). We found that we could temporally monitor the macrophages' polarization by measuring luminescence. How previous polarization can affect macrophages' polarization response to new stimulus was also explored. Cells polarized towards a TAM phenotype had a similar response to LPS, a TLR4 agonist known to induce a M1 phenotype, as nonpolarized macrophages. However, macrophages that had been previously M1 polarized showed a decrease sensitivity to LPS stimulation. Further studies demonstrated how repeated polarization changes can cause a decreased ability to repolarize, suggesting that macrophages have a repolarization limit. These results both show the importance of a thorough understanding of what factors affect TAM polarization as well as the feasibility of using bioluminescence to monitor polarization changes. This work is the first step in utilizing this platform as a way of studying how therapeutics affect macrophage polarization within the tumor microenvironment.

Lipid nanoparticles elicit structure-dependent innate and adaptive immune response

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The vast majority of literature on RNA delivery vehicles focuses on efficacy [1]. The immune response of delivery vehicles is often not studied until they are tested in higher-order animal models. Nevertheless, the interaction of a drug with the immune system is just as important as efficacy for bringing it to the market. By understanding how delivery vehicles provoke the immune system, we can engineer well-tolerated vehicles during the initial phases of drug development. Additionally, we can identify delivery vehicles that are best suited for their intended therapeutic application. For example, inflammatory delivery vehicles can improve the potency of RNA vaccines, whereas immunosilent delivery vehicles will be a better choice for protein replacement therapy [2]. This research intends to understand the effect of lipid nanoparticle chemistry on immune response. We show that the amine group of the lipids regulates the magnitude of adaptive immune response. We identify the immune receptors that are triggered by lipid nanoparticles. Finally, we use molecular dynamics to identify the specific motifs in the lipids that enable immunostimulation. This work broadens our understanding of the impact of lipid structure on the immune response.

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[2] K. A. Hajj, K. A. Whitehead, *Nat. Rev. Mater.* 2 (2017)

Shear Stress Modulation of BMP/ALK1 Signaling in Endothelial Cells Revealed by an All-in-One Multi-Shear Stress Microfluidic Device

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Vascular networks are shaped by early proliferation, vessel growth, and collective movement of endothelial cells (ECs). Organized networks emerge during angiogenesis and mature as blood vessels remodel. Defects in angiogenesis and remodeling, via overgrowth or inadequate network elaboration, lead to many diseases. Defects can arise from altered signaling through diffusible growth factors, altered mechanical microenvironments, or may arise when synergy between growth factor signaling and mechanical cues is disrupted, e.g. arteriovenous malformations (AVMs). Here, we focus on one type of AVM, hereditary hemorrhagic telangiectasia (HHT), which is thought to arise from defects in ALK1-receptor growth factor signaling and fluid shear stress (SS) mechanosensing. Since SS is well known to polarize and direct EC migration during vascular morphogenesis, we sought to understand how cell responses are altered when co-stimulated with the ALK1-ligand BMP9. To directly test flow and BMP9 interactions, we developed a microfluidic device and a custom image analysis pipeline to quantify end-point immunofluorescence staining. Our "All-in-One" microfluidic system delivers 14 levels of physiological laminar and gradient SS in a single device, allowing direct quantitation of EC responses.

To target the most sensitive concentration range of the BMP9-mediated ALK1 signaling, we measured a dose-response curve under static and flowed, and identified the dose which achieves 50% maximal response (EC50). In brief, serum-starved confluent human umbilical vein endothelial cells (HUVECs) in multiple devices are exposed to different BMP9 doses or control medium for 45 mins under flow or static culture. To assess the immediate level of BMP9, we measured nuclear translocation of phosphorylated SMAD1/5/8 (pSMAD), a target of the ALK1 signaling cascade. The EC50 for pSMAD translocation was ~ 46 pg/ml without flow and dropped to ~ 3 to 4 pg/ml with SS. We are narrowing the range BMP9 to the SS EC50 range, to quantify the effect of SS and SS-gradient on the ALK1 signaling pathway. In order to understand the impact of HHT on vascular remodeling, we have also begun live-cell imaging, to assess the acute changes to EC polarity and migration during SS/ALK1 activation. Future work will include analysis of other ALK1-ligands, how EC mechanosensing detects SS and SS gradients, and how these pathways may be targeted for clinical treatment of HHT and other defects in vascular remodeling

Role of Microcalcifications in Breast Cancer Bone Mimicry

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Breast microcalcifications (MCs) are insoluble calcium deposits, that are used as a diagnostic tool to detect breast cancer (BC) where up to 93% of cases of ductal carcinoma in situ (DCIS) are determined based on the presence of MCs. Breast MCs are categorized into type I (benign, calcium oxalate, OX) and type II (malignant, hydroxyapatite, HA) MCs. Breast lesions with the presence of MCs show an increase in the expression of mesenchymal and bone markers, also termed as “bone mimicry”. Breast cancer cells with bone mimicry are known to exhibit bone propensity. Despite correlative evidence linking MCs to bone mimicry and bone mimicry to bone metastasis, the biological role of MCs in promoting invasive phenotype, bone mimicry and subsequent metastasis remains unknown. To address this, our laboratory has developed collagen-inspired extracellular matrices (“ECM-Mimics”) recapitulating benign or malignant MC composition observed in MC-positive DCIS patients. We hypothesize that the presence of malignant microcalcifications in the primary breast tumor can promote invasive and bone mimicry phenotypes and further enhance homing and survival of breast cancer cells in the bone microenvironment leading to bone metastases.

In this project, we show that the ECM-mimics are able to deposit and recapitulate the composition of benign (OX) and malignant (HA) microcalcifications found in breast biopsies of cancer patients. When seeded onto the ECM-mimics containing different microcalcifications, non-metastatic T47D breast cancer cells exhibit higher mRNA expression of mesenchymal and bone markers by qPCR analysis. The conditioned media collected from T47D cells exposed to malignant MCs show higher ALP and TRAP enzyme activity, which are markers for osteoblast and osteoclast activity, respectively. Furthermore, ex vivo human bone explants exposed to conditioned media from the T47D cells seeded on malignant MCs also show significant increase in the bone metabolic activity, ALP enzyme activity, and TRAP enzyme activity of the bones compared to bones exposed to conditioned media from the T47D cells seeded on non-mineralized or benign MC containing ECM-mimics. These results suggest that malignant MCs may play a critical role in metastatic breast cancer progression.

Immunomodulatory matrix-bound nanovesicles mitigate acute and chronic pristane-induced rheumatoid arthritis

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation and destruction of synovial joints affecting ~7.5 million people worldwide. Disease pathology is driven by an imbalance in the ratio of pro-inflammatory vs. anti-inflammatory immune cells, especially macrophages. Modulation of macrophage phenotype, specifically an M1 to M2, pro- to anti-inflammatory transition, can be induced by biologic scaffold materials composed of extracellular matrix (ECM). The ECM-based immunomodulatory effect is thought to be mediated in part through recently identified matrix-bound nanovesicles (MBV) embedded within ECM. Isolated MBV was delivered via intravenous (i.v.) or peri-articular (p.a.) injection to rats with pristane-induced arthritis (PIA). The results of MBV administration were compared to intraperitoneal (i.p.) administration of methotrexate (MTX), the clinical standard of care. Relative to the diseased animals, i.p. MTX, i.v. MBV, and p.a. MBV reduced arthritis scores in both acute and chronic pristane-induced arthritis, decreased synovial inflammation, decreased adverse joint remodeling, and reduced the ratio of synovial and splenic M1 to M2 macrophages ($p < 0.05$). Both p.a. and i.v. MBV reduced the serum concentration of RA and PIA biomarkers CXCL10 and MCP-3 in the acute and chronic phases of disease ($p < 0.05$). Flow-cytometry revealed the presence of a systemic CD43^{hi}/His48^{lo}/CD206⁺, immunoregulatory monocyte population unique to p.a. and i.v. MBV treatment associated with disease resolution. The results show that the therapeutic efficacy of MBV is equal to that of MTX for the management of acute and chronic pristane-induced arthritis and, further, this effect is associated with modulation of local synovial macrophages and systemic myeloid populations.

Incidence and Function of Matrix-bound Nanovesicles through Evolution

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Matrix-bound nanovesicles (MBV) are a recently described class of extracellular vesicles embedded within the extracellular matrix (ECM) and containing cytokines, chemokines, RNA and other signaling molecules, and a lipid membrane that is distinct from other vesicle types. Previous studies suggest that MBV are released from the ECM following tissue injury and serve to modulate inflammation and promote recruitment and differentiation of tissue stem cells —properties that make MBV attractive as a potent and systemically deliverable therapeutic. Initially isolated from porcine urinary bladder, dermis, and small intestine, MBV have since been found in virtually all tissues examined from porcine, bovine, murine, and human sources. The physiologic significance, evolutionary origin, and potential theranostic applications of MBV have yet to be explored. The objectives of this study will determine the phylogenetic distribution of MBV and characterize differences in MBV composition and function over evolutionary history.

Tissue samples were obtained from 38 animal species representing 7 phyla and 18 classes. All tissues were subjected to a standardized, mild decellularization protocol. Decellularized samples (i.e. ECM) were digested with LiberaseTM to release the embedded MBV. MBV were then isolated and purified by ultracentrifugation and size exclusion chromatography. MBV size and concentration were quantified by nanoparticle tracking analysis, and protein concentration was measured by BCA. Transmission electron microscopy was performed to visualize MBV. Bioactivity was quantified by a macrophage challenge assay, in which mouse bone marrow-derived macrophages were activated toward an M1-like (pro-inflammatory) phenotype by exposure to LPS and IFN γ for 6h, then treated with MBV for 24h, followed by analysis of M1-like and M2-like (anti-inflammatory) marker expression. To determine MBV miRNA cargo, total RNA was isolated using a miRNeasy Mini Kit (Qiagen), and RNA sequencing was conducted using 500 ng of RNA from each sample. Lipidomic analysis was conducted by liquid-chromatography-mass spectrometry.

Of the 38 species, all contained MBV, though differences were observed across species in the yield of MBV per unit weight of ECM. The size of isolated MBV ranged from approximately 70-140 nm, similar to previous reports. MBV showed typical cup-shaped morphology upon TEM visualization. Effects on macrophage phenotype showed similarities and differences across phyla and classes, as did miRNA content and lipid membrane composition. Our results suggest that MBV are an evolutionarily conserved mechanism for cell:cell communication, tissue repair, and immunomodulation following tissue injury. Further study will identify common markers and bioactive molecules that can be exploited for theranostic applications.

Smart Delivery of Epirubicin to Cancer Cells Using Aptamer-modified Ferritin Nanoparticles

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Purpose: Epirubicin is a chemotherapy agent which is commonly used in the treatment of cancers. However, despite being efficient, the tendency to use this drug is declining mostly due to its myocardiopathy and drug-resistance of tumor cells. Such side effects could be prevented using targeted nanocarriers. This study aims to evaluate targeted delivery of epirubicin (Epi) to colon cancer cells using ferritin nanoparticles (Ft NPs) and MUC1 aptamer (Apt) and formation of Apt-Epi Ft NPs.

Methods: In the current study, Apt-Epi Ft NPs were prepared. Then, physicochemical properties of nanoparticles, including size and zeta potential, morphology, drug loading, drug release from nanoparticles, drug uptake of cancer cells, cytotoxicity, and in vivo results were collected.

Results: The results showed that the nanoparticles were synthesized with a mean size of 37.9 nm and encapsulation efficiency of 67%. The drug release from these nanoparticles was about 90% within 4 h in the acidic medium. Also, targeted delivery of Epi enhanced its anticancer effects in both in vitro and in vivo.

Conclusion: In this study, targeted delivery of Epi using aptamer-modified ferritin nanoparticles improved in vitro and in vivo results which indicates that it could be useful as a successful drug delivery system against cancer cells.

Keywords: Epirubicin; Ferritin; MUC-1 Aptamer; Smart drug delivery system; pH-sensitive

The Effect of Profilin Expression on Intratumoral Infiltration of CD8+ T Cells in Breast Cancer

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Intra-tumoral infiltration of CD8+ T lymphocytes is associated with good prognosis in breast cancer. As immunotherapy becomes a more common treatment option in triple negative breast cancer, it will be important to develop predictive clinical markers of immune infiltration. Profilin-1 (Pfn1) is a key regulator of actin polymerization that is downregulated at the protein level in human breast cancer, more prominently in invasive breast cancer. Mouse model studies further demonstrate restoring Pfn1 expression suppresses tumorigenic ability of breast cancer cells. The goal of this study was to investigate whether Pfn1 expression in breast cancer cells has any effect on immune microenvironment in human breast cancer.

By multiplexed quantitative immunohistochemistry of a tissue microarray of clinical breast cancer specimens, we discovered a strong positive correlation between tumor cell-specific Pfn1 expression and intra-tumoral infiltration of CD8+ T cells (Pearson's $r = 0.755$, p value <0.0001). Using QuanTIseq, an algorithm which deconvolutes RNA seq data into proportions of immune cells, we obtained a similar correlation between Pfn1 mRNA and the CD8+ T cell fraction in the METABRIC transcriptome dataset of human breast cancer (Pearson's $r = 0.3018$, p value <0.0001). The CD8+ T cell fraction was estimated using the T cell inflamed gene expression profile, a metric which scores cytotoxic T cell activities including antigen presentation, chemokine expression, and adaptive immune resistance, also showed a positive correlation with Pfn1 expression in METABRIC data analyses (Pearson's $r = 0.4798$, p value <0.0001).

Transwell migration experiments further showed that stable silencing of Pfn1 expression dramatically reduces chemotactic migration of T-cells stimulated by conditioned media derived from MDA-MB-231 breast cancer cells. Analyses of cytokines and chemokines showed evidence for Pfn1-dependent modulation of several key immunomodulatory molecules in MDA-MB-231 cells including CCL2, VEGFA, CCL5, TNF α , IL-1RA, and PDGF- $\alpha\beta/\beta\beta$, a subset of which we further validated by bioinformatic analyses of METABRIC dataset. Based on these findings, we propose that Pfn1 increases intratumoral infiltration of CD8+ T cells in breast cancer by altering the expression and secretion of certain immunomodulatory cytokines, a hypothesis that we will test in our future mouse model studies.

Characterization of endothelial Profilin-1 depleted mice to assess health and long-term viability

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Endothelial cell (EC) dysfunction is an important contributor to the pathogenesis of atherosclerosis, as increased microvascularization, perturbed cell adhesion marker (CAM) expression, inflammatory cytokine/chemokine signaling, and monocytic adhesion and extravasation are classically reliant on disrupted endothelial function. Global heterozygous knockout of actin-binding protein gene profilin1 (Pfn1) leads to reduced macrophagic accumulation and CAM expression conferring protection against atherosclerosis. To what extent reduced expression of Pfn1 specifically in EC relates to these phenotypes are not clear. We have utilized a novel mouse model engineered for inducible EC-selective Pfn1 knockout which shows defects in post-natal developmental angiogenesis in various organs (e.g. retina and kidney). The overall goal of this study is to further study the effect of endothelial Pfn1 gene deletion on vascular-immune cell crosstalk in adult mice. Our studies suggest that total loss of Pfn1 in EC is detrimental to animal health as evidenced by labored breathing, lethargy and abnormal fluid accumulation in Pfn1 KO (knockout) mice. Analyses of serum cytokines/chemokines showed changes in select circulating inflammatory cytokines in Pfn1 KO mice relative to wild-type (WT) animals, findings partly recapitulated in similar analyses of cell-culture supernatants from control vs Pfn1-silenced EC. These findings pave the way for future studies to investigate specific immune cell profile of EC-Pfn1 perturbed mice, in either basal state or after acute inflammation in disease settings.

Design and Development of Smart Cell-Based Therapeutics Aimed at the Precision Treatment of Liver Fibrosis

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Chronic liver disorders are the leading cause of liver-related deaths globally. Liver fibrosis, characterized by activation of hepatic stellate cells (HSCs) and an increased deposition and stabilization of extracellular matrix caused by factors TIMP1 and LOXL2 is the main cause of cirrhosis and hepatocellular carcinoma if left untreated. Currently, there are no well-established therapeutic strategies to cope with liver fibrosis, and the development of antifibrotic therapeutics that can halt fibrosis or reverse it is urgently needed. We propose the development of cell-based therapeutics including antifibrotic/fibrolytic CAR T cells that sense the liver environmental cues and then actuate a user-defined antifibrotic response. The proposed antifibrotic CAR T cells are able to sense multiple rather than one signature of HSCs (i.e., CD146 and DLK1) and eliminate them specifically. In addition, as an assistant to the CAR T cells, fibrinolytic CARs will serve as vehicles for the specific delivery to the liver of a well-established antifibrotic antibody against TIMP1. Specific inhibition of TIMP1 in the liver will unleash matrix metalloproteases and enable them to remove the fibrotic matrix.

Universal SNAP CAR T cells that can be post-translationally modified with different adaptor antibodies to recognize different targets were used as effector cells. As a first step, these universal effector cells were armed with CD19 or CD20 adaptors and their switchability and target specificity were evaluated. The proposed antifibrotic CARs will be further improved to sense multiple signatures of HSC (based on AND, NOT gates) and eliminate them specifically or to deliver therapeutic payloads (anti-TIMP1). Fetal liver organoid (FeLo) was generated based on the heterogeneous expression of GATA6 in iPSCs. FeLo produces a multicellular structure with features similar to the human liver and will be used as a testbed for evaluating the antifibrotic potential of smart cellular machines.

Universal CAR T cells against CD20+/CD19+ targets were confirmed to be both switchable and specific as indicated by flow cytometry. In addition, the universal CARs were compared to conventional CAR T cells and found as effective based on flow cytometry results. Fetal liver organoid was generated and shown to be an ideal liver niche and testbed based on imaging and other techniques.

Based on our preliminary results, the universal CARs are promising to be used and further improved as smart therapeutics for targeting cellular species behind liver fibrosis. Further engineering will enable the generation of antifibrotic living devices that can actuate a response (killing targets or delivering therapeutic payloads) only inside the liver milieu.

Testicular Tissue Cryopreservation in a Coordinated Network of Academic Centers

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Testicular tissue cryopreservation is the only fertility preservation option for prepubertal boys who are at risk of infertility as a side effect of their medical treatment. The Fertility Preservation Program at Pittsburgh has cryopreserved almost 500 patient samples since 2011. We used the donated tissues to evaluate the impacts of alkylating chemotherapy for cancer patients, hormone affirming treatments for gender dysphoria patients, or cryogenic storage duration on testicular histology and function.

Immunohistochemistry was used to quantify germ cell number in all samples. Testicular tissues undergo enzymatic digestion to derive single-cell suspension that was transplanted into immunodeficient nude mice. Eight weeks post transplantation, recipient mouse testes were retrieved for whole-mount staining to evaluate the donor cells' colonization potential.

Between 2011 – 2021, the University of Pittsburgh Fertility Preservation Program has cryopreserved testicular tissues for 472 patients. The average age of patients was 7.6 years (3 months old – 34 years old). Indications for TTC include cancers (63.2%), myeloablative conditioning prior to bone marrow transplant (28.4%), gender dysphoria (3.9%) and others (4.5%). More than 40% of our patients had initiated their chemotherapy and/or radiation prior to TTC.

Immunohistochemistry data showed no difference in total VASA+ germ cell number but reduced UTF1+ undifferentiated spermatogonia in samples that had previous alkylating chemotherapy compared to those that did not. Upon testicular cell transplantation, colony formation was observed in all transplanted samples but was reduced in the alkylating-treated group compared to the non-chemotherapy group. In our transgender patient cohort, 14 out of 18 had initiated androgen blocker and/or cross-sex steroid treatments prior to TTC. Immunohistochemistry results showed presence of VASA+ germ cells in all testicular samples of hormone-treated and hormone-free patients. Testicular cell transplantation data showed no difference in the colonizing potential among the hormone-treated groups versus the hormone-free group. Lastly, testicular cell transplantation data revealed no difference in colonizing potential upon testicular cell transplantation from samples frozen for 0-2 year, 2-4 years, 6-8 years, or 8-10 years.

Our results showed while it is best to cryopreserve tissues prior to the initiation of gonadotoxic treatments, even testicular samples which have undergone early stage of alkylating chemotherapy or gender affirmative therapy still possess some reproductive function to be utilized in the future. Cryostorage duration does not impact the number of transplantable spermatogonia in patient samples.

A Crystalline Adenosine Formulation for Localized Delivery and Immunomodulation

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Adenosine (Ado) is an endogenously produced metabolite and an FDA approved cardiac modulator and imaging agent. Preclinical studies have demonstrated that Ado can be repurposed as an immunomodulator in respiratory distress and brain injury. However, delivery of Ado to target tissues is limited by the nucleoside's short plasma half-life (<10 seconds) and toxicities due to ubiquitous expression of Ado receptors in the cardiovascular system. While off-target effects can be circumvented through localized drug administration, only limited volumes (< 5 ml) can be delivered through intratracheal and intrathecal routes of administration. These limitations create the need for highly concentrated Ado formulations for localized administration to attain high doses of Ado in small volumes of injection.

Herein a previously unreported crystalline form of Ado (cAdo) by which the compound is concentrated 2X above solubility will be presented. Sustained release can be attained by loading cAdo into hydrogels made from the self-assembly peptide (SAP) [AEAEAKAKEAK]₂, or EAK, which forms supramolecular structures in physiological conditions. The EAK hydrogel system is well characterized and found to be non-toxic for use in animals, self-assembling nature, and its ability to retain drug content at injection site for days in vivo. Our group have developed and characterized bioaffinity EAK drug delivery systems that can be readily tested in disease specific animal models. In cAdo loaded EAK gels we found the drug release extended upto 3 days. In addition, evidence will be presented for the new crystalline form of Ado and biological activity in cultured macrophages.

Design and development of folate decorated nanoemulsions as the new pain nanomedicines – in vitro study

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Peripheral nerve injury (PNI) triggers neuropathic pain, which is caused by lesions or disorders of the somatosensory nervous system. Effective treatment of neuropathic pain remains a challenge, with poor outcomes and continued reliance on opioids as primary treatment. The need for an alternative became acutely apparent during the opioid overdose epidemic, which resulted in a staggering number of opioid overdose-related deaths in recent years. Our team pioneered a targeted nanomedicine platform, formulated as a drug loaded near-infrared dye labeled nanoemulsion, that specifically targets COX-2 enzyme in the macrophages during nerve injury. These are activated macrophages (M1 phenotype) and serve as major drivers of chronic neuroinflammatory processes leading to neuropathic pain. The folate receptor beta (FR- β) is preferentially expressed on these macrophages during inflammation. Because folic acid has a nanomolar affinity for the FR- β (Kd 0.1–1.0 nM) thus the goal was to design, develop, and conduct preliminary in vitro assessment on a theranostic nanoemulsion anchored with a folic acid derivative. Here we show that the folic acid derivative surface conjugated nanoemulsion leads to enhanced receptor-mediated macrophage uptake over unconjugated nanoemulsions. Furthermore, the conjugate had no influence on colloidal stability and did not cause macrophage cytotoxicity. The presented formulation is also laden with perfluorocarbon (an MRI agent) making these M1 macrophage targeted nanoemulsions into potentially personalized treatments for neuropathic pain. The presented approach could lead to effective pain relief while also providing real-time feedback on macrophage migration patterns to study inflammatory responses through both near infrared and magnetic resonance imaging.

Evaluating Cellular Stress and Phototoxicity of Photothermal Stimulation on Neurons

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The modulation of neural activity with high resolution has advanced the investigation of brain functions and therapeutics for neurological diseases. Photothermal stimulation is a remote and genetic-free technique to modulate neural activity with high spatiotemporal resolution and cell-type specificity. During the stimulation, light pulses illuminated on the bio-interface between photothermally active nanomaterials and neurons will result in local temperature rise and change the electrical properties of the neuronal membrane. However, the clinical translation of this technique necessitates a comprehensive evaluation of its potential cytotoxicity and cellular stress effects in advance.

Recently, we have demonstrated that two-dimensional nanoflakes, Ti₃C₂Tx (MXene), have outstanding photothermal responses to modulate neural activity due to their near-infrared absorption, high photothermal conversion efficiency, low needed stimulation energy per pulse, and large batch production ability. Here, we systematically investigate the cytotoxicity of photothermal stimulation with Ti₃C₂Tx on dorsal root ganglion (DRG) neurons. We demonstrate the cell viability, mitochondria membrane potential, and reactive oxygen species (ROS) generation with different light illumination conditions ($\lambda = 635$ nm, 1 ms pulses, different illumination powers, and frequencies). We also investigate instant response and long-term effects of neurons' health illuminated with light. We benchmark the cytotoxicity with a local temperature rise of Ti₃C₂Tx induced by light using micro-pipettes. Our results demonstrate that light pulses with high incident power (larger than 14 mW) will generate cell stress and reduce cell viability irreversibly. Stimulating neurons with 1 single pulse, 1 Hz and 10 Hz pulses with low powers did not show detectable phototoxicity effects. These evaluations will provide insights and guidance toward further in vitro and in vivo use of photothermal stimulation, especially for clinical translation. It will eventually allow for remote control of the nerve system to expand our understanding of fundamental neural signaling pathways and the development of innovative therapeutics.

Islet-on-Chip Model for Type 2 Diabetes

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Diabetes is an increasingly prominent disease that develops from the dysfunction of the insulin production within pancreatic islets. This dysfunction stems from autoimmune elimination of the insulin producing beta cells (Type 1) or a toxic environment that hinders insulin production or effectiveness (Type 2). Testing of possible treatments for preventing or mitigating the complications of diabetes is hindered by expensive and inadequate animal models. The human-on-chip project seeks to replace these models and aid in the transition from initial testing to clinical trials by mimicking the biochemical and biophysical environment of organs on a fluidic device. This project aims to develop a type 2 diabetic islet-on-a-chip model utilizing both primary islets and human induced pluripotent stem cells (hiPSCs).

The islet-on-chip platform is based on the commercial fluidic device from Micronit that utilizes three glass layers to form a 2-chamber system partitioned by a polyester membrane. The chip design was altered to maintain pancreatic islets 3D morphology due to structure's association with maintained function. Alternate seeding methods were investigated for primary human islets, leading to the development of a novel technique for hydrogel-supported islet micropatterning on the membrane. A COMSOL-based in silico flow field model informed this system design for optimizing flow rate and micropattern design to retain adequate oxygen level in the islet vicinity, while minimizing shear stress. Our islet-on-chip system retained high viability and glucose stimulated insulin secretion (GSIS) of primary human islets over 4 weeks of perfusion culture under normal 'fasting' condition. This extended culture of healthy islets is expected to facilitate disease induction in our system with the capability of forming a toxic environment over time to damage cell function while still maintaining viability and widening the window for therapy testing. For inducing type 2 diabetes within the formed chip, the diseased states of glucotoxicity, lipotoxicity, and glucolipotoxicity were respectively simulated through long term exposure to pathological glucose levels, elevated free fatty acids, and combinations of the two. In parallel, a method for forming hiPSC-derived islet organoids was developed to replace the primary human islet with a regenerative cell source. As with the primary islets, the fluidic culture system maintained survival and functional glucose-stimulated insulin responsiveness of the hiPSC-derived islets over extended culture period. The end goal of this project is to simulate treatment of disease patients and test type 2 diabetes treatments to determine the reversibility of the toxic states.

The Impact of Placental Hypoxia on Monocyte Recruitment

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During pregnancy, monocyte populations increase and become more pro-inflammatory. It is believed that multi-nucleated placental cells called syncytiotrophoblasts play a significant role in monocyte activation. The syncytiotrophoblast exists at the maternal/fetal interface, and comes into direct contact with maternal circulation. Consequently, the syncytiotrophoblast is able to both interact with monocytes directly and via the release of molecular signals or extracellular vesicles into the bloodstream.

The interactions between syncytiotrophoblasts and monocytes become dysregulated in the context of maternal diseases such as preeclampsia, which is a leading cause of maternal morbidity and mortality. It is known that in preeclampsia, the placenta undergoes prolonged hypoxia. This hypoxic stress increases the production of the syncytiotrophoblast extracellular vesicles and alters their bioactivity, making them more pro-inflammatory.

Due to the challenges of researching pregnancy, the communication between the placenta and the immune system is poorly understood. Placental diseases such as preeclampsia or other preexisting inflammatory conditions further complicate these interactions. The aim of this study is to understand how monocyte recruitment and adhesion to the syncytiotrophoblast changes in the context of preeclampsia. First, we created an in vitro model of the preeclamptic placental syncytium via the induction of hypoxia. The syncytial stress was quantified via qPCR analysis of hypoxic and cellular stress genes. To assess monocyte adhesion, THP-1 monocytes tagged with a GFP marker were co-cultured with hypoxic BeWo cells, and the number of adhered cells was quantified via fluorescence microscopy. To assess monocyte recruitment, a Boyden chamber assay was used. Extracellular vesicles isolated from the stressed syncytium were placed in the bottom chamber as the chemoattractant for the monocytes. The results of this study will provide greater insight into PE mechanisms, risk factors, and potential biomarkers.

Using a cell-based model to investigate cellular mechanics during convergent extension and apoptotic cell extrusion in epithelial tissues

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It is often difficult to distinguish between active and passive responses that shape epithelial tissues. Cells may adopt a shape in response to external stresses, or they may actively change to the same shape autonomously. Here, we ask what cell shape changes are possible when cells are fully passive or interact with passive neighboring cells. To distinguish between active and passive responses we have developed a computational modeling approach where cells are represented as interacting particles with repulsive or attractive potentials. Cells move and remodel the epithelium in response to the potentials and positions of neighboring cells and confining boundaries.

Our first model depicts *Xenopus laevis* neural epithelia undergoing convergent extension (CE) where the tissue narrows, i.e. converges, in one direction and lengthens, i.e. extends, in the orthogonal direction. To isolate the passive responses to CE field elongation from contributions from active cellular processes, we simulate epithelial responses to external forces. In the model, cells are represented as interacting particles in a rectangular bounding box. Boundaries are moved such that the field of cells undergoes CE but does not change area.

Our second model depicts the extrusion of apoptotic cells from the larval epidermis of *Danio rerio*. In the case of extrusion, we populate a field with repulsive cells with fixed potentials as above and add apoptotic cells with decreasing potentials that oscillate. Neither the size nor the shape of the field change in the extrusion models. to extrude with that matching features of cells in vivo.

Time series of tessellated polygonal cell networks in both models are analyzed to quantify deformation and strain both globally at the tissue scale and locally by considering the domain of a cell and its immediate neighbors. We have identified potential signatures of active cell mediated CE such as low local tissue strain and divergence of cellular and tissue strain over the time course of CE. Extrusion simulations reveal correlation between initial size/potential of an extruding cell and its area loss. Additionally, cell extrusion dynamics appear to be cell-autonomous based on in silico experiments where cell features are kept fixed while the local environment is allowed to vary. Taken together, we show how a simple model of an epithelial tissue can represent the mechanics involved in two distinct developmental processes. Quantifiable morphological outcomes from our simulations can inform the development of interventions by shedding light on factors driving potential defects.

Geometric Characteristics of the Pelvic Floor Muscles Associated with Anatomic Recurrence After Prolapse Repair

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The pelvic floor muscles (PFMs) are essential for providing structural support to the vagina. Defects and injury of the PFMs have been linked to a higher risk of anatomic recurrence following prolapse repair. Geometric characterization of the PFMs and how PFM geometry is associated with anatomic recurrence is lacking but necessary for bettering surgical outcomes.

Therefore, the objective of this study was to determine geometric differences of the PFMs during rest and maximal strain between women with and without anatomic recurrence after native tissue repair (NTR) vs vaginal mesh repair (VMR). We hypothesized that PFM geometry significantly differs by surgery (NTR vs VMR), anatomic outcome (recurrence vs success), and maneuver (rest vs strain).

Eighty-eight women surgically treated for prolapse (43 NTR, 45 VMR) underwent 30-42-month postoperative pelvic MRI during rest and maximal strain. Anatomic recurrence was specified on MRI as vaginal protrusion (prolapse) beyond the hymen during strain. The PFM complex was traced in the mid-sagittal plane to create a 2D curve aligned and normalized across patients.

After defining point correspondence between all PFM curves, Principal Component Analysis (PCA) was performed to determine significant modes of geometric variation. The PCA scores were then assessed via a Three-Way Mixed MANOVA.

Of the 88 women evaluated, anatomic recurrence was observed in 24 (56%) NTR and 13 (29%) VMR patients. There was significant interaction between anatomic outcome and maneuver on overall PFM geometry ($p=0.002$) that was also observed in Modes 1 ($p=0.025$), 2 ($p=0.036$), and 3 ($p=0.002$). Qualitatively, Mode 1 described PFM relaxation (straightening) and perineal body descent; Mode 2 described anterior distension of the PFMs; Mode 3 described anterior distension of the PFMs and straightening of the perineal body during strain.

For Mode 1, the posterior portion of the PFM complex was straighter and the perineal body was lower at strain in recurrences vs successes ($p=0.009$). For Mode 2, there was more anterior distension of the PFMs from rest vs strain in recurrences ($p=0.005$); PFM shape did not differ by maneuver in successes ($p=0.887$). For Mode 3, there was anterior distension of the PFMs and straightening of the perineal body during strain in both groups.

Hyper-relaxation and anterior distension of the PFMs, as well as descent/hyper-mobility and straightening of the perineal body, during maximal straining were associated with anatomic recurrence after prolapse surgery. These geometric characteristics are indicative of PFM defects and warrant prospective investigation in their mechanistic role in prolapse recurrence.

Identification Of Intramural Stress Distribution In Cerebral Aneurysms Using Patient-Specific Wall Thickness Maps

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Background: Large variations in wall thickness are found within and between intracranial aneurysm (IA) tissue samples. The availability of accurate thickness measurement in-vivo is currently not feasible with typical clinical imaging protocols, however, the measurement of ex-vivo tissue thickness is possible with high-resolution MicroCT imaging. While aneurysm wall thickness can be modulated over a healthy growth and remodeling cycle, we hypothesize stress and thus failure of intracranial aneurysm tissue is modulated by variations in wall thickness.

Objective: The primary objective of this work is to explore the thickness variation within and between human aneurysm samples. Second, to understand the significance of the intramural stress state using patient-specific wall thickness.

Methods: Twenty-two IA specimens were resected perioperatively. We used high-resolution MicroCT imaging to segment and reconstruct the tissue surface with high fidelity. Then used industry-standard software to calculate a thickness map for each sample. For samples that we had intraoperative video and pre-surgery 3DRA derived luminal surface, we used intra-observer agreement to orient the MicroCT surface to the 3DRA derived luminal surface. Next, using a combination of open-source and custom software we mapped the thickness to the luminal surface and extruded a 3D mesh from the luminal surface to create a patient-specific thickness model. To elicit the effect of a variable thickness model we also constructed a model using a constant wall thickness from literature. Finally, to calculate intramural wall stresses we pressurized to 120 mmHg using a custom finite element code.

Results: Average sample wall thickness ranged from 50 μm to 800 μm with a mean and standard deviation of 310 $\mu\text{m} \pm 180 \mu\text{m}$, respectively. The average sample thickness was not normally distributed, with 77% of samples less than 300 μm . The sample average maximum principal stress varied from 40 kPa to 240 kPa. The 95th percentile of the normalized (by the average maximum principal stress in the mapped-on region) maximum principal stress was elevated in the patient-specific wall thickness model for nine out of ten samples, compared with constant wall thickness models (mean value of 2.03 vs 1.64%).

Conclusions: Thickness varies greatly both between samples and within samples and our cohort falls within reported thickness ranges. While the constant thickness model can predict locations of high and low stress, governed by curvature, prediction of peak stress magnitude without thickness proves to be difficult with no clear relationship between thickness and curvature.

Development and Scaleup of Perfluorocarbon Nanoemulsions: Adoption of Quality by Design

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Perfluorocarbon materials have undergone decades of development as potential oxygen carrier therapeutics in biomedical applications. Despite the promising pre-clinical results in a variety of disease states, there has been limited success translating and commercializing perfluorocarbon-based oxygen carriers. One possible reason for the difficulty is the existence of a tradeoff between perfluorocarbon-based therapeutic product stability and effectiveness which has been observed experimentally. The traditional approaches of pharmaceutical product development focus on one factor at a time. Conversely, Quality by Design as an innovative strategy aims to systematically build high-quality attributes into a finished product. Here, we report on the adaptation of the Quality by Design methodology to formulation of perfluorocarbon-in-hydrocarbon-in-water nanoemulsions for oxygen therapeutics with the overall goal of building process knowledge that will be utilized to design a stable and effective oxygen therapeutic. In this work, we establish a formal risk assessment of the formulation and processing operations of a previously developed high-energy nanoemulsion formulation. The risk assessment allows the identification of potential critical material attributes and critical processing parameters inherent to our process. We then study the impact of these potentially critical parameters on the critical quality attributes of our final perfluorocarbon nanoemulsion preparation. The selection of critical quality attributes are various measures of nanoemulsion stability and oxygen transport. Application of multivariate data analysis as part of describing the quality attributes in terms of the critical processing/material parameters mathematically formalizes the tradeoff between stability and oxygen transport effectiveness. Importantly, our study highlights the dependence of both stability and oxygen transport attributes on composition only and suggests there may be an intermediate formulation space to manage the stability-applicability tradeoff.

Isolated Support with an Extracorporeal Perfusion System Deters Ischemia-related Metabolic Derangement of a Rat Fasciocutaneous Free Flap

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Background: Machine perfusion of vascularized tissues can positively impact the future of microsurgery by enabling isolated perfusion of composite tissues such as free flaps. The goal of perfusion in this setting is to temporarily perfuse tissues until fully supported by neovascularization. For different tissue types and components, an improved understanding of the type and level of support is needed to develop an optimal perfusion system. Although they are more cost-effective, rodent free flaps have been employed with limited success in this setting due to challenges with small vessels and relatively delicate tissues. This study aimed to establish a rodent model of machine perfusion in a fasciocutaneous free flap to serve as an affordable testbed and determine the potential of the developed protocol to deter ischemia-related metabolic derangement.

Methods: A 2x3 cm rat epigastric fasciocutaneous free flap was harvested and the vessels were cannulated. The flap was transferred to a closed circuit that provides circulatory support via a peristaltic pump and respiratory support via a custom gas exchanger. Fresh, heparinized whole rat blood (~40 mL) was used as the perfusate. Outflow from the flap vein was recirculated during 8 hours of support. Blood flow rate was adjusted to maintain arterial-like perfusion pressures. Continuous papaverine infusion (1 mg/hr) was used to mitigate vascular spasm and improve maintenance of flow stability. Blood samples were drawn during support for measurement of gases and metabolites. Extracellular tissue lactate and glucose levels were characterized with a custom microdialysis probe placed in the flap tissue. Lactate to glucose ratio (L/G) was used as an indicator of tissue metabolism and compared with warm ischemic, cold ischemic and anastomosed free flap controls at the same timepoints.

Results: Maintenance of physiologic arterial pressures (85-100 mmHg) resulted in average pump flow rates of 300-450 uL/min with minimal flap bleeding. Blood-based measurements showed maintained glucose and oxygen consumption throughout support, indicating sustained metabolic activity. Average normalized L/G for the perfused flaps was 5- to 32-fold lower than that for the warm ischemic flap controls during hours 2-8 ($p<0.05$).

Conclusion: We developed a rat model of extended machine perfusion of a fasciocutaneous free flap. Ex vivo machine perfusion maintained stable perfusion and tissue metabolic activity out to 8 hours of support. This model can be used to further assess critical elements of support in this setting as well as explore other novel therapies and technologies to improve free tissue transfer.

Large-Scale Manufacturing of Artificial Oxygen Carriers for Organ/Limb Preservation

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Artificial Oxygen Carriers (AOCs) are materials that are used to transport oxygen into tissues and ideally should replenish oxygen in lieu of whole blood without the risk of infection. These materials should be chemically and biologically inert and remain stable at elevated temperature (>50C) and at prolonged conditions of storage. Perfluorocarbon-Nanoemulsions (PFC-NEs) are effective candidates for use as AOCs, however challenges exist on manufacturing them on large scale while maintaining their colloidal stability and oxygen loading/release performance. Here we present novel PFC-NE formulations which are successfully manufactured at volumes >0.5L under controlled processing parameters with reproducible quality (droplet size distribution, oxygen loading, stability under storage and stress). We also show initial evaluations of the PFC-NEs for oxygen delivery in solid organs and limbs ex vivo. The presented PFC-NEs efficacy for tissue oxygenation and preservation were evaluated using a novel Cardio-Vascular-Emulation-System-Waveform Perfusion System, an automated “smart” system for ex vivo tissue perfusion and preservation for the liver, kidneys, lungs and VCAs.

Diastolic Blood Pressure at the Fingertip using Pulse Arrival Time

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Towards furthering development of nearables for healthcare monitoring, oscillometric finger-pressing promises to detect blood pressure without the need for bulky arm-cuffs. In 2020, Chandrasekhar et al. showed possible evidence for a phenomenon whereby a trend in pulse arrival time (PAT) between an ECG and photoplethysmography (PPG) signal markedly changed when measuring near diastolic pressure.

Currently, we have performed a study of PPG, ECG, and pressure signals from 19 subjects. This study seeks to interrogate this PAT phenomenon in diverse individuals.

Extending on the oscillometric finger-pressing method, subjects pressed their fingertips and contained arteries against a pressure sensitive PPG sensor. We hypothesize that increased contact pressure and the nonlinear compliance of arteries led to narrower and effectively later arrival of measured volume pulses. This resulting change in PAT associates with the diastolic pressure due to definition of wave arrivals as the beginning of the volume upstroke.

Targeted Therapeutic Electrical Stimulation for Accelerated Wound Healing

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The neuromuscular junction (NMJ) serves as the interface between individual neurons of the peripheral nervous system (PNS) and distal skeletal muscle myofibers. When neuromuscular tissue is damaged, the region undergoes an endogenous regenerative process marked by growth factor synthesis. These signaling molecules' upregulation triggers the activation and proliferation of resident satellite and Schwann cells, replenishing depleted cellular populations to restore healthy function. However, following large scale neuromuscular trauma, fibrotic tissue formation impedes endogenous stem cell proliferation, compromising complete functional recovery. Previous studies have demonstrated that therapeutic electrical stimulation following neuromuscular injury can significantly increase the rate of tissue innervation leading to increased functional recovery relative to untreated conditions. The biological mechanism for accelerated NMJ formation is hypothesized to be the upregulated synthesis of systolic Ca^{2+} correlated growth factors including Pax-7+, Myogenin, and Raspin which enhance satellite cell proliferation, fusion, and differentiation into synaptic mononuclei, respectively. Here, we demonstrate a novel platform to interface with damaged nerves and injured muscle at a wound bed through the application of customized high-density ultra-compliant microelectrode arrays. These modular electrode arrays allow us to provide therapeutic electrical stimulation and detect muscle recruitment in a multisite manner across the wound bed. Muscle and nerve regeneration in a canine wound bed model has been illustrated through evoked post-threshold potential propagation and neuroelectronic latency quantification at four time points over fourteen days of recovery. We are currently continuing our work with in-vivo models to optimize electrical stimulation conditions for neuromuscular regeneration. We anticipate our work will lead to expansive in vivo studies to further elucidate the potential of electrical stimulation to enhance functional neuromuscular recovery to assist patients with traumatic injuries that, if untreated, would result in non-functional scar tissue.

Designing and testing a novel stent graft developed using superelastic nitinol and stretchable ePTFE to Isolate Blood Flow in Donation after Cardiac Death

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Shortage of healthy donors' organs has appeared as one of the main challenges for organ transplantation. Consequently, a novel endovascular device has been developed to increase the number of available organs from cardiac death donors. The device consists of two primary materials: 0.005 inch thick highly stretchable expandable polytetrafluoroethylene (ePTFE) tube and 0.015 inch diameter superelastic nitinol wire. To prepare this novel device, a dual chamber nitinol backbone was fabricated via a low-energy laser joining process followed by the attachment of a highly stretchable expandable polytetrafluoroethylene (ePTFE) to create a fully collapsible stent graft with two chambers and one docking zone.

A functional prototype stent graft has been developed via device design iteration process of the nitinol backbone and ePTFE membrane. The cardiac flow dropped by at least 30% after the device deployment in the in vitro anatomical aorta model. Both in vitro and in vivo study results have demonstrated the device's performance on the flow separation within the anatomical silicone model and animal (here, swine model). The perfusion sheath influenced the perfusion flow rate, to be maximum at 9Fr. sheath. The perfusion flow rate was maximum at 50mm perfusion length when the perfusion outlet was located distal to the heart. The new stent graft successfully isolated the cardiac flow from the branches to major abdominal organs. This new stent graft could substantially increase the number of available organs from the cardiac death donors minimizing potential organ ischemic complications.

Fully degradable endovascular embolic coil for saccular aneurysm treatment

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INTRODUCTION: Cerebral aneurysms are at risk of rupture resulting in catastrophic subarachnoid hemorrhage often leading to morbidity or death. Cerebral aneurysm embolization with appropriate materials is a therapeutic approach to stabilize the lesion, prevent rupture, and avoid clinical sequelae from the disease. Non-bioabsorbable metallic coils (platinum or tungsten) are a typical, practical option to secure cerebral aneurysms. However, numerous clinical studies indicate that up to 30% of aneurysms recur within 1 year after coiling, leading to frequent retreatment and risks associated with the additional surgery and/or hemorrhage. Thus, complete and durable aneurysm occlusion following coiling remains an important unmet clinical need. In this regard, it would be ideal for the aneurysm coiling material to induce acute occlusion via thrombosis, contribute to a tissue development process to fortify the degenerated vessel wall, and ultimately resorb to avoid leaving a permanent foreign body.

APPROACH, RESULTS and DISCUSSION: We proposed and developed here a fatty amide-based polyurethane (PU) elastomer coated magnesium (Mg)-alloy (e.g., AZ31, WE43B) coil as a bioabsorbable cerebral saccular aneurysm embolization coil. The chemical structure of the synthesized fatty amide-based polyether-urethane urea (PHEUU) was confirmed by ¹H-nuclear magnetic resonance. PHEUU showed comparable physical properties to elastomeric biodegradable PUs lacking fatty amide immobilization. Degradation profiles of the fatty amide-based PUs were evaluated in 100 U/mL lipase or 100 U/mL esterase solution. The degradation profiles show modest enzymatic weight loss in the first 8wk, with expected increased weight loss beyond that point. PHEUU showed inherent antioxidant activity at DPPH assay (>70% at 48h). In vitro cell viability tests by indirect contact method using rat aortic smooth muscle cells (rSMCs) and rat aortic endothelial cells (rECs) showed no cytotoxicity. Coating stability tests using polymer-coated Mg-alloy showed better protection with PHEUUs than PGLA against surface corrosion and cracking of the underlying Mg-alloy. In vitro rSMC attachment was higher with the PHEUUs compared to bare or PGLA coated Mg-alloy. In vitro platelet deposition was higher on PHEUU-coated Mg-alloy compared to uncoated and PGLA coated surfaces. PHEUUs showed their potential use in aneurysm coiling in terms of moderating underlying acute alloy corrosion and supporting moderate platelet deposition and cell attachment and proliferation. To create radiopacity, FDA-approved water-soluble contrast agent iohexol (OmnipaqueTM) was mixed with PHEUU followed by coil dip-coating. The coated coil was visualized with DSA angiography or X-ray. An in vitro aneurysm model was used for blood-contact testing of PHEUU coated Mg-alloy coils (pure Mg, AZ31, WE43B). Greater thrombus deposition was observed on the PHEUU coated Mg alloy coils vs. bare or coated pure Mg coils (Fig. 1). From these preliminary data, we plan to move forward to the fabrication of a fully bioabsorbable aneurysm coil system and then onto animal studies.

Two-color Nanoemulsion for Near-infrared Fluorescent Imaging in Rodent Pain Models

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A major limitation of current non-opioid and local analgesics is their lack of molecular and cellular targeting to effectively engage key cellular players in the neuronal injury milieu. Immunomodulating theranostic pain nanomedicines offer unique advantages to standard non-opioid treatments by directly engaging these targets and by doing so achieve >1000 fold increases in efficacy. Molecular imaging is essential for successful development in pain nanomedicine. In previous studies we demonstrated broad utility of multimodal (NIRF/19MRI) nanoimaging agents for imaging inflammation and establishing direct correlation between inflammation severity and resultant pain in rodent models of inflammation and injury. Here we present novel two-color fluorescent nanoemulsions as tools for imaging macrophages in multiple rodent models of nerve injury pain, inflammatory pain and pain associated with diabetic neuropathy. We also show how theranostic nanomedicine can reveal the role of immune cells in pain biology. Utilization of the theranostic features of presented two color nanoemulsions in animals uncovered unique molecular and cellular signatures of the immune responses in both sexes in response to insult, whether inflammatory or physical nerve injury. We found that neuroinflammation is distinct between males and females and this seems to be shared across pain models, both in mice and rats. Specifically, we demonstrate that immune cell targeted nanoemulsions can produce novel insights into pain biology in multiple pain models in rodents.

Assessing the Macrophage-Directed Remodeling Kinetics of a Peripheral Nerve Matrix Hydrogel

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Peripheral nerve injuries (PNI) are a frequent result of traumatic injury and can cause deficits in sensory or motor function including paralysis. Acute nerve gap injuries (when the nerve is severed) very rarely recover spontaneously and require surgical intervention to restore function. Despite advances in PNI treatment, recovery remains largely unsatisfactory and functional deficits become permanent.

This study investigates the regenerative nature of a decellularized nerve hydrogel (peripheral nerve matrix; PNM) as a therapeutic for gap injury. PNM has demonstrated improved recovery in a nerve gap, however the mechanism responsible for functional returns remains unclear. Macrophage polarization is believed to enhance regeneration in the presence of PNM as alterations to the microenvironment are more conducive to an anti-inflammatory, more pro-regenerative/M2-like phenotype. To understand their contribution to PNM-induced nerve healing, a Macrophage fas-induced apoptosis (MaFIA) mouse model is being used to eliminate macrophages at the time of injury. Firstly, this model was assessed to determine the extent of macrophage elimination, specifically at the systemic level and at the tissue level within injured nerves, to evaluate depletion. Flow cytometry on a peritoneal lavage and CD11b staining on nerve sections demonstrated near complete ablation of macrophages upon depletion and showed limited recovery to the macrophage population 1 week after depletion.

Experimentation is still ongoing to determine how regeneration with and without PNM is affected with and without macrophage depletion. Small gap nerve injuries are being assessed histologically, electrophysiologically, and functionally for nerve recovery with PNM and macrophage depletion as factors to observe if PNM retains its regenerative capacity when macrophages are not present in the acute healing response at 1 and 8 weeks post-injury.

A Hollow Fiber Membrane Oxygenator for Extremely Premature Infants

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Prematurity is the number one cause of both neonatal and under-5 pediatric mortality. Extremely premature infants (EPIs), neonates born at 22-28 weeks of gestational age, are at the greatest risk of morbimortality. The lung development of an EPI has not progressed to the stage at which it can sustain life, therefore mechanical ventilation (MV) must be used for respiratory support. The use of MV in this patient population results in respiratory insufficiencies that can last into adulthood and may result in heart failure. Research has turned to the investigation of extrauterine environments in which the EPI is submerged in an artificial amniotic fluid bath with an oxygenator taking the place of the placenta, via cannulation of the umbilical vasculature, as the gas exchanging organ. In-vivo studies of these systems have been plagued by complications stemming from the relatively large volumes and resistances of the circuit oxygenators. Our group employed mathematical modeling and computational fluid dynamics to design a hollow fiber membrane (HFM) oxygenator, the Preemie-Ox, capable of supporting the metabolic needs of a fetus with a resistance and priming volume comparable to that of the native placenta. The in-vitro performance of the device was characterized through benchtop studies of gas exchange, resistance, and hemolysis. The Preemie-Ox achieved the targeted 12 mL/min CO₂ removal rate target while completely saturating hemoglobin at a blood flow rate of 165 mL/min. Measured resistance was 27 mmHg/L/min, 36% smaller than the targeted value, and no detectable plasma free hemoglobin was recorded over 6 hours of device use. Bench top results favor the progression of the device to in-vivo studies with a fetal ovine model. Future work involves the use of zwitterions to mitigate the immune reaction against the hydrophobic device surfaces.

Novel Collagen behaviour mimicking synthetic material for artificial tissues

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Introduction: Fibrous collagen in the tissues exists in crimped form with periodic crimps. This structure affects the mechanical and load-bearing properties of these tissues. Due to these crimps the tissues act as soft materials at low strains and the fibers then uncrimp as the tensile strain is increased, thus bearing more load and resulting in increased stiffness. (fig. 1). Artificial tissue conduits are used to replace the damaged tissues in the body. For example, vascular grafts are used to replace damaged blood vessels due to cardiovascular diseases. However these conduits have low patency rates. A major cause of failure is the inability of the conduits to match the mechanical properties in the native tissue. The aim is thus to create a material which mechanically mimics the behavior of collagen containing tissues.

Idea: To create a hyper-elastic silicone material with chopped crimped fibers embedded inside. These fibers will be chopped so as to mold the material into various shapes of the required tissues.

Materials and Methods: Materials chosen for the hyper-elastic matrix is soft bio-compatible silicone and the embedded fibers are polypropylene fibers with a diameter of 15 microns. These fibers are then crimped using a novel setup (fig. 2). In this setup, they are placed between two grooved plates, perpendicular to the grooves. A vacuum is then applied to pull the grooves in place with the fibers in between, and then heated to 150 deg C to retain their crimps. The crimped fibers are then chopped and mixed in the soft silicone, after getting plasma treated. After curing the material, mechanical behavior of the composite is observed by tensile stretch tests in order to obtain the contribution of the fibers. This is verified by computational analysis of a single crimped fiber in a soft matrix.

Results: Fig.3 shows the stress-strain plot for a composite with 0.1% weight fraction of fibers. This plot compares embedded crimped thread samples to the samples without threads and samples with uncrimped threads. Tensile tests show a marked increase in the stiffness of the material with crimped fibers with an increase in applied strain. This is verified by the simulation results showing that the fibers bear load at the center (fig.4a) and the strain developed in the fibers corresponds to the toe region of collagen containing tissue behavior (fig.4b). Numerous tests are then done for then assessing the effect of crimp level, fiber concentration and fiber stiffness on the composite mechanics.

Real-time Nitric Oxide Monitoring for Wound Healing with Flexible and Multiplexed Electrode Array

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Volumetric muscle loss (VML) injuries from trauma or surgery overwhelm the regenerative capacity for muscles and lead to permanent loss of skeletomuscular function. After injuries, neutrophils and macrophages infiltrate the wound site, release cytokines and growth factors to allow proliferation and innervation of muscles. Nitric oxide (NO) released by induced nitric oxide synthase (iNOS) from macrophages is a key regulatory biomarker of wound inflammatory response. Monitoring NO concentration over time allows for interpreting stage of wound healing and providing guidance for clinical interventions. However, the detection of NO on wound bed is challenging due to its short half-life time (6 - 50s) and low concentration on the wound (1 nM to 1000nM for rodent model). Current NO probes are limited with low number of detecting nodes, device sensitivity and long-term stability. Here, we demonstrate multi-point and real-time NO detection using flexible electrode arrays on rodent wound model. Our sensing devices show high NO sensitivity (5 nA/ μ M for 1mm-diameter electrode), and high selectivity against nitrites (10000-fold), ascorbic acid (10000-fold) and uric acid (1000-fold). We monitor NO concentration and iNOS expression over time (Day 1, 3, 5, 7 and 14) on rodent wound. NO concentration measurement from sensors is highly correlated with histological analysis results. We exhibit NO mapping using multiplexing functionality to investigate the location-dependent healing process. Future work will entail continuous and chronic recording of NO concentration for long-term monitoring. This multiplexed platform will further the understanding of wound healing mechanism and provide guidance for targeted therapeutic intervention to accelerate healing.

Tissue engineered nerve guide containing GDNF microspheres improves recovery after facial nerve injury in rats

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Background: Injury to the facial nerve and the resulting facial nerve palsy leads to devastating functional, psychological, and cosmetic challenges. Rapid functional recovery after facial nerve injury is critical to prevent muscle atrophy and restore expression. Bioengineering plays an important role to create artificial scaffolds that can enhance the recovery. This can be improved by addition of exogenous neuro-supportive agents such as glial-derived neurotrophic factor (GDNF). GDNF is a promoter of axonal elongation and branching and has been shown to promote Schwann cell proliferation and migration. In this study, we evaluated efficacy of a composite poly(caprolactone) nerve guide containing double-walled GDNF microspheres on functional, electrophysiological, and histological outcomes in a rat facial nerve injury model.

Methods: GDNF was encapsulated within double-walled poly(lactic-co-glycolic acid)/poly(lactide) microspheres and embedded in the walls biodegradable poly(caprolactone) nerve guides. This nerve guide capable of providing a sustained release of GDNF for >50 days was used to repair a facial nerve injury model in male Lewis rats. After transection and primary repair of the buccal branch of the facial nerve, the rats were divided as follows: a) transection and repair only, b) empty guide, c) GDNF-guide. Marginal mandibular branch of the facial nerve was also transected and ligated to prevent innervation of the whiskers. Weekly measurements of the whisking movements for protraction, retraction and amplitude angles were recorded. At the endpoint of 12-weeks, compound muscle action potentials at the whisker pad were assessed and nerve, muscle, and whisker pad were collected for histomorphometric analysis, including Schwann cell analysis.

Results : GDNF-guide treated rats displayed earliest peak and achieved the highest whisking amplitude with 36% recovery compared to the baseline. Weekly whisking amplitude measurements demonstrated both time and the treatment groups were independently associated with the recovery ($p<0.001$) and GDNF treatment had the highest impact versus all others ($p<0.05$). Compound muscle action potentials were significantly higher after GDNF-guide placement versus all others ($p<0.001$). Mean muscle fiber surface area at the levator labii superioris muscle was the highest ($p<0.01$). The axonal integrity loss was less prominent within the GDNF-guides. Gross morphology of the whisker pad was not different across the groups.

Conclusion: The novel tissue engineered nerve guide containing double-walled GDNF microspheres enhances recovery after facial nerve transection. Results support the clinical viability of these guides to enhance recovery after nerve injury and hold promise to facilitate recovery in defects with larger gaps.

Manipulating Poly Lactic-co-Glycolic Acid (PLGA) End-Cap Functional Group Reduces Electrostatic Peptide-Protein Interactions

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Introduction: A significant challenge in the field of regenerative medicine is effectively delivering bioactive molecules in a controlled manner for a sustained duration. Many researchers have devised novel methods to encapsulate and administer several types of bioactive molecules including chemokines, cytokines, growth factors, and nucleic acids, and many of these molecules are cationic. Poly Lactic-co-Glycolic Acid (PLGA) matrices are often employed to control the release rate of cationic proteins; however, negatively charged carboxylic acid end groups (-COOH) in low molecular weight PLGA introduce significant challenges due to protein-polymer interactions. These interactions can result in acylation reactions with the N-terminus or lysine residues on these bioactive molecules leading to significantly diminished release rates or protein denaturing. Consequently, significant research has been devoted to modifying proteins or peptides to be more resilient or to identify acylation inhibitors that can be co-encapsulated in PLGA delivery devices. The former can require individual processing and custom design of each biomolecule to be encapsulated and the latter can complicate encapsulation while reducing biomolecule loading capacity. Rather, this research seeks to identify approaches to alter PLGA properties to reduce electrostatic interactions and subsequent acylation of peptides and proteins to yield a robust formulation capable of releasing cationic proteins and peptides. This work is widely applicable to the fields of regenerative medicine and cardiovascular bioengineering.

Methods: CCL22 (Protein) microparticles (MP) were prepared through a double emulsion (water-oil-water) process in which the encapsulating polymer (oil) was adjusted between experimental groups. In vitro release behavior was characterized by suspending MP in PBS supplemented with 1% bovine serum albumin and taking routine samples.

Results: In this work, PLGA formulations with various end-cap groups; including acid (PLGA-COOH), ester (PLGA-OH), di-hydroxyl (HO-PLGA-OH), and di-amine (H₂N-PLGA-NH₂); were evaluated for their effects on recombinant mouse CCL22. Encapsulation of CCL22 in PLGA-COOH resulted in severely diminished release rates. Formulations with PLGA-OH significantly increased the burst release but resulted in an extended lag phase of release due to increased polymer hydrophobicity. Similarly, HO-PLGA-OH increased burst magnitude followed by an extended lag phase. H₂N-PLGA-NH₂ formulations exhibited diminished burst release with shortened lag phase release followed by stable, linear release kinetics. Polymer blends were also evaluated, in which synergistic effects were observed.

Conclusion: These data suggest that utilizing PLGA with positively charged end-cap groups can reduce electrostatic interactions between the anionic polymer surface and the cationic protein in a predictable and controllable manner.

Matrix Bound Vesicles modulate inflammation in an imiquimod-induced psoriasis C57BL/6 mice model

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Psoriasis is a skin disease characterized by the infiltration of immune cells such as macrophages and T-cells in the dermal tissue. These immune cells show an inflammatory profile producing cytokines such as IL-6, IFN γ or TNF- α , resulting in the common symptoms of psoriasis: inflammation, pruritis, redness and squamous/flakey skin. In imiquimod-induced psoriasis mice models, these symptoms are generally mediated by the IL-17/IL-23 axis, similar to human psoriasis. Matrix bound nanovesicles (MBV) have recently been identified as an inherent component of the extracellular matrix (ECM) and can modulate the immune cell phenotype, specifically a reduction in proinflammatory (M1) phenotype and induction of a M2 or remodeling phenotype. The aim of the present study was to assess the immunomodulatory effect of porcine urinary bladder (UBM) MBV in the inflammatory context of psoriasis.

All experiments were conducted with approval of the IACUC (Protocol #19116023). The dorsum of 6–8-week-old C57BL/6 and Balb/c mice was shaved, and hair was completely removed with depilatory cream at day -1. At day 0, animals were randomly assigned to one of the following groups: i) no treatment control, ii) imiquimod cream application, iii) imiquimod cream application and intravenous MBV injection, and iv) imiquimod cream application and intraperitoneal MBV injection (N=4). In all treated groups, approximately 5 mg of imiquimod cream was topically applied daily. For those animals in MBV groups, MBV were injected with MBV suspended in sterile saline at a total dose of 10×10^{12} particles at days 2, 4 and 6. Evaluation of the skin was conducted by macroscopic observation and measurement of skin thickness. At day 7, mice were euthanized, blood samples were collected, spleens were weighed and samples of skin and spleen were harvested for mRNA analysis and presence of cytokines.

Macroscopic analysis and biologic metrics showed MBV reduced inflammation in C57BL/6 mice until Day 4 ($p < 0.05$). In contrast, this anti-inflammatory effect was not observed in BALB/c mice ($p > 0.05$). It is hypothesized that these results could be associated with the higher activation of the IL17 axis in C57BL/6 mice than in BALB/c, and that MBV have a modulatory effect upon the IL17/IL23 pathway. Gene expression as measured by RT-qPCR in C57BL/6 mice showed both MBV-treatment groups (IV and IP) had increased the expression of IL-4 in skin samples at day 7 ($p < 0.05$). TBET expression was also downregulated in MBV groups ($p < 0.01$). This transcription factor is responsible for Th1 differentiation of CD4+ T cells, where its downregulation elicits a Th2 commitment. Also, IFN- γ expression was less when MBV were injected, although not at statistically significant values. Cytokine analysis in C57BL/6 mice skin, showed a decrease in IL-17 at day 7 in both IV ($p < 0.01$) and IP ($p < 0.05$) MBV-treated groups (Figure 1), which would indicate the modulation of MBV in the IL-17/IL-23 axis. Interestingly, these differences were not observed at systemic level (serum). Results of the present study suggest the therapeutic potential of MBV in a murine imiquimod-induced psoriasis.

The present study demonstrates the potential of MBV derived from UBM to mitigate the inflammatory response elicited by imiquimod in a murine psoriasis model in C57BL/6 mice. MBV prevented the inflammatory symptoms of imiquimod until day 4, showing also an upregulation of IL4 and downregulation of TBET and IL-17 at day 7. Further analysis on T-cell population and comparison with a non-responsive mouse strain (Balb/c) will confirm these results and help elucidate the mechanisms behind the observed outcomes.

Histological characterization of myofiber architecture in ventricular myocardium to elucidate functionality of ultrasound myofiber imaging

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Ventricular myocardium is composed of laminar sheets of myofibers, the orientations of which vary in a helix through the tissue thickness. The helical transmural fiber organization results in anisotropic tissue stiffness and enhances the biomechanical efficiency of the heart. Many heart diseases, such as pulmonary hypertension, are accompanied by chronic remodeling of the fiber architecture such that the helical fiber organization is lost. Fiber remodeling affects the biomechanical properties of the myocardium and thus the hemodynamic performance of the ventricles. It is thought that fiber remodeling contributes to heart failure in diseases like pulmonary hypertension, but the underlying mechanisms that drive remodeling are not well understood.

Biaxial mechanical testing is a powerful tool for evaluating the myocardium biomechanical properties. For the elucidation of mechanisms for tissue remodeling, it is of interest to also observe the behavior of the fibrous microstructure during biaxial testing. Current optical imaging techniques have a limited penetration depth of around 1 mm (e.g., multi-photon microscopy), and cannot yield measurement of the fiber orientation through the tissue thickness (~5 mm). Tissue microarchitecture must then be examined post-hoc with destructive histological sectioning, and mechanical behavior determined using finite element modeling.

Ultrasound imaging can penetrate more than 10 mm into tissue, and thus is able to image the entire thickness of ventricular myocardium simultaneous with biaxial testing. Although individual myofibers are too small to be resolved in the ultrasound images, Backscatter Tensor Imaging (BTI) is an ultrasound-based technique that determines the fiber orientation through the entire tissue thickness by detecting variations in the spatial coherence of ultrasound echoes from the tissue, which is directly related to the spatial autocorrelation of the tissue microstructure.

Preliminary studies show that BTI is unable to accurately estimate the fiber orientation in some cases. This study attempts to elucidate this limitation by investigating the fiber microstructure. Samples of excised porcine myocardium were imaged using BTI to determine the transmural fiber orientation, and then fixed in formalin. Histology was performed using H&E stain, and the sections were viewed under a microscope to obtain images of the fibrous layers. Each image was digitally analyzed to obtain measurements of fiber orientation, microstructural autocorrelation lengths, and fractional anisotropy. While data demonstrate structural differences between right and left ventricular samples, they do not suggest significant differences in the investigated parameters between samples for which BTI was successful and unsuccessful, indicating that some other microstructural parameters account for the limitation.

Comparison of Extracellular Vesicles Present in Bone, Blood and Extracellular Matrix

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The term “extracellular vesicle” (EV) is broadly used to describe naturally released cellular vesicles with a lipid bilayer and without a nucleus. EV represent a heterogeneous population that is generally categorized into subpopulations based on characteristics such as size, biogenesis, function, and composition. The field of extracellular vesicle (EV) research is growing rapidly, but current metrics to delineate differences between vesicle subpopulations are limited. Recently, a type of EV embedded within the extracellular matrix of soft tissues termed matrix-bound nanovesicles (MBV) has been described but defining characteristics of MBV relative to other types of EV have not been established. The present study compared three distinct subpopulations of EV, namely mineralization-competent matrix vesicles (cMV), exosomes (Exo), and MBV.

MBV were isolated from mouse skeletal muscle that was decellularized, minced, and digested enzymatically to release the MBV from the extracellular matrix. cMV were isolated from the matrix of mineralizing mouse 17IIA11 pre-odontoblast cells. Exo were isolated from mouse plasma. The three types of vesicles were quantified using NanoSight Nanoparticle Tracking Analysis. The vesicle size, physical characteristics, protein cargo, miRNA cargo, and lipid membrane composition were compared. The immunomodulatory activity on naïve and pro-inflammatory bone-marrow derived macrophages was also evaluated.

NanoSight and transmission electron microscopy (TEM) showed similar size and morphology of each vesicle type. Characterization of protein cargo showed that MBV contain low levels of proteins commonly associated with both Exo and cMV, including CD63, CD81, Annexin V, and alkaline phosphatase. Each vesicle type was associated with a unique profile of cytokine cargo. Lipidomic analysis showed clear differences in membrane composition of MBV relative to both Exo and cMV. The miRNA cargo of MBV and cMV were more similar while Exo-associated miRNA were highly distinct. Characterization of the immunomodulatory activity of each vesicle type showed that cMV induce pro-inflammatory activation of macrophages, while MBV induced a more anti-inflammatory phenotype.

The present study shows that despite similar physical characteristics, these three types represent distinctly unique subpopulations of EV. These results inform metrics for proper categorization of EV and guides choice of EV based on desired functional parameters.

Scaffold-free conduits formed from dental pulp stem cell sheets provide neurotrophic and directional support for regenerating axons

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Current treatments for peripheral nerve injuries (PNIs) result in slow and inefficient nerve regeneration, yielding poor clinical outcomes. The delivery of neurotrophic factors (NTFs), growth factors specialized in supporting axon growth and repair, to sites of PNIs can enhance this regeneration. Moreover, extracellular matrix (ECM)-scale nanofibers have been shown to orient axon extension towards the end organ, thus improving functional recovery. The dental pulp is a soft, innervated within the tooth that contains a population of stem/progenitor cells embryonically derived from the neural crest. These dental pulp cells (DPCs) express higher levels of NTFs than other mesenchymal stem cells, likely because of their developmental origin. We have recently shown that DPCs can form scaffold-free cell sheets that act as NTF delivery vehicles and promote regeneration when wrapped around PNIs in rats. The goal of this study is to now engineer a bioactive, scaffold-free nerve conduit using DPC sheets with an endogenous, aligned ECM capable of providing both neurotrophic and guidance cues to regenerating axons. To accomplish this, we formed DPC sheets on a substrate comprised of linear microgrooves to induce the cells to align and deposit a linearly aligned ECM. Histological analysis and scanning electron microscopy showed that the resulting DPC sheets were solid and cellular, and immunofluorescent analysis indicated that these sheets were composed of an aligned, collagenous ECM. Additionally, the aligned DPC sheets expressed high levels of NTFs (brain-derived NTF, glial cell-derived NTF, and neurotrophin-3), similar to that of the un-aligned DPC sheets previously shown capable of inducing axon regeneration. Furthermore, we found that the aligned cell sheets were able to functionally induce and orient neurite extension in co-cultured neuronal cells in vitro. The neurites cultured on the aligned cell sheets also contained fewer branches than those on the un-aligned sheets, further highlighting the potential of the aligned DPC sheets to reduce aberrant reinnervation. We assembled these bioactive cell sheets into robust, scaffold-free cylindrical constructs approximately 7mm in length and 500 μ m in diameter. Multiphoton and scanning electron microscopies showed that the DPC sheets maintained their aligned ECM, and thus their ability to orient extending axons, when formed into conduits. Overall, scaffold-free nerve conduits formed from aligned DPC sheets are capable of enhancing nerve regeneration by providing both a continuous supply of NTFs, to promote axon regeneration, and guidance cues from an aligned ECM, to direct axon extension, thus improving the clinical outcomes of PNI treatment.

Method of decellularization alters the native structure and function of porcine vagina

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Decellularization involves selective removal of cells from a tissue to isolate the intact extracellular matrix or its components. Isolated matrix has invaluable research and clinical uses. For the vagina, little is known of the impact decellularization processes have on native structure, which impedes development of materials aiming to recapitulate native matrix structure and function. Here we aimed to compare the impact of 3 common decellularization methods on critical structural properties of porcine vaginal extracellular matrix (vECM). Vagina from 20 nulliparous adult domestic swine were divided into four treatment groups: no treatment (NT, N=5), 5 freeze-thaw cycles in hypotonic solution (FT, N=5), trypsin/Triton-X-100/sodium deoxycholate solution immersions (SDC, N=5), or freeze-thaw followed by Triton-X-100 (FTT, N=5). Full-thickness sections were isolated from each vagina representing proximal, midsection, and distal vagina on both anterior and posterior aspects (total N=120). Tissues were subjected to ball burst testing (rate 10 mm/min, ball diameter 10 mm), sulfated glycosaminoglycan (sGAG) content (N=116, Blyscan), and dsDNA content (N=24, PicoGreen). Separate tissue sections treated with SDC (N=3) were also analyzed for residual cells (H&E). After confirming normality, 1-way ANOVA followed by Bonferroni correction was performed. H&E analysis showed nuclear clearing after decellularization. PicoGreen analysis demonstrated significant reductions in DNA content (average 63.1% FT, 62.4% FTT, 59.2% SDC) after all treatments ($p < 0.001$). No significant reduction in sGAG content was observed between groups. Maximum stiffness (N/mm) for each group varied: 41.79 ± 17.04 (NT), 53.96 ± 20.24 (FT), 41.43 ± 17.46 (FTT), and 60.23 ± 18.51 (SDC). SDC treatment induced an increase in structural stiffness for all vaginal regions combined ($p < 0.01$). Further analysis showed that samples from the posterior ($p < 0.05$) and proximal ($p < 0.05$) vagina had significantly stiffened after SDC treatment. FT and FTT decellularization did not alter matrix stiffness relative to NT. Decellularization methods have varying effects on matrix properties based on tissue type and reagents used. The strategies analyzed in this study significantly reduced DNA content, although not to the 50ng dsDNA/mg dry weight benchmark used for delineating successful decellularization. sGAG content was preserved for all treatments relative to NT. SDC treatment induced location-specific stiffening of the tissue versus NT controls, suggesting that vaginal homogeneity should not be assumed when using this method to create vECM for regenerative medicine applications. Further characterization of matrices yielded from these methods is ongoing to define tissue and location-specific treatment effects on collagen content, retention of growth factors, and microscopic matrix stiffness.

The AxoDip: a novel machine for consistent manufacturing of nerve guides in clinical trials

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Background: Severely damaged peripheral nerve injuries require long recovery times. Often, the motor function never fully restores to original capability, leading to a decrease in the patient's quality of life. Recent studies have examined the functional recovery of severed nerves using autologous nerve grafts, empty polymer conduits, and polymer conduits that release growth factors via a slow-release mechanism. While an empty conduit may provide a path for the nerve to regenerate, the conduits with growth factors, such as Glial Cell Line-Derived Neurotrophic Factor (GDNF), stimulate axonal growth across the gap. Our lab has developed a protocol for encapsulating a desired concentration of GDNF into polymeric double-walled microspheres (DW MS). These GDNF DW MS are then incorporated into porous polycaprolactone (PCL) conduits through a repeated dipping process that results in five layered walls. To ensure consistent reproduction of the nerve guides for human clinical trials, our lab has designed and built a machine called the AxoDip™ (patent pending). The AxoDip has been shipped to a GMP Biomaterials Lab at the Mayo Clinic for fabrication of the GDNF-containing nerve guides for the first-in-human clinical trial.

Methods: Biodegradable GDNF DW MS were prepared using a water-oil-water emulsion technique. GDNF DW MS were characterized using scanning electron microscopy (SEM) and MS drug concentration was assessed using enzyme-linked immunosorbent assay (ELISA). The AxoDip was designed using SolidWorks design software and constructed from poly (lactic acid) (PLA) 3D printed parts. The electronics were operated via LabView and Arduino software.

Results: SEM imaging revealed two distinct layers in the microspheres. ELISA revealed that the GDNF DW MS were consistently loaded with the targeted dose of GDNF. The AxoDip operated on both a manual and automated setting, with the automated setting allowing the user to set the number of dips and the spin-drying time. Nerve guides fabricated using the AxoDip demonstrated consistency between guides, yielding wall thicknesses of 660-790um.

Conclusions: GDNF DW MS were successfully made, achieving the desired drug concentration. GDNF DW MS can now be used in conjunction with our PCL solution and our newly created AxoDip machine to fabricate consistent nerve guides with wall thicknesses in the desired range. Final optimization is being conducted for clinical trials and FDA submission. These studies represent an important milestone in the translation of this research to clinical trials.

Magnetic Microrobots that Demonstrate Liquid-Gel Penetration for Biomolecule Delivery

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The ability to induce changes in cellular behavior using biomolecules is an important tool for both scientific discovery and medical therapy. Precise and quantitative manipulation of the cell's biomolecular environment is more readily implemented in vitro but requires drug delivery systems in vivo. Current drug delivery systems typically rely upon direct implantation in situ or passive circulatory transport to reach their intended targets where they deliver their payloads via passive diffusion. These drugs must cross protective barriers including endogenous liquid-gel interfaces (e.g., the gastric mucosa). Ideal drug delivery systems would allow for ex vivo guidance to targets and tunable payload delivery. One class of next-generation drug delivery systems is based upon biomolecule-laden microrobots, which can be externally guided to release their payload at a site of interest. Here, we present a biomimetic microfluidic platform in which magnetically guided, hydrogel-based microrobots can probe a liquid-gel interface and deliver antibiotics to bacteria encapsulated within a hydrogel matrix. Our microfluidic arena enables localized delivery to fluidically interconnected cultures, allowing different regions to be targeted individually. Both prophylactic and therapeutic antibiotic delivery investigations were conducted, demonstrating the precision achievable with this biomolecular delivery system. These results showcase the potential use of biomimetic microrobots to deliver biomolecules for scientific studies or localized therapy in the future.

Therapeutic Potential of Matrix Bound Nanovesicles in Rodent Model of Ulcerative Colitis

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Ulcerative colitis (UC) is an inflammatory bowel disease that results in ulceration of the colon and rectum. Current treatments for UC vary from immunosuppression to surgical intervention (e.g. colectomy) both of which fail to maintain long-term remission and have a negative impact on quality of life. Extracellular matrix (ECM) hydrogels have been shown to facilitate the restoration of epithelial barrier function and mitigate a pro-inflammatory state of macrophages in that occurs in a dextran sodium sulfate (DSS) model of UC. Recently discovered matrix bound nanovesicles (MBV) embedded within the ECM have been found to play a central therapeutic role in the immunomodulatory properties of ECM-based materials. The objective of the present study was to determine the efficacy of MBV for the treatment of UC, specifically the effect of MBV treatment on the healing rate of DSS-induced UC. A DSS model of UC was used in male Sprague Dawley rats. Animals were induced for six days with 5.5% DSS in their drinking water. After induction, animals were treated either daily with ECM hydrogel and/or MBV via enema or every other day with MBV via intravenous (IV) injection. Clinical in-life observation including water and food consumption, stool consistency, blood presence, and body weight was conducted daily. Colonic tissue was evaluated histologically after 4 days of treatment. In-life observations showed faster recovery in both ECM and MBV treatment groups compared to the disease control group. Gross scoring and histological analysis confirmed the in-life observations showing a lower disease score in treated groups with macroscopic and microscopic analysis. The cell density within the mucosa and submucosa was lower in treated groups than disease control consistent with the observed decreased inflammation. Treatment groups showed greater recovery of the mucosa compared to the disease control group shown by the increased presence of mucosal crypts. This mucosal recovery was greatest in the MBV IV Injection treatment group. Macrophages in ECM and MBV treated groups showed a greater shift toward a remodeling phenotype than the disease control group. The results of the present study show the efficacy of MBV in the treatment of DSS-induced ulcerative colitis. A comprehensive look at the local and systemic effects of MBV in this model needs to be studied further to unlock the true potential of MBV as a UC therapy. The present study has shown exciting evidence that MBV may be beneficial therapy to enhance the quality of life of UC patients.

Immune Cells Activating Biotin-decorated PLGA Protein Carrier

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Nanoparticle formulations have long been proposed as protein carriers owing to their ability to entrap proteins. In addition, nanoparticles can be modified to promote immunostimulatory or immunosuppressant responses. Poly(lactic-co-glycolic acid) (PLGA) remains one of the most studied polymers for controlled release and nanoparticle drug delivery, and numerous studies exist proposing PLGA particles as protein carriers. In this work we report using PLGA nanoparticles modified with biotin (bNPs) to deliver proteins via adsorption, and stimulate professional antigen-presenting cells (APCs). We present evidence showing bNPs are capable of retaining proteins through the biotin-avidin interaction. Surface accessible biotin bound both biotinylated catalase (bCAT) through avidin as well as streptavidin-horse radish peroxidase (HRP). Analysis of the HRP found that activity on the bNPs was preserved once captured on the surface of bNP. Further, bNPs were found to have self-adjunct properties, evidenced by bNP induced IL-1 β , IL-18, and IL-12 production in vitro in APCs, thereby licensing the cells to generate T helper type 1 (Th1)-type T cell responses. Th1 responses have favorable indications in infectious disease, cancer and in early wound healing. For bNPs, we found that we can modulate cytokine production by blocking biotin on the particle surface. Indeed, cytokine production was reduced in avidin pre-coated bNPs (but not with other proteins). These data, in addition to the observed increase in cytokine release as the ratio of biotin: PLGA increased, provide evidence that the pro-inflammatory response from bNPs is specific to the amount of exposed biotin on the surface of bNPs. We present evidence that the bNPs promote these responses, in part, through the mechanistic target of rapamycin (mTOR) pathway given the significant reduction of cytokines in cultures incubated with rapamycin. bNPs were internalized by Bronchoalveolar lavage dendritic cells and macrophages in mice in a dose-dependent manner when delivered intranasally. Taken together, these data provide evidence that bNPs are suitable for characterization and optimization for use in infectious diseases, cancer, and early wound healing.

Modelling articular cartilage post-traumatic changes using human cell-based hydrogel constructs

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Background: Traumatic impacts on the articular joint surface are known to lead to degeneration of the cartilage, as in post-traumatic osteoarthritis (PTOA). While animal-based systems have been instrumental in understanding pathogenic progression of PTOA, they have not served to develop effective treatments for the disease. The limited progress in the development of disease-modifying medications (DMMs) may be due to insufficient mechanistic understanding of human disease onset/progression that can, in part, be attributed to insufficient in vitro models for disease and therapeutic modeling. To overcome this insufficiency, we are testing hydrogel-based models using adult human mesenchymal stromal cells to examine the effects of traumatic impacts on human cell-based engineered cartilage constructs. We hypothesize that cells encapsulated within biomimetic scaffolds will respond to traumatic impacts in a manner congruent with early PTOA pathogenesis in animal models.

Methods: Engineered cartilage constructs were fabricated by encapsulating adult human mesenchymal stromal cells (hBM-MSCs) in a photocrosslinkable, biomimetic hydrogel (15% methacrylated gelatin, GelMA) that were chondrogenically differentiated for 28 days using TGF-beta3. Constructs were subjected to traumatic impacts with 30% strain or 10ng/ml IL-1beta. Cell viability and metabolism, mechanical property, gene expression, matrix protein production and activation of catabolic enzymes were assessed.

Results: Live and dead staining results showed that traumatic impacts of 30% strain caused massive cell death in engineered cartilage constructs. Elastic modulus of engineered cartilage constructs decreased significantly after traumatic impacts. CCK8 assay results also showed significant cell death and metabolism decrease in the constructs. GAG production decreased 1 day after impacts but recovered 7 days after impact, as was observed in safranin O staining and GAG assay. RT-PCT results and IHC results showed that anabolic activities were depressed and catabolic enzymes (MMP13, ADAMTS4, ADAMTS5) were activated after impact.

Conclusion: Traumatic impacts delivered to engineered cartilage constructs induced PTOA-like changes in the encapsulated cells. The development of in vitro PTOA model contribute to development of disease-modifying medications (DMMs) of OA.

Study of Ovarian Cancer Clearance Dynamics Utilizing A Novel Tumor-Mesothelial Assay

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Ovarian cancer is the leading cause of death among gynecological cancers and metastasis to the peritoneum occurs in over 60% of patients. Cancer cells in the peritoneum attach and invade through a layer of mesothelial cells that form the peritoneal membrane. Clearance of the mesothelium is defined as the displacement of mesothelial cells by the invading cancer cells. The signaling pathways in mesothelial cells that are necessary for effective clearance are poorly understood. This is partially due to limitations of existing experimental models to recapitulate the metastatic environment. The goal of this study is to explore how modulation of the mesothelial barrier alters clearance dynamics.

The clearance assay consists of a monolayer of ZTGFP (mesothelial cells) on top of which serous ovarian cancer spheroids are seeded (OV90, OVCAR3, OVCAR8, OVCA432). Mesothelial barriers are treated with Forskolin, a CAMP activator, and using an imaging processing pipeline, we identify the number of clearance events and quantify their temporal evolution.

Treatment with Forskolin significantly reduced the number of clearing spheroids compared to the control untreated condition. Removing Forskolin at the time of spheroid addition reverted the clearance dynamics to control conditions. When Forskolin was added with the addition of spheroids, without pretreatment, clearance dynamics were inhibited after 12 hours. Treating mesothelial cells with protein kinase inhibitor peptide (PKI), PKA inhibitor, in the presence of Forskolin restored the high clearance rate. Lastly, staining with phalloidin revealed a reduction in actin stress fibers in the Forskolin treated cells when compared to control.

In this study we found that treatment with Forskolin decreased the metastatic potential of spheroids through the mesothelial monolayers. Understanding the signaling pathways in mesothelial cells that enable fast clearance will provide us with new insights on how ovarian cancer causes distant metastasis and potentially identify targets for improved treatments.

Effects of Poly(vinyl alcohol) and Triacetin on Surface Morphology and Encapsulation Rate in Biodegradable Double-Walled Microspheres

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Introduction: Double-walled microspheres (DWMS) provide enhanced microencapsulation efficiency, therapeutic agents protection, and a controlled release of drugs over time. DWMS can be fabricated from different biodegradable polymers and combined with or incorporated into other tissue scaffolds in order to support wound healing and facilitate tissue repair and regeneration.

Materials and Methods: In this study, double-walled polymer microspheres were fabricated using a modified water-oil-water emulsion solvent evaporation method. The core was composed of 17.4% Poly(lactic-co-glycolic acid) (PLGA) solution, protected with the outer layer fabricated from 10% Poly(lactide) (PLLA) solution. Lysozyme was used as a model protein for the purpose of studying the effects of additives on encapsulation efficacy and surface morphology in DWMS, due to its small size of 14 to 15kDa, a molecular weight nearly equivalent to size of some neurotrophins, including the Glial Cell Line-Derived Neurotrophic Factor (GDNF). Microspheres' size, shape, and morphology were evaluated using Scanning Electron Microscopy (SEM) while the lysozyme encapsulation rate in DWMS was quantified with the Pierce Bicinchoninic Protein Assay Kit (BCA).

Results: SEM analysis showed that PLGA/PLLA DWMS with lysozyme encapsulated in the core, fabricated with 1% Poly(vinyl alcohol) (PVA) and 0.1% Triacetin, had uniform particle sizes with the mean diameter of $172 \pm 52 \mu\text{m}$. The external structure was intact with a smooth surface and no apparent surface cracks. The internal structure of polymeric DWMS was solid and free of air-pockets, holes, and cracks that were evident in the lysozyme loaded DWMS when a 0.5% PVA concentration was added to the emulsion with no Triacetin. The presence of PVA and Triacetin resulted in slight increase in yields of Lysozyme DWMS and clear suspension hence shortening the collecting and washing times of hardened microspheres. However, the protein concentration that was determined spectrophotometrically, showed no significant change in lysozyme encapsulation rate when incorporating 1% PVA emulsifier and 0.1% Triacetin.

Conclusions: Successfully establishing and optimizing the lysozyme loaded DWMS protocol was an essential step for creating Standard Operating Procedures and in vivo assessment of GDNF microencapsulation in PLGA/PLLA DWMS.

Comparing extracellular matrix from the epicardium and the perfusion decellularized whole cardiac tissue

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Epicardium, composed of mesothelial cells, is the outermost layer of the heart adjacent to the myocardium. The role of the epicardium is well appreciated as a source of trophic signals and specialized cells that contribute toward the survival and healing of both the endocardium and myocardium. However, the composition and functional benefit of epicardial extracellular matrix (ECM) is not yet explored. In the present study, the epicardium was mechanically separated from the porcine heart and decellularized via a detergent-free method. Epicardial ECM was compared with whole cardiac ECM decellularized by pulsatile retrograde aortic perfusion of porcine hearts using different concentrations of detergents. The quality of decellularized ECM was assessed by DNA quantification, histology analysis, glycosaminoglycan quantification, and cardiac cell proliferation. Moreover, matrix-bound nanovesicles (MBV) isolated from the whole cardiac ECM or epicardial ECM were compared for yield and composition. Results showed that compared to perfusion decellularized whole cardiac tissue, epicardial ECM contained significantly higher glycosaminoglycans and elastin. Ongoing studies will continue to examine the effect of epicardial ECM on macrophages, cardiomyocytes, and cardiac fibroblasts at normal and hypoxic cell culture conditions.

Ascending and Descending Aortic ECM Hydrogels to Study Microvascular Function in Aortic Disease

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Biomechanical weakening of the vessel wall, medial degeneration, and vasa vasorum remodeling are all hallmarks of aneurysmal diseases via mechanisms that are not fully understood. In particular, an adventitial microvascular network known as the vasa vasorum (“vessels of the vessels”) provides oxygen and nutrients to the outer aortic wall as a key component of inter-layer vascular health. In the ascending aorta, the vasa vasorum originate from the coronary and brachiocephalic arteries whereas in the descending aorta, the vasa vasorum stem from the intercostal arteries. The different origins of the vasa vasorum correspond to the anatomically specific functions of the aortic regions, which can further pertain to the differing origins of vascular wall cells and putative differences in the composition of extracellular matrix (ECM). In the present study, we investigated putative differences in composition, structure, and bioactivity between ascending and descending aorta-derived ECM to better understand intra- and inter-layer cell-matrix interactions relevant to vasa vasorum function in the aorta. Decellularization of porcine aortic ECM was validated via determination of DNA content through molecular and histological measure to meet stringent requirements. Optical density of hydrogels from ascending and descending aorta-derived ECM revealed a logarithmic curve during the gelation period at 37°C. Peak gelation of ECM hydrogels was achieved within 30 minutes while collagen alone gelled within 1 hour. Ascending aorta-derived ECMs gelled faster when compared with descending aorta-derived ECMs. A collagen compaction assay using vasa vasorum-associated pericytes confirmed similar bioactivity for ascending or descending pAdv or pMed ECMs when compared with gels lacking aortic ECM (cell only controls). SEM imaging of ECM hydrogels revealed acellular fibrous architecture for all ECMs. Interestingly, pAdv hydrogels exhibited single fiber microarchitecture, whereas pMed hydrogels were comprised of collagen fiber bundles.

In conclusion, ascending and descending aortic ECM hydrogels retain bioactivity to influence human perivascular cells and can potentially be used as disease models for investigating aortic aneurysms. A comprehensive understanding of the influence of layer-specific ECM on cells in different aortic regions could help uncover novel disease mechanisms and serve as less invasive treatments for aortic aneurysms.

Revealing pathogenic effectors of ischemic injury to donor lungs using chemoselective profiling of newly synthesized glycoproteins during ex vivo lung perfusion

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Lung grafts from Donation after the Circulatory Death (DCD) are valuable organ sources for lung transplantation to patients with end-stage pulmonary diseases but suffer from high incidence of primary graft dysfunction due to warm ischemic injury. Ex vivo lung perfusion (EVLP) is widely used to assess DCD lung quality and has the potential of serving as a therapeutic platform. However, new therapeutic and diagnostic developments are hampered by our limited understanding of pathogenic effectors and reliable biomarkers and their effects on mediating ischemic injury to donor lung grafts and predicting graft quality respectively. In particular, protein effectors newly induced by ischemic injury is usually in extremely low abundance compared to the bulk pre-existing protein background. Herein, we generated a rat DCD lung model with 1-hour warm ischemia and used bioorthogonal tagging to selectively label newly synthesized glycoproteins (NewS-glycoprotein) during EVLP with unprecedented temporal resolution. By administering azido monosaccharide probe (Ac4GalNAz) into EVLP perfusate, robust and selective labeling of NewS-glycoproteins can be detected with as short as 4-hour EVLP. Following chemoselective desthiobiotinylation using click chemistry, we affinity-enriched the labeled NewS-glycoproteins, which enabled sensitive proteomic detection with mass spectrometry. Comparison between the enriched proteome of Ac4GalNAz and vehicle control groups demonstrated specific purification of azido-tagged NewS-glycoproteins. Comparing the enriched NewS-glycoproteome in DCD lungs versus control lungs without warm ischemia, we captured six protein candidates with DCD-associated upregulation in their synthesis, with five of them having known linkage to ischemia/reperfusion injury. As the next step, we intend to extend our approach to and validate our findings in donor human lungs to identify novel predictive markers and therapeutic targets during EVLP to detect and attenuate ischemic damage in DCD lungs, respectively.

Joint Pain on a Chip: Mechanistic Analysis, Therapeutic Targets, and an Empirical Strategy for Personalized Pain Management

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Osteoarthritis (OA) is a painful and debilitating disease, as well as the 11th global contributor to disability. However, the correlation between OA and pain is not well understood. To date there are not any disease modifying osteoarthritis drugs that have reached FDA approval leaving pain management to non-steroidal anti-inflammatory drugs or invasive surgeries such as total joint arthroplasty (TJA). Due to the lack of appropriate models to recapitulate the whole-joint disease nature of OA in humans, there is a clinical need to establish safe and effective methods for the treatment OA-associated pain. Here we report the creation of an innervated organ-on-chip model to enable the dynamic interplay between the nervous system and the synovial joint.

1) Establishing the Neu-microJoint: Human bone marrow-derived mesenchymal stem cells (hBMSCs) were encapsulated in gelatin scaffolds to engineer the two primary tissue types in this model; cartilage and fibrous tissue. A two-chamber microfluidic tissue chip was two dimensionally seeded with human dorsal root ganglion (DRG) neurites in one chamber and the other was used for the fibrous tissue construct. 2) Neuronal Fluorescence and imaging: Fibrous cells were stained with DiO prior to encapsulation with the hopes of generating a green fluorescence. Neurites were treated with Dil and virally with GFP for fluorescence detection. 3) Creation of a macrophage-fibrous tissue construct: To further mimic the synovial tissue preliminary studies were conducted to create an M1 macrophage-fibrous tissue. Tissue constructs were treated with M1 macrophage polarizing factors.

Cartilage tissue constructs treated with IL-1 β showed robust inflammation compared to the negative control. Fluorescence imaging of DRG neurites showed neurite extension through the microchannels. Live imaging was used to track neuronal activity and electrical stimulation of the neurite-evoked calcium transients in the corresponding soma. Importantly, neurons continue to respond to allogenic stimuli including "synovium fluid" from the "osteoarthritic" microJoint. Furthermore, the macrophage-fibrous tissue constructs showed an increase in tumor necrosis factor α (TNF- α) after two days of treatment with INF- γ and LPS, indicating the successful polarization of the macrophages into M1 macrophages.

The relationship between pain and OA complex and not well understood. The use of advanced technologies can help elucidate the complexities of pain and OA. The end objective of this study is to use the Neu-microJoint to serve as a platform for personalized medicine for the assessment and treatment of OA-patients.

Topical Elastase Administration and β -aminopropionitrile (BAPN) Decrease Elastin and Collagen Content and Alters Mechanical Properties in an Abdominal Aortic Aneurysm Mouse Model

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Introduction: Abdominal aortic aneurysm (AAA) is diagnosed after dilatation of the aortic diameter by 50% and characterized by a loss of elastic fibers and their inherent recoil properties. During progression of the disease, degradative enzymes within the inflammatory environment of the AAA continue to destroy both elastin and collagen fibers, leading to further expansion and potentially rupture. Recapitulating AAA in animal models has included proteolysis of native elastin or lysyl oxidase inhibition with BAPN to inhibit stabilization of new matrix; regardless of model, the process of mechanical weakening is not fully understood. We hypothesize that mouse aortas exposed to a combination of elastase and BAPN for 2 weeks will both stiffen and weaken coincident with reduced elastin.

Methods: Mouse aorta tangent modulus, ultimate tensile strength (UTS), elastin content, collagen content, and diameter were measured at the 2-week endpoint after a combination of topical elastase exposure with or without BAPN supplementation in the drinking water (4 days or all 2 weeks). Tangent modulus and UTS were evaluated using uniaxial ring testing to failure while ninhydrin and hydroxyproline assays to quantify elastin and collagen content.

Results: The final aortic diameters were larger in all elastase treated groups regardless of BAPN ($2.02\text{mm} \pm 1.03$, $1.88\text{mm} \pm 0.57$, and $1.61\text{mm} \pm 0.55$) compared to heat-inactivated elastase controls ($0.98\text{mm} \pm 0.34$). Tangent modulus increased in the elastase only group compared to controls ($32.60\text{N/cm}^2 \pm 9.83$ vs $17.99\text{N/cm}^2 \pm 5.76$, $p=0.046$). UTS was higher in 2-week BAPN treated vs. 4-day BAPN treated mice ($35.18 \pm 18.60\text{N/cm}^2$ vs $18.05\text{N/cm}^2 \pm 4.95$, $p=0.029$). Elastase-treated and 2-week BAPN aortas had lower elastin ($7.41\% \pm 2.43\%$ and $7.37\% \pm 4.00\%$, $p=0.035$ and $p=0.021$) and collagen ($4.25\% \pm 0.79\%$ and $5.86\% \pm 1.19\%$, $p=0.0012$ and $p=0.0052$) content compared to controls ($15.29\% \pm 7.67\%$ and $12.44\% \pm 6.04\%$, respectively).

Discussion: The topical elastase alone and 2-week BAPN supplemented groups exhibited larger aortic diameter and reduced elastin and collagen content after 14 days compared to controls. Tangent modulus was higher in elastase treated mice while UTS was lower in mice with longer exposure BAPN treatment compared to the shorter exposure BAPN group. Ultimately, understanding the biomechanics of these AAA mouse models can pave the way for development of other models to better mimic human AAA progression and rupture. Future work will investigate other time points, other AAA mouse models, and potential regenerative treatments to restore elastic fiber integrity and vessel wall mechanics.

3D Bioprinting of iPSC Derived Islet Organoids in Hydrogel Constructs

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One of the largest medical problems in the world is the lack of organs available for transplantation. In the US alone, as of February 2021, over 107,000 people were waiting for a transplant, and the organ need increases while the supply remains stagnant (1). 3D bioprinting is designed to fight this issue by printing biomaterial layers embedded with cells that can synergistically recreate a tissue construct. Primarily bioprinting has focused on recreating hard tissues, with less work being done to recreate soft tissue environments (2). Soft tissue requires bioinks that can act as support structures, while maintaining the correct biophysical properties for human cells. Another technology used to address organ shortages is induced pluripotent stem cells (iPSCs), as they can be differentiated to any tissue and tailored to patients. The current aim of this project is combining the advantages of bioprinting and iPSC organoids to form a complex soft tissue environment. In this study, iPSC islets were bioprinted with the goal of engineering endocrine pancreas tissue.

The ideal bioink was determined by optimizing the ink composition to produce a structure that provided the desired stability for iPSC culture. A combination of 3% w/v alginate, and 6% w/v methylcellulose crosslinked with 50 mM CaCl₂ was able to achieve this stability. After determining the optimal bioink, undifferentiated iPSCs in both single cell and aggregate form were printed and cultured. The SC iPSCs formed small aggregates within the print and retained high viability over a 9-day culture. The aggregate iPSCs were printed at an average size of 350 µm and cultured for 3 days. LiveDead imaging showed the aggregates maintained viability, while fluorescent staining showed the presence of Oct4 and Nanog, confirming pluripotency. Having established successful printing with undifferentiated iPSCs, the next focus was printing iPSC derived islet organoids. These organoids were derived using an organoid culture method established previously, where the islets exhibit pancreatic and islet markers, c-peptide and glucagon (3). iPSC islets were printed using the same protocol as the undifferentiated iPSCs and cultured for 7 days. LiveDead imaging showed the iPSC islets sustaining viability, while fluorescent staining indicated that they also retained expression of the pancreatic markers, NKX6.1 and PDX1. Work is ongoing to test for printed islet functionality, and to see if iPSC aggregates can undergo differentiation post-printing. This project acts as proof of concept for bioprinting a functional pancreatic environment using iPSC islets, with future work focused on organ transplantation.

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Evaluation of the Host Response to Galatea Surgical Scaffold

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Statement of Purpose: This project sought to evaluate the host response to Galaflex Surgical Scaffold as compared to two competitive products; Bard Mesh and Alloderm Tissue Matrix. Galaflex mesh is made of P4HB, a resorbable biologic monofilament used for medical applications like hernia or reconstructive surgeries. Studies have shown increased strength and improved tissue integration at the site of implantation. With increasing knowledge and growing popularity of this material, fully understanding the immune response to Galaflex as compared to meshes commonly used in similar surgical applications: Bard, a polypropylene mesh, and Alloderm, a human dermal tissue matrix.

Methods: Mesh types were subcutaneously implanted (1.5X1.5cm) in C57BL/6 mice, which were then sacrificed with the mesh tissue complex being explanted at days 3, 7, 14, and 90. Following explantation, the mesh-tissue complex was divided and analyzed by histology and flow cytometry.

Results: Recent research has suggested that an early immune response, followed by a phenotypic transition to a more M2 healing environment suggests ideal remodeling outcomes. With an initial influx of cells, as shown by H&E histologic analysis (Day 3: $p=0.0420$, Day 7: $p=0.0119$), followed by a resolve at day 90 ($p=0.0352$), suggests an early immune response that is somewhat resolved by 3 months post implantation. Quantifying collagen deposition showed an increase of density at 7 days surrounding Galaflex compared to both Alloderm and Bard ($p=0.0431$ and $p=0.0275$, respectively). Characterization of these cells in the granulation tissue causing these remodeling outcomes showed differences in early immune cell recruitment of neutrophils, monocytes, and macrophages. An increase in monocytes is seen in the Galaflex group at 3 ($p=0.0061$, $p=0.0003$), 7 ($p=0.0008$) and 14 days ($p=0.0114$), with an increase with Alloderm ($p<0.0001$) and Bard ($p=0.0008$) at 90 days as compared to Galaflex. At day 3 Galaflex had more macrophages than both Alloderm ($p=0.0095$) and Bard ($p=0.0072$). Further phenotyping of these early macrophages showed an increase in M1 macrophages at day 3 in response to Bard ($p=0.0102$), with an increase in M2 macrophages with Galaflex compared to both Alloderm and Bard ($p=0.0018$). Days 7, 14, and 90 have significantly more macrophages with Bard and Alloderm than Galaflex. At days 14 and 90 these macrophages are more phenotypically M1 in Galaflex than Alloderm ($p=0.0034$, $p=0.0099$). However, at day 90, Galaflex has more M2 macrophages than Bard mesh ($p<0.0001$). These results suggest an early influx of cells recruited for immune and structural support, with resolve in these responses by 90 days.

Engineering 3D skeletal muscle constructs to model age-related regenerative defects and responses to intervention

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Aging is associated with impaired skeletal muscle function and regenerative capacity. To date, the bulk of our understanding of such impairments has relied on two-dimensional (2D) cell culture systems and animal models. While 2D systems provide a highly valuable platform to evaluate aged muscle stem cell phenotypes, culture artifact limits the utility of this model to faithfully represent the behavior of cells under in vivo conditions. Aging mammalian models, while essential, are time-consuming, costly, and often limited in availability. Here, we present a novel three-dimensional (3D) muscle construct system designed to overcome the limitations of these traditional platforms and to model the effects of aging on muscle structure, functional regeneration, and response to intervention. Young muscle constructs (YMC) and old muscle constructs (OMC) are fabricated using muscle progenitor cells (MPCs) isolated from young or aged mice, respectively. Whereas both young and old constructs form aligned myotubes after maturation, OMC manifest a sarcopenic phenotype, as evidenced by hypotrophic myotubes and limited calcium handling capacity and force production, when compared to YMC. OMC also display a signature of impaired healing capacity, including delayed MPC amplification, defective myotube regeneration, and reduced force recovery after injury. We further show that the age-related delay in MPC regenerative response can be rescued by exposing OMC to the conditioned medium of injured YMC, raising the novel hypothesis that age-related factors released by damaged myofibers regulate MuSC activation. Finally, we show that treating injured OMC with either recombinant α -Klotho protein or extracellular vesicles isolated from young mouse serum phenocopies the in vivo regenerative responses of aged skeletal muscle to intervention. These collective data reveal that our aging muscle platform can be used both to investigate mechanisms underlying aged muscle dysfunction and to evaluate the efficacy of interventions to enhance muscle healing in a geriatric population.

Engineering Rotating Apical-Out Airway Organoids for Assessing Respiratory Cilia Motility

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Motile cilia project from the airway apical surface and directly interface with the inhaled external environment. However, prevailing airway organoid models have an apical-in conformation with cilia facing organoid's interior. Further, due to cilia's nanoscale dimension and high beating frequency, quantitative assessment of their motility remains a sophisticated task. Here we described a robust approach for reproducible engineering of apical-out airway organoid (AOAO) of defined size. Propelled by exterior-facing cilia beating, the mature AOAO exhibited stable rotational motion when surrounded by Matrigel. We developed a computational framework leveraging computer vision algorithms to quantify AOAO rotation and validated its correlation with direct measurement of cilia motility. We further established the feasibility of using AOAO rotation to recapitulate and measure defective cilia motility caused by chemotherapy-induced toxicity and by CCDC39 mutations in cells from primary ciliary dyskinesia patient. We expect our rotating AOAO model and the associated computational pipeline to offer a generalizable framework that can be adopted to develop high-throughput assays to expedite modeling of and therapeutic development for genetic and environmental ciliopathies.

Engineering pro-angiogenic biomaterials via chemoselective extracellular vesicle immobilization

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Nanoscale extracellular vesicles (EVs) represent a unique cellular derivative that reflect the therapeutic potential of mesenchymal stem cells (MSCs) toward tissue engineering and injury repair without the logistical and safety concerns of utilizing living cells. However, upon systemic administration in vivo, EVs undergo rapid clearance and typically lack controlled targeted delivery, thus reducing their effectiveness in therapeutic regenerative therapies. Here, we describe a strategy that enables long-term in vivo spatial EV retention by chemoselective immobilization of metabolically incorporated azido ligand-bearing EVs (azido-EVs) within a dibenzocyclooctyne-modified collagen hydrogel. MSC-derived azido-EVs exhibit comparable morphological and functional properties as their non-labeled EV counterparts and, when immobilized within collagen hydrogel implants via click chemistry, they elicited more robust host cell infiltration, angiogenic and immunoregulatory responses including vascular ingrowth and macrophage recruitment compared to ten times the higher dose required by non-immobilized EVs. We envision this technology will enable a wide range of applications to spatially promote vascularization and host integration relevant to tissue engineering and regenerative medicine applications.

Is aging accompanied by ‘biological wisdom’ at the single-cell level?

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A tendency towards disorder is a quintessential expression of time’s arrow. In biology, aging manifests as progressive organismal dysfunction and increased vulnerability to death. The maintenance of proper functioning requires highly coordinated communication of trillions of biomolecules. However, over time, noise accumulates, thereby disrupting effective communication flow. Here, we employed an unbiased Information Theory approach to quantify whether and how aging affects the transmission of useful information within individual cells. For this, we used mononuclear cells from aging skeletal muscle as our model system. We applied Shannon’s Noisy Channel Coding Theorem to transcriptional regulatory networks (TRNs) within single cells. TRNs were constructed using single-cell RNA seq data from the hindlimb muscles of young, middle aged, and aged mice. Each cell is represented as a network of communication channels between transcription factors (TF) and their target genes (TG). TFs provide the input message that is received by the downstream TGs as an output. We found that the mutual information (MI), or the overall “useful information” relayed from input to output, progressively declined with age. Moreover, aged cells displayed an increase in biological noise, defined as the conditional entropy of TG expression given TF expression. When we further probed individual TF:TG pairs, we found that a small subset of communication channels were tightly maintained over time. Remarkably, enrichment analysis revealed that MI was preserved in gene pairs associated with tissue homeostasis at the expense of tissue adaptability and plasticity. Taken together, the data suggest that aging is associated with an overall increase in biological noise, which compromises the transfer of ‘useful information’ over time. However, a distinct set of messages crucial to homeostatic functions are preferentially preserved with increased age. An enhanced understanding of how certain biological functions are given priority (i.e., ‘biological wisdom’), may aid in the development of therapeutics designed to preserve organismal health and function in an aging population.