2020 McGowan Retreat
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Investigation of the role of monocyte activation in intracellular trafficking of ultra-small nanoparticles

Dasia Aldarondo (1) and Elizabeth Wayne (1)

(1) Department of Chemical Engineering, Carnegie Mellon University, Pittsburgh, PA

Monocyte recruitment plays a decisive role during disease pathogenesis and in wound healing/muscle regeneration. Monocyte and macrophages have the potential to be targeted carriers for small molecule drugs. The goal of this research is to characterize an ultra-small nanoparticle loaded monocyte delivery system. Initial research aims to understand how the ultra-small nanoparticles uptake into the monocytes and to track their intracellular trafficking using live cell imaging. Along with studying monocyte interaction, we also aim to understand the effects these nanoparticles have on monocyte differentiation to macrophages and the markers they present. This research will better characterize the potential use of monocyte mediated drug delivery of ultra-small nanoparticles and give information important to utilize moving into in vivo testing.

Profilin1 as an interventional target in clear cell renal cell carcinoma

Abigail Allen (1*), David Gau (1*), Yue Wang (2), Ryan Martin (3), Jodi Maranchi (4), Anette Duensing (5), Sunder Sims-Lucas (6), Adam Kaczorowski (7), Stefan Duensing (7), Lily Wu (8), Michael T. Lotze (1,2,9), Walter Storkus (1,2,9,10) and Partha Roy (1,5); * Equal contribution

Departments of (1) Bioengineering, (2) Surgery, (3) Biology, (4) Urology, (5) Pathology, (6) Pediatrics, (7) Biostatistics, (9) Immunology and (10) Dermatology at the University of Pittsburgh (8) Department of Urology, University of California, Los Angeles, and the (7) Department of Urology,at the Heidelberg School of Medicine, Heidelberg, Germany

A distinguishing feature of clear cell renal cell carcinoma (ccRCC), the most common subtype of renal cancer, is a highly-vascularized tumor microenvironment due to upregulation of proangiogenic pathways. Analyses of the Cancer Genome Atlas datasets demonstrated increased expressions of several important actin-regulatory proteins including profilin-1 (Pfn1), Arp3, cofilin1, Ena/VASP and CapZ correlated with features of advanced-stage disease, and adverse outcome amongst ccRCC patients. We provide further evidence for dysregulation of expression of a subset of these proteins (Pfn1, Mena and CapZ) by tumor-associated vascular endothelial cells (TA-VEC) in ccRCC, in addition to increased levels of soluble Pfn1 in the serum of ccRCC patients when compared to normal donors. VEC-selective conditional knockout of the Pfn1 gene in mouse demonstrated Pfn1-dependency for blood vessel formation in the kidney. In vitro studies further suggested Pfn1's important role in proliferation and migration of RCC cells, and VEC-tumor cell crosstalk as an extracellular agent. Additionally, a novel small molecule inhibitor of pfn1:actin interaction was found to reduce proliferation and migration of RCC cell lines, and reduced in vivo tumor progression. Based on these findings, we propose a potentiating role for Pfn1 in tumor progression, with Pfn1 also serving as a prognostic biomarker and a potential interventional target in the setting of ccRCC.

Therapy Induced Extracellular Matrix Drives Melanoma Progression by Activating the Tumor Microenvironment

Andrew M. Bradshaw (1), Erica Kuo (1), Jelena Grahovac (3), Kyle Sylakowski (1), Amanda Clark (1,2), Cindy Sander (2), Howard Edington (2,4), John M Kirkwood (1,2,5) and Alan Wells (1,2,6)

(1) Department of Pathology, University of Pittsburgh, (2) University of Pittsburgh Cancer Institute, University of Pittsburgh, (3) Institute of Oncology and Radiology of Serbia, National Cancer Research Center, Belgrade, Serbia, (4) Department of Surgery, Allegheny Health Network, Pittsburgh, PA, (5) Division of Hematology and Oncology, University of Pittsburgh Medical Center, Pittsburgh, PA, (6) VA Pittsburgh Healthcare System, Pittsburgh, PA

Therapies designed to stall tumor progression often fail after an initial response or perversely accelerate progression. Here, we probe how therapies directed toward melanoma induce inflammation to generate a pathological extracellular matrix (ECM) via transforming growth factor beta (TGFβ). The drastic remodeling and presence of aberrant ECM derived from fibroblasts in the tumor microenvironment is mirrors the composition of advanced melanoma tissue. We examined the expression of ECM and cell adhesion molecules and found Tenascin-C (TNC) and alpha-smooth muscle actin (aSMA) are upregulated and the small leucine-rich proteoglycan Decorin (DCN) decreased in advanced malignant melanoma. In a skin organ model of melanoma invasiveness, TNC promoted, while DCN decreased melanoma invasiveness. The opposite and almost mutually exclusive pattern of ECM expression was observed in response to melanoma treatment. Using clinically relevant inhibitors of TGFβ receptor we were able to suppress αSMA expression and abrogate the effects of melanoma cell stress during targeted therapy, resulting in decreased proliferation and invasion, an effect that was also mediated by DCN. These findings indicate the novelty of dual targeting to suppress melanoma and the pro-progressive ECM that results from treatment stress.

Improved RBC Rheology of Sickle Cells Following Transfer of Intracellular Hemoglobin

Dan Crompton (1,2), David Chan (1,2), Jonathan Waters (3) and Marina V. Kameneva (1,2,4)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Anesthesiology, (4) Department of Surgery, University of Pittsburgh Medical Center

Introduction: Sickle cell disease (SCD) is an autosomal recessive genetic condition in which mutant hemoglobin (Hb) pathogenically polymerizes within red blood cells (RBCs) under deoxygenated conditions. Intracellular Hb polymerization reduces RBC deformability, which results in vaso-occlusion, hemolysis, hypoxia, and severe pain. A primary treatment for SCD is exchange transfusion, however repetitive transfusions may lead to alloimmunization which results in difficulties in finding compatible donor blood. This lab has pioneered a novel cellular therapy in which pathological sickle Hb is replaced with healthy donor Hb in sickle RBCs (S-RBCs), eliminating their ability to sickle, improving RBC rheology, and removing the risk of alloimmunization with the use of autologous RBC membranes.

Methods: Donor Hb solution was prepared by lysing healthy bovine or human blood samples in sterile water and the free Hb purified and concentrated to approximately 30 g/dL. S-RBC “Ghosts” (devoid of Hb) and S-RBC “Refills” samples were created from SCD blood samples using specific intracellular Hb replacement therapy and compared them with healthy human RBCs and initial SCD RBC samples. Rheological properties including viscosity, elasticity and deformability of these RBCs were measured, and total Hb capture efficiency of “refilled” RBCs calculated, and SickleScreen Hb solubility tests were conducted to determine the presence of residual sickle Hb.

Results: Following intracellular Hb replacement, all S-RBC derived samples were found to be devoid of sickle Hb and non-sickling. Refilled S-RBCs were found to contain up to 8 g/dL encapsulated Hb and were significantly less viscous and more deformable than their corresponding S-RBC samples. This work serves as a proof-of-concept that intracellular Hb replacement therapy may potentially be used in the future as a means to prevent alloimmunization for SCD patients by refilling a patient’s own RBCs with donor Hb, and transfuse those cells back to the patient.

Diploid hepatocytes resist acetaminophen induced acute liver injury and drive compensatory liver regeneration

Evan R. Delgado (1,2,3), Madeleine Leek (1,2,3), Kerollos Kamel (1,2,3), Patrick Wilkinson (1,2,3), Frances Alencastro (1,2,3), Nairita Roy (1,2,3) and Andrew Duncan (1,2,3,4)

(1) Department of Pathology, (2) McGowan Institute for Regenerative Medicine, (3) University of Pittsburgh School of Medicine, (4) Pittsburgh Liver Research Center

Background: Acetaminophen (APAP) is a commonly used analgesic that can be safely used at doses under 4 g/day. When taken in excess, APAP causes acute liver injury, which leads to acute liver failure and death. APAP overdose leads to 56,000 emergency room visits and 26,000 hospitalizations annually and is the leading cause of acute liver failure in the United States. The only available pharmacological treatment, N-acetyl cysteine, is effective within hours of overdose. The only other treatment option is orthotopic liver transplantation, and this is limited by the availability of donor organs. Considering the paucity of therapeutic options, there is an urgent need to understand mechanisms of liver regeneration and repair. Most mammalian somatic cells are diploid and contain pairs of each chromosome, but there are also polyploid cells, such as hepatocytes that contain additional sets of chromosomes. Polyploid hepatocytes are among the best described, and in adult humans and mice comprise more than 25% and 90%, respectively, of the hepatocyte population. The cellular and molecular mechanisms that regulate polyploidy have been well-characterized; however, it is poorly understood if diploid and polyploid hepatocytes play specialized roles in liver injury and repair.

Methods: Our lab recently found that diploid hepatocytes are inherently more proliferative than polyploid hepatocytes. Additionally, we can specifically study diploid hepatocytes using mice lacking E2f7 and E2f8 in the liver (referred to as “LKO” mice), which are functionally normal, but livers are depleted of polyploid hepatocytes. Considering that liver regeneration affects the outcome of APAP-medicated injury, we asked if LKO mice enriched with highly proliferative diploid hepatocytes would respond differently than control livers. We fasted LKO and control mice for 16 hours, injected intraperitoneally 300 mg/kg (the LD50 dose) APAP and harvested livers after 0.5, 3, 6, 12, 24, and 48 hours. We used these tissues to investigate differences in liver injury, necrosis, and proliferation.

Results: Liver injury enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were higher in control mice compared to LKO mice beginning at 3 hours. Necrotic liver tissue was abundant in control mice and was markedly reduced in LKO mice. We also measured more apoptotic, TUNEL positive hepatocytes in control mice. We next assessed the proliferation response after APAP-induced liver injury by staining tissues for PCNA which was induced by 6 hours in LKO mice compared to 24 hours in control mice. Finally, we treated wild-type 19-day old hepatocytes with 5 mM APAP for 12, 24, and 36 hours in vitro and found that diploid hepatocytes proliferate more readily compared to polyploids as marked by BrdU incorporation.

Conclusion: Based on these data, diploid hepatocytes appear to be the main contributors of early-stage compensatory regeneration following APAP-mediated liver injury. These data suggest that, compared to polyploid hepatocytes, diploids initiate and drive liver healing/regeneration after acute injury.

Developing tools to assess spatial and temporal dynamics of macrophage polarization in response to biomaterials

Hannah Fox (1) and Elizabeth Wayne (1,2)

(1) Carnegie Mellon University Department of Chemical Engineering, (2) Carnegie Mellon University Department of Biomedical Engineering

Macrophages maintain tissue homeostasis through polarization, or ability to switch between many cellular phenotypes ranging from a M1, pro-inflammatory phenotype to an M2, anti-inflammatory wound healing phenotype on the other. What is known about macrophage polarization is typically quantified using qPCR or ELISA measurements of total mRNA and cytokine levels respectively. While very sensitive, these measurements are done in absentia, they require homogenization of tissue which removes all potential for correlation of environmental determinants with individual macrophage polarization response. Moreover, these measurements only offer one snapshot and may not reflect the full spectrum of activity. Because of this, we need adequate cellular and animal models to observe this change in activation state real-time. Our goal is to understand the factors behind macrophage polarization and to obtain dynamic measurements to show how this occurs within a physiological system. We will do this by developing macrophage sensors that can provide bioluminescent feedback. The method of lentiviral transduction will be used to enable the macrophage cell lines to constitutively express luciferase and produce luminescence upon activation. The results of this study will provide increased understanding of how macrophage polarization is affected by interaction with biomaterials in diseased tissue environments, i.e. tumors.

Identifying 5hmC-Dependent Gene Networks in the Developing Zebrafish Retina

Shea Heilman (1,2,5) and Jeff Gross (1,2,3,4,5)

(1) University of Pittsburgh Department of Ophthalmology, (2) University of Pittsburgh Department of Molecular Genetics and Developmental Biology, (3) The Louis J. Fox Center for Vision Restoration, (4) The McGowan Institute for Regenerative Medicine, (5) The University of Pittsburgh School of Medicine

Tet-mediated DNA hydroxymethylation (5hmC) is associated with global gene expression changes and terminal differentiation in many developmental contexts. Our lab recently showed that loss of tet2/3 function led to global loss of 5hmC and poor terminal differentiation of all seven retinal cell types in zebrafish. While this and many related studies characterize the genome-wide impact of 5hmC loss, the disrupted gene networks that promote cell-type specific phenotypes, like those in the retina, are unknown. To identify tet-responsive genes and gene networks required for zebrafish retinal development, we utilize two approaches: (1) building transcriptomic lineage trajectories that will reveal cell type-specific, tet-responsive gene networks and (2) investigating the developmental roles of putative tet2/3 targets identified from bulk-RNA-seq datasets. To identify developmental gene networks disrupted in tet2/3 mutant retinae, we will isolate single cells from tet2/3 mutant retinae at several developmental timepoints, perform single-cell RNA-sequencing, build computational lineage trajectories, and compare these data to those from sibling control retinae. Here, we demonstrate our protocol for isolating retinal single-cell suspensions for single-cell RNA-sequencing, which we adapted from existing work in adult retina and neural tissues. Additionally, we investigate lhx3, a lim homeobox gene involved in motor neuron development, whose expression was altered in the bulk RNA-seq dataset. We demonstrate that lhx3 is downregulated in the ganglion cell layer of the tet2/3 mutant retina at 36 hours post fertilization, a time when most ganglion cells are fully differentiated. We also assess the effects of lhx3 knockout in a ganglion cell reporter fish, isl2b: GFP. When completed, our results will identify tet-responsive genes required for retinal development, and will determine how DNA hydroxymethylation regulates gene expression networks that underly retinal lineage specification in developing retinal progenitor cells. This work is critical for informing stem-cell based strategies to replace a wide array of cell types that are often damaged in retinal disease.

Determining the effects of Wnt signaling in the alleviation of cholestasis via the promotion of hepatocyte transdifferentiation

Karis Kosar and Kari Nejak-Bowen

Department of Cellular and Molecular Pathology, University of Pittsburgh

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by bile duct inflammation and fibrosis, which results in end-stage liver disease and reduced life expectancy. Therefore, an effective treatment for PSC is a major unmet clinical need. Hepatocytes exhibit remarkable plasticity and are known to be capable of transdifferentiating into cholangiocytes in models of biliary injury. This phenomenon, known as transdifferentiation, may create de novo ducts for bile flow, repair damaged cholangiocytes, or contribute to bile detoxification. Previous studies utilizing in vitro organoid culture systems and genetic mouse models found that β-catenin and downstream targets are upregulated in hepatic organoid cultures, and mice expressing excess β-catenin in the liver had an increased number of A6-positive hepatocytes expressing cholangiocyte markers compared to wild type (WT) when subjected to cholestatic injury. These findings led to the hypothesis that Wnt/ß-catenin signaling drives hepatocyte-to-cholangiocyte transdifferentiation during biliary injury. To test this hypothesis, we utilized lineage tracing in in vitro hepatic organoid cultures and in TG mice expressing a mutated non-degradable form of β-catenin (S45D) in liver. We show that hepatocytes transdifferentiate to cholangiocytes in organoid cultures, and that the cholangiocytes present are not from native contaminating cholangiocytes. We determined that TG mice fed DDC diet, which induces bile stasis, have: 1) improved serum ALP over time, indicating less biliary injury; 2) increased bile output compared to WT mice fed DDC diet; and 3) bile ducts that are populated with hepatocyte-derived cholangiocytes. Through these studies we demonstrate that Wnt/ß-catenin signaling catalyzes hepatocyte-to-cholangiocyte transdifferentiation, and activation of this pathway alleviates cholestasis in mouse models of PSC.

Pregnancy-associated Plasma Protein A (PappA) Knockout Mice: In Vivo Disc Study

Rebecca Kritschil (1), Shouei Zhao (1), Abbe N. De Vallejo (3), Qing Dong (1), Joon Lee (1), Gwendolyn Sowa (1,2) and Nam Vo (1)

(1) Department of Orthopaedic Surgery, University of Pittsburgh (2) Department of Physical Medicine and Rehabilitation, University of Pittsburgh (3) Department of Pediatrics and Immunology, Children's Hospital of Pittsburgh

Past research in disc biology has highlighted the beneficial effects of growth factors which can stimulate matrix synthesis, in vitro. Based on this research, it was proposed exogenous IGF-1 be used as a therapy for intervertebral disc degeneration (IDD). In the aging research field, reducing the IGF-1-mediated signaling pathway has consistently been shown to reduce age-related disorders and increase lifespan of mammals and rodents. PappA knockout mice are one of the animal models used to study how lowered IGF-1 bioavailability affects different tissues. Here, we examined the discs of aged PappA-/- mice versus wild type to determine whether there was an effect on age-associated IDD.

This study was conducted according to IACUC approved protocols at University of Pittsburgh. Spine segments were harvested from WT and PappA-/- mice at 23 months. Disc proteoglycan content was assessed by safranin-O-histology and DMMB assay was used to quantify sulfated GAG. Western blotting was performed to measure the disc aggrecan proteolytic fragments, and the catabolic markers MMP-3 and ADAMTS-4. Levels of disc cellular senescence was assessed by immunohistochemistry and western blot for p53. Student’s t-test was used to test significance between groups ($p<0.05$).

Compared to Wt, aged PappA-/- mice showed two times less disc GAG content. Aged PappA-/- mice also showed significantly decreased aggrecan fragmentation and decreased protein levels of MMP-3 and ADAMTS-4 in their discs compared to those in aged Wt. Lamin B1 expression was qualitatively more in aged Pappa-/-, suggesting reduced disc cellular senescence. Less p53 expression in aged Pappa-/- further supports this finding.

The role of insulin like growth factor (IGF) in disc health and aging is currently unresolved. PappA-/- mice have reduced matrix content but the aggrecan matrix present after aging appears to be less fragmented based on our findings. Levels of cellular senescence were positively impacted by decreasing IGF-1 signaling in aged discs. Further studies are needed to determine if the decrease in anabolism following reduced IGF-1 signaling is balanced by a similar decrease in catabolism to determine how IGF-1 signaling impacts matrix homeostasis during aging.

Stem cell secretome induces tissue regeneration for glaucoma treatment

Ajay Kumar (1), Siqi Xiong (1), Enzhi Yang (1) and Yiqin Du (1,2)

(1) Department of Ophthalmology, University of Pittsburgh, (2) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Purpose: Glaucoma is a serious cause of irreversible blindness worldwide. Stem cell-free therapy using stem cell secretome offers great hope for vision restoration. We investigated the potential of stem cell secretome in treating a transgenic mouse glaucoma model with myocilin mutation (Tg-MyocY437H) and explored the regeneration mechanism.

Methods: Human Trabecular Meshwork Stem Cell (TMSC) secretome was isolated in serum free conditions. Animals were divided into five groups: WT group, Tg-MyocY437H mice, Tg mice with periocular injection of the basal medium and TMSC 1/2 secretomes (n=16 each). 20µl of secretome or basal medium was injected periocularly. IOP was measured using I-care tonometer. Pattern Electroretinography (PERG) was performed for retinal ganglion cell (RGC) function. Optic nerve axons were counted to evaluate RGC loss. Proteomics of TMSC secretome was performed using LC-MS/MS. The statistical differences were analyzed by ANOVA followed by Tukey posttest.

Results: Tg-MyocY437H mice had elevated IOP at 4-months age when we injected secretomes (0 week). Secretome from both TMSCs reduced IOP significantly (12.6±2.7mmHg and 13.2±2.5 respectively) after one week of secretome injection as compared to Tg-Myoc mice (18.5±2.8). Tg-Myoc mice showed elevated IOP at week 10 also (14.8±2.5). which was reduced to normal by TMSC secretomes (9.7±1.9 and 9.4±2.2 respectively) similar to WT mice (10.3±2.2). RGC function was found to be lost in Tg-Myoc mice with P1 amplitude 5.85 ± 2.24μV. Taking average, TMSC secretomes preserved about 90% of the RGC function with P1 amplitude 9.07 ± 2.5μV. TMSC secretome also rescued RGC damage in optic nerve. LC-MS/MS analysis of TMSC secretome uncovered 74 proteins related to axon guidance pathway, 78 proteins involved in neurogenesis, and 12 proteins involved in the regulation of neuron apoptotic process.

Conclusion: TMSC secretome was effective in treating Tg-MyocY437 glaucoma mice and preserved vision loss. It opens new avenue for stem cell-free therapy for glaucoma with minimum invasive procedures.

Nuclear Phosphoinositide 3-kinase delta (PIK3CD) Increases with Conditional Deletion of Hepatocellular Integrin Linked Kinase

Nicole J. Martucci (1), Mary-Claire Cotner (1), Bharat Bhushan (1), Wendy M. Mars (1) and George K. Michalopoulos (1)

(1) Division of Cellular and Molecular Pathology, Department of Pathology, University of Pittsburgh School of Medicine

Introduction: Previous data from our lab has shown that with hepatocyte specific knockout of integrin linked kinase (hILK KO), there is hepatocyte proliferation, increased matrix deposition, unorganized biliary cell/ductal proliferation, and possible transdifferentiation of hepatocytes to cholangiocytes; this led us to investigate the signaling pathways downstream of hILK KO that might be responsible for the observed phenotype. We uncovered a potential central role for Phosphoinositide 3-kinase (PI3K) delta (PIK3CD), a variant that is considered leukocyte specific.

Methods: Mice with germ-line loss of hepatocellular ILK were generated by crossing double floxed ILK mice (ILKfl/fl) with mice expressing cre recombinase under an afp enhancer-albumin promoter (Cre+/-) to obtain ILKfl/fl:Cre+/- (hILK KO) and ILKfl/fl:Cre-/- (WT). Livers from 5 and 14-week old chronic hILK KO and WT mice were analyzed for RNAs of interest and then further examined by western blot analyses, immunohistochemical staining, and induction of acute loss of ILK using AAV8 cre in ILKfl/fl mice.

Results: Using immunohistochemistry, we detected prominent staining of PIK3CD in hILK KO hepatocytes. Inhibition of PIK3CD resulted in a decrease in both pAKT (a downstream target) and Cyclin D1. Western blot analyses of whole livers also uncovered a size variant of PIK3CD that is only observed in isolated nuclei. Additionally, at 7 days after the acute removal of ILK, we can observe staining for the cholangiocyte specific marker, CK19, in hepatocytes as well as strong PIK3CD staining in regions with spontaneous duct formation.

Conclusion: Our data has 1) revealed PIK3CD, a leukocyte specific protein, to be hepatocellular 2) shown that a size variant of PIK3CD exists in the nuclei of hepatocytes and 3) uncovered a potential relationship between PIK3CD in hepatocytes and their transdifferentiation to biliary cells. Currently, we are using an in vitro model of trans-differentiation in an attempt to further explore these preliminary findings.

CRISPR-based transcriptional repression to perform immunomodulation in vivo

Farzaneh Moghadam (1,2,3), Jeremy J Velazquez (1,2,3), Ryan LeGraw (1,2,3), Nan Cher Yeo (4,5), Chenxi Xu (6), Jin Park (6), Alejandro Chavez (7), Mo R Ebrahimkhani (1,2,3,8) and Samira Kiani (1,2,3)

(1) Pittsburgh Liver Research Center, (2) Division of Experimental Pathology, Department of Pathology, University of Pittsburgh, (3) School of Biological and Health Systems Engineering, Ira A. Fulton Schools of Engineering, Arizona State University, Tempe, AZ, (4) Wyss Institute for Biologically Inspired Engineering, Harvard University, Cambridge, MA, (5) Department of Genetics, Harvard Medical School, Boston, MA, (6) Center for Personalized Diagnostics, Biodesign Institute, Arizona State University, Tempe, AZ, (7) Department of Pathology and Cell Biology, Columbia University College of Physicians and Surgeons, New York, NY, (8) McGowan Institute for Regenerative Medicine

Recent repurposing of the Clustered Regularly Interspace Short Palindromic Repeat (CRISPR) system for transcriptional modulation has opened new avenues for developing strategies to confer protection against pathogens or other environmental exposures and modulate the course of many acquired diseases. Here, we developed a potent CRISPR-based transcriptional repressor. Our engineered repressor relies on simultaneous employment of two repressor-domains, fused to MS2 coat protein and truncated guide RNA (gRNA) from 5' end, which enables Cas9 protein to perform transcriptional modulation of the targeted gene. Our data shows that using this system we could efficiently achieve a functionally relevant phenotype through transcriptional repression of targeted genes in vivo (above 60% in different organs), in normal condition and in response to lipopolysaccharide (LPS) induced inflammation. We have shown that transcriptional modification of inflammatory genes generates less immunoglobulin against Adeno-associated virus (AAV) and modulates general immunoglobulin expression patterns, which addresses a major challenge involved with AAV-based clinical gene therapies. We report that this is a promising strategy for application of CRISPR-based transcriptional modulators to reprogram immune response against AAV vectors, as well as inflammatory responses implicated in innate immunity.

Hepatocyte Stress Increases Expression of YAP and TAZ in Hepatocytes to Promote Parenchymal Inflammation and Fibrosis

Meghan Mooring (1), Brendan H. Fowl (2), Shelly Z.C. Lum (2), Ye Liu (1), Kangning Yao (1), Samir Softic (2,3), Rory Kirchner (4), Aaron Bernstein (5), Aatur D. Singhi (6), Daniel G. Jay (5), C. Ronald Kahn (3), Fernando D. Camargo (7,8) and Dean Yimlamai (1,2,6)

(1) Division of Gastroenterology and Nutrition, Department of Pediatrics, Children's Hospital of Pittsburgh of UPMC, (2) Division of Gastroenterology and Nutrition, Department of Pediatrics, Boston Children's Hospital, Boston, MA, (3) Section on Integrative Physiology and Metabolism, Joslin Diabetes Center and Department of Medicine, Harvard Medical School, Boston, MA, (4) Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, (5) Department of Developmental, Molecular, and Chemical Biology, School of Medicine, Tufts University, Boston, MA, (6) Pittsburgh Liver Research Center, University of Pittsburgh, (7) The Stem Cell Program, Boston Children's Hospital, Boston, MA, (8) Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA

Activated hepatocytes are hypothesized to be a major source of signals that drive cirrhosis, but the biochemical pathways that convert hepatocytes into such a state are unclear. We examined the role of the Hippo pathway transcriptional coactivators, YAP/TAZ in hepatocytes to facilitate cell-cell interactions that stimulate liver inflammation and fibrosis. Using a variety of genetic, metabolic and liver injury models in mice, we manipulated Hippo signaling in hepatocytes and examined its effects in non-parenchymal cells to promote liver inflammation and fibrosis. YAP expressing hepatocytes rapidly and potently activate the expression of proteins that promote fibrosis (COL1A1, TIMP1, PDGFc, TGFb2) and inflammation (TNF, IL1b). They stimulate expansion of myofibroblasts and immune cells followed by aggressive liver fibrosis. In contrast, hepatocyte-specific YAP and YAP/TAZ knockouts exhibit limited myofibroblast expansion, less inflammation, and decreased fibrosis after carbon tetrachloride injury despite a similar degree of necrosis as controls. We identified CYR61 as a chemokine that is upregulated by hepatocytes during liver injury but is expressed at significantly lower levels in mice with hepatocyte-specific deletion of YAP or TAZ. Gain and loss of function experiments with CYR61 in vivo point to it being a key chemokine controlling liver fibrosis and inflammation in the context of YAP/TAZ. There is a direct correlation between levels of YAP/TAZ and CYR61 in liver tissues of high-grade NASH patients.

Conclusion: Liver injury in mice and humans increases levels of YAP/TAZ/CYR61 in hepatocytes, thus attracting macrophages to the liver to promote inflammation and fibrosis.

Functional impact of ex vivo culture expansion of adipose stem cells

Feng Qin (1), Benjamin Schilling (2), Alexander Stavros (3), Sarah Seman (4,5), Lauren Kokai (6,7)

(1) Department of Plastic surgery, Peking Union medical collage hospital, Beijing, China, (2) Department of Bioengineering, School of Engineering, University of Pittsburgh, (3) University of Pittsburgh School of Medicine, (4) Department of Science and Mathematics and (5) the School of Engineering and Computer Science, Cedarville University, Cedarville, OH, (6) Department of Plastic Surgery, University of Pittsburgh, (7) McGowan Institute for Regenerative Medicine

Introduction: Therapeutic cells isolated from adipose tissue are being used clinically with increasing frequency due to their potential regenerative benefits. Within the mixed population of adipose stromal cells reside adipose stem cells (ASCs), which are generally regarded as the most therapeutically beneficial component and which have demonstrated anti-inflammatory, pro-angiogenic and anti-oxidative properties. Though pure ASCs are not FDA approved in the United States, there is significant interest in future therapeutic use and a commercial entity already exists in Denmark that utilizes culture-expanded ASCs for volumizing soft tissue, at a concentration of 20 million cells per mL tissue scaffold. The oncologic risk of ASCs is currently under debate, with both tumor-promoting and no-effect results reported with culture expanded cells. We hypothesize that the phenotype of ASCs is significantly impacted by culture expansion and the variability in cell culture protocols may account for discrepancies reported in literature. The aim of this study was to determine how key variables in cell culture expansion impact ASC tumorigenicity.

Methods: Adipose stromal cells were obtained through enzymatic digestion of human adipose tissue obtained under IRB exemption. Cells were expanded after inoculating at different plating densities (5K cells/cm2 or 15K/cm2) in proangiogenic (high FGF2) or standard culture media. Equal numbers of passage 3 ASCs were then co-culture with breast cancer MCF7 cells to determine their modulatory capacity of cancer proliferation and invasion.

Results: The most significant culture variable affecting ASC phenotype was media concentration of FGF2, which significantly decreased ASC secretion of collagen 1 ($p = 0.00028$). The expression of COL1A1 was significantly predictive of thrombospondin-1 (TSP1) expression ($p < 0.00001$). Cell plating density inversely impacted ASC autocrine expression of FGF2 ($p = 0.00227$) and surprisingly, cell passage had no significant effect on any of the measured genes. When co-cultured with MCF7 cells, ASCs which express high TSP1 significantly suppressed MCF7 invasion ability.

Conclusions: To date, there has been little research on TSP expression by adipose stem cells despite the important roles for TSPs in cancer, immunomodulation, wound healing and fibrosis. This study investigated how ex vivo culture impacts ASC phenotype and particularly, expression levels of ECM and TSP1. Our results showed that FGF2 is critical for ASC culture expansion toward a low TSP-1 secreting phenotype, which we further show has durable impact on tumorigenicity. Considering that TSP-1 play an important role in regulating cancer progress, modulation of ASC-TSP1 through ex vivo expansion could significantly affect tumor growth or invasiveness when used as a cell therapy in the context of tissue reconstruction after cancer.

Regulation of Smooth Muscle Cell Proliferation and Function by Mitofusin-1

Christopher Reyes (1,2), Li Mo (2,3), Danielle Guimaraes (2), Andrea Braganza (2), Yinna Wang (2) and Sruti Shiva (2,3,4)

(1) Department of Bioengineering, (2) Pittsburgh Heart, Lung and Blood Vascular Medicine Institute, University of Pittsburgh, (3) Department of Pharmacology & Chemical Biology, (4) Center for Metabolism & Mitochondrial Medicine, University of Pittsburgh

Vascular smooth muscle cells (VSMC) are the primary mediators of vessel tone within the vasculature. In numerous cardiovascular-related pathologies such as restenosis following balloon angioplasty, VSMC undergo phenotypic switching from a quiescent, contractile phenotype to a synthetic, proliferative phenotype. These cells migrate into the intimal layer and aberrantly proliferate which consequently decreases vessel luminal diameter and potentiates myocardial infarction and stroke. We have recently shown that nitrite, an endogenous signaling molecule, inhibits VSMC proliferation and attenuates restenosis after vascular injury. However, the mechanism of nitrite-dependent inhibition of VSMC proliferation is unknown. Interestingly, emerging evidence has suggested mitochondrial fusion may mitigate VSMC phenotypic switching and proliferation. Here, we show that nitrite inhibits PDGF-induced proliferation of RASMC in vitro in a concentration dependent manner. Mechanistically, this effect was dependent on the nitrite-mediated upregulation of mitochondrial dynamics fusion protein mitofusin-1 (Mfn1). Further investigation revealed that nitrite treatment of RASMC inhibited Mfn1 protein degradation and decreased Mfn1 protein ubiquitination by E3 ligase March5. To determine the role of Mfn1 in VSMC function in vivo, a smooth muscle-specific Mfn1 knockout (KO) mice was generated. In ex vivo wire myography experiments, deletion of Mfn1 blunted vasodilation by sodium nitroprusside and acetylcholine. Additionally, Mfn1 KO mice exhibited increased neointimal hyperplasia following carotid artery ligation compared to injured control animals. In sum, these data elucidate a novel mechanism by which nitrite regulates Mfn1 to attenuate VSMC proliferation and provide evidence for a role in Mfn1 in the maintenance of VSMC function and vessel tone.

Extracellular vesicles within young blood reverse aged-related declines in stem cell and tissue function

Amrita Sahu (1,2), Amin Cheikhi (1,3), Sunita Shinde (1), Zachary Clemens (1), Abish Pius (1), Nicholas Fitz (2), Hikaru Mamiya (1,4), Silvia Picciolini (5), Cristiano Carlomagno (5), Alice Gualerzi (5), Marzia Bedoni (5), Bennett Van Houten (6), Iliya Lefterov (2), Aaron Barchowsky (2,6), Radosveta Koldamova (2) and Fabrisia Ambrosio (1,2,4,7)

(1) Department of Physical Medicine & Rehabilitation, University of Pittsburgh, (2) Department of Environmental Health and Occupational Safety, University of Pittsburgh, (3) Department of Medicine, University of Pittsburgh, (4) Department of Bioengineering, University of Pittsburgh, (5) Laboratory of Nanomedicine and Clinical Biophotonics (LABION), Fondazione Don Carlo Gnocchi, Milan, Italy, (6) Department of Pharmacology and Chemical Biology, University of Pittsburgh, (7) McGowan Institute for Regenerative Medicine, University of Pittsburgh

While heterochronic parabiosis studies have linked age-related impairments to changes in circulating proteins, whether and how circulating extracellular vesicles (EVs) mediate these age-associated changes is unknown. We find that the beneficial effect of young blood on both skeletal muscle and cognitive function in aged mice is diminished when serum is depleted of EVs. Aging caused an overall decline in a sub-population of EVs, CD63 and shifted the EV molecular fingerprint as well. Supervised machine learning using Raman scattering based analyses revealed that EV nucleic acid, but not protein, content decreased over time. Further interrogation revealed that young EVs enhanced mitochondrial integrity and function of target muscle and neural stem cell progeny. Aged EVs, however, failed to induce a benefit in target cells. In vivo, injection of young EVs improved histological and functional muscle regeneration in aged mice. These studies demonstrate that circulating EVs mediate intercellular communications crucial for maintenance of muscle and brain function over time.

A non-retinoid small-molecule chaperone of rhodopsin and its potential for the treatment of RHO-associated Retinitis Pigmentosa.

Abhishek Vats (1,2), Serge Picaud (2) and Yuanyuan Chen (1,2)

(1) Department of Ophthalmology, Department of Pharmacology and Chemical Biology, University of Pittsburgh, (2) McGowan Institute of Regenerative Medicine, (3) Department of Visual Information Processing, Institut de la Vision, Paris, France

Retinitis pigmentosa (RP) is an inherited retinal disease affecting more than one million people worldwide. Genetic mutations in the RHO gene account for 25-30% of autosomal dominant RP (adRP). Mutations in the RHO gene leads to rhodopsin protein misfolding and causes rod cell death, resulting in the loss of peripheral vision and RP. The RHO-P23H mutation alone accounts for ~10% of adRP. We recently identified a non-retinal ligand (YC-001) of rhodopsin by a cell-based high-throughput screening. YC-001 works as a potent molecular chaperone and rescues the cellular transport of P23H and many other rhodopsin mutants in vitro. However, the half-life of YC-001 in vivo is as short as 40 min, thus we cannot determine the efficacy of YC-001 via systemic treatment. The goal of this study is to address the efficacy of YC -001 to the P23H rhodopsin expressing retinae. We established an ex-vivo retinal culture system to test YC-001 at constant concentration. We used RhoP23H/+ knock-in mouse as an adRP model and the WT mice as normal control. First, we did a time-lapse ex-vivo retinal culture for 1,3,7,14&21 days, and found an faster reduction of the outer nuclear layer (ONL) thickness of RhoP23H/+ retinae than WT control, but such difference was not seen for the thickness of outer and inner segments (OS/IS). This result suggests the ex-vivo model can be used for safety and efficacy test of YC-001. Further, a ten-day treatment with YC-001 significantly increased the rhodopsin intensity, OS/IS thickness and ONL thickness in the RhoP23H/+ retinae, compared to DMSO control. These changes by YC-001 were also observed in WT retinal explant. Our results showed that YC-001 is a safe and effective drug candidate to improve rhodopsin homeostasis and photoreceptor survival in the retinae of the adRP animal model, providing a therapeutic potential for this currently untreatable disease.

Viral Control Over Host Nuclear Architecture

Daniel B. Whitefield (1), Jonathan S. Minden (2), Fred L. Homa (3) and Kris Noel Dahl (1,4)

(1) Carnegie Mellon Department of Biomedical Engineering, (2) Carnegie Mellon Department of Biological Sciences, (3) University of Pittsburgh Department of Microbiology and Molecular Genetics, (4) Carnegie Mellon Department of Chemical Engineering

Herpes Simplex Virus 1 (HSV-1) enters nuclei of host cells and destroys the intricate organization of chromosomes and other structures in favor of an architecture suited to producing more virus particles during lytic infection. Cellular defense mechanisms attempt to silence HSV-1 gene expression by enriching the viral genome with heterochromatin marks, however, HSV-1 circumvents this by exerting exquisite control over the chromatin state of its own DNA. Furthermore, during the course of a lytic infection, HSV-1 exerts control over nuclear organization by displacing host chromatin to the periphery of the nucleus through a process called margination. Whether this process is primarily biochemical or biophysical in nature remains unclear. To better understand HSV-1's reorganization of the nucleus, chromatin mobility was measured within infected cells utilizing a particle tracking technique developed in our lab. This revealed a consistent decrease in chromatin mobility throughout the course of infection. Interestingly, this decrease in mobility began before chromatin margination was recognizable. This indicates that chromatin architecture is modified by some mechanism in addition to simple nuclear crowding caused by replication and assembly of viral capsids within the nucleus. In light of this evidence, a purely biophysical mechanism for margination seems unlikely. Therefore, future work will include investigation of changes to gene expression and post-translational modifications that could influence chromatin mobility and organization. A more detailed understanding of HSV-1's ability to modulate the host chromatin architecture may open the door for a treatment that could interrupt margination and break the infection cycle, which could potentially then be generalized to disrupt infections of a variety of nuclear-replicating DNA viruses such as Human Papilloma Virus.

Corneal stromal stem cells release specific microRNAs to reduce corneal fibrosis and inflammation

Gary Yam, Martha L Funderburgh, Kishan Patel, Irona Khandaker, Moira L Geary, James L. Funderburgh and Yiqin Du

Department of Ophthalmology, University of Pittsburgh School of Medicine

Corneal stromal stem cells (CSSC) have been shown to prevent corneal fibrotic scarring (reduced expression of collagen 3a1, alpha-smooth muscle actin and tenascin C) and regenerate transparent stromal tissue in a murine corneal wounding model. CSSC exert anti-inflammatory effect to block neutrophil infiltration into the injured stroma via the release of extracellular vesicles (EV). This study investigated the mechanisms by which CSSC EV suppress corneal scarring and inflammation. Conditioned medium from primary human CSSC cultures were collected for EV isolation. RNA content of EV fractions was determined by RNAseq and results showed the differential expression of mRNA, lncRNA and microRNAs in association with CSSC with regenerative potential, compared to HEK293 cells. Using Alix knockdown to block microRNA cargo (>85% reduction), the modified CSSC EV failed to suppress corneal scarring and inflammation in murine corneas after injury. Application of microRNA mimics to wounded corneas has validated that specific microRNAs are capable to reduce inflammation (uPAR, Cxcl7, myeloperoxidase expression). Our results support the idea that CSSC release diffusible factors (specific microRNAs) to prevent corneal scarring and to induce the regeneration of transparent corneal tissues after wounding. The delivery of microRNAs appears to require the presence of EV loaded with microRNAs.

foxm1 is required for cardiomyocyte proliferation after zebrafish cardiac injury

Daniel A. Zuppo (1), Maria A. Missinato (1), Lucas Santana dos Santos (2), Panayiotis Benos (2) and Michael Tsang (1)

(1) Department of Developmental Biology, University of Pittsburgh, (2) Department of Computational and Systems Biology, University of Pittsburgh

Mammalian hearts fail to regenerate damaged tissue after cardiac injury because adult cardiomyocytes (CM) do not proliferate sufficiently. However, recent findings demonstrate these CMs can dedifferentiate, proliferate, and redifferentiate near the site of injury. It is imperative to discern the mechanisms regulating CM proliferation and to determine how they affect regeneration. Unlike mammals, adult zebrafish CMs can robustly proliferate after injury which allows for the identification of genes and pathways that control heart regeneration. We compared the transcriptome profile of uninjured and ventricular resectioned hearts and identified a number of candidate genes implicated in cell cycle regulation. Expression of foxm1, a forkhead-binding transcription factor, was identified in CMs at 3 days post-amputation (dpa) when CM proliferation initiates. Foxm1 is a mitotic regulator and is expressed during mammalian cardiac development, but its role in cardiac regeneration has not been characterized. We hypothesized that foxm1 is critical for CM division, and that loss of its activity will result in heart regeneration failure. Indeed, we observed a significant decrease in PCNA+ CMs at 7dpa and increased scar tissue at 30dpa in foxm1 mutants compared to WT controls. Transcriptome profiling of foxm1 mutant hearts at 3dpa showed decreased cell cycle gene expression. Specifically, several G2/M phase cell cycle genes were decreased in mutant hearts suggesting that Foxm1 is required for mitotic progression through activation of genes involved in cytokinesis. This was supported by the decreased expression of centromere protein f (cenpf), a gene involved in chromosome segregation, g2/m-phase specific E3 ubiquitin protein ligase (g2e3), and protein regulator of cytokinesis 1 (prc1) in foxm1 mutant hearts. Previous research revealed cenpf expression and CM proliferation in the developing heart persists until P7 in neonatal mice, so we hypothesized cenpf is required for mitosis. To confirm that cenpf is critical for CM cell division, we analyzed cenpf mutant hearts after ventricular resection and noted a failure to regenerate after injury. Moreover, by 20dpa cenpf mutant hearts showed an abundance of binucleated CMs indicative of incomplete cytokinesis. These findings reveal that foxm1 is critical for proper CM proliferation after injury through activation of G2/M phase cell cycle genes.

Mechanics of passive cell rearrangement during epithelial convergent extension: understanding experimental observations through theory

Sommer Anjum (1) and Lance Davidson (1,2,3)

(1) Department of Bioengineering, (2) Department of Developmental Biology, (3) Department of Computational and Systems Biology, University of Pittsburgh

Convergent extension (CE) is a critical process in shaping embryos and organs, driving their growth. CE is a common trope in animal morphogenesis for narrowing tissue in one direction and lengthening it in the orthogonal direction. One strategy of CE involves directed cell intercalation. For instance, the vertebrate neural epithelium elongates via directed cell intercalation as it forms the neural tube, the precursor of the spinal cord. Failure of this process leads to birth defects such as spina bifida. CE is proposed to be the result of a coordination between biochemical patterning that establishes and maintains the direction of intercalation and mechanical responses from passive and active elements. However, it has been difficult to delineate these contributors with traditional experimental design, so we have adopted a computational modeling approach inspired by ongoing studies of Xenopus neural CE.

The relative contributions from active and passive processes during directed cell intercalation remain unclear. For instance, passive jamming transitions have been implicated in epithelial morphogenesis. To isolate the passive responses to tissue field elongation from contributions from active cellular processes, we simulate epithelial responses to external CE forces. In the model, cells are represented as interacting particles in a square bounding box. Boundaries are moved such that the field of cells undergoes area-conserving CE. Time series of polygonal cell networks are analyzed to quantify deformations, cell neighbor exchanges, and cell area fluctuations. We quantify local tissue strain by analyzing the dynamics of the strain experienced by domains consisting of a cell and its neighbors. Recapitulating endogenous rates of Xenopus neural CE reveal neighbor exchanges and directional remodeling that mimic in vivo observations.

Extending our simple simulations by incorporating active cell elements such as actomyosin contractility and junctional remodeling will enable quantitative assessment of specific biochemical and mechanical drivers of tissue shape change.

Evaluation and Prediction of Anatomical Outcomes Following Uterovaginal Prolapse Surgery

Shaniel Bowen (1), Pamela Moalli (1,2,3,4) and Steven Abramowitch (1,3)

(1) Department of Bioengineering, University of Pittsburgh, (2) Magee-Womens Research Institute, University of Pittsburgh Medical Center, (3) Department of Obstetrics, Gynecology & Reproductive Sciences, University of Pittsburgh Medical Center, (4) McGowan Institute for Regenerative Medicine, University of Pittsburgh

By the age of 80, 11-19% of women will undergo surgery for pelvic organ prolapse (POP). Of the 300,000 POP surgeries performed each year that use patients' own tissues, up to 30% will fail within 5 years due to prolapse. In response to the high failure rate of native tissue repair (NTR), synthetic mesh has been used to augment POP surgery. While synthetic mesh repair (SMR) was found to improve anatomical outcomes in the short-term, its long-term outcomes were not superior to those of traditional NTR.

Failure of POP repair is fundamentally a biomechanical process that is currently ill-defined. Thus, a biomechanical understanding of how and why repairs fail is needed to better treat POP and prevent its recurrence. To solve this problem, we will create a new anatomy-based assessment tool to evaluate and predict surgical outcomes of POP repairs.

First, we will characterize the relationship between pelvic floor muscle geometry and failure of POP repairs to create a predictive model of surgical outcome based on statistical shape modeling (SSM). The SSM results will identify site-specific, anatomical descriptors and predictors of surgical outcomes. Second, we will measure changes in the mechanical demand of POP repairs in response to differences in pelvic floor anatomy using finite element modeling (FEM). The FEM outcomes will quantify the relationship between the mechanical demand required for POP repair to correct prolapse and pelvic floor anatomy.

To accomplish this, we will use pelvic MRIs of 89 women with POP that underwent NTR or SMR 30-42 months post-surgery. With these MRIs, we will use SSM and FEM to test our hypothesis that abnormal morphology and function of the pelvic floor muscles are primarily involved in failure of POP repairs. Our findings will build the framework for a tool to assess and predict outcomes of POP surgeries.

Effect of macro-calcification on the failure mechanics of intracranial aneurysmal wall tissue

Ronald Fortunato (1), Anne M. Robertson (1,2), Chao Sang (1), Xinjie Duan (3) and Spandan Maiti (1,2,4)

(1) Department of Mechanical Engineering and Materials Science, (2) Department of Bioengineering, (3) Intelligent Automation Group, PNC Bank, (4) Department of Chemical and Petroleum Engineering, University of Pittsburgh

Spontaneous rupture of intracranial aneurysms (IA), the pathological enlargement of the cerebral arterial wall, is a devastating disease with high mortality and disability rates. Mechanisms of IA rupture are poorly understood, thereby making it difficult for clinicians to confidently choose aggressive and somewhat risky treatment protocols over careful observation. As IA rupture ultimately involves the structural failure of the wall tissue, biomechanics is expected to play an important role in mechanistic understanding of this disease. Different structural pathways such as anomalous remodeling of the wall collagen architecture, inflammation, the presence of lipid pools or calcified regions within the wall tissue have been variously implicated as wall-weakening mechanisms leading to the IA rupture. Using a combined experimental and computational approach, we investigated the multifactorial nature of IA rupture to understand how the calcification and also its surrounding wall tissue environment influence rupture risk. We found that the material composition of the near-calcification region of the calcified tissue influences the mechanical stress distribution within the tissue, and thus impacts the overall failure properties of the vessel wall. Our work implies that biomechanical pathways of IA tissue rupture can be altered by the ultrastructural remodeling in the vicinity of the calcifications. The study presented herein is an important first step towards understanding the failure propensity of the calcified IA wall tissue.

Brain Abnormalities and Neurobehavioral Deficits in a Mouse Model of Hypoplastic Left Heart Syndrome

George C. Gabriel (1), Hisato Yagi (1), Nathan Salamacha (1), Tuantuan Tan (1), William T. Reynolds (1,2), Abha Bais (1), Marla Shaffer (1), Dennis Simon (3), Ashok Panigrahy (2), Yijen Wu (1) and Cecilia Lo (1)

(1) Department of Developmental Biology, University of Pittsburgh, (2) Department of Pediatric Radiology, Children's Hospital of Pittsburgh of UPMC, (3) Department of Critical Care Medicine, University of Pittsburgh

Introduction: Hypoplastic left heart syndrome (HLHS) is a severe congenital heart defect associated with poor neurodevelopmental outcome. We recently generated the first HLHS mouse model, the Ohia HLHS mutant line, which exhibited not only congenital heart defects but also brain abnormalities. The cardiac and head defects are elicited by mutations in two genes: Sap130 encoding Sin3A-associated protein 130, a component of the HDAC repressor complex, and Pcdha9 encoding the protocadherinA9 cell adhesion protein known to regulate synaptic connections. We hypothesize mutation in either or both HLHS causing mutations can contribute to brain abnormalities and neurobehavioral deficits.

Methods and Results: HLHS mutant mice exhibited neuroanatomical defects involving forebrain structures including the cortex, hippocampus, and olfactory bulb. Transcriptome profiling with RNAseq showed Ohia mutant brains have perturbations in pathways regulating neurodevelopment and learning and memory. To determine the contribution of Sap130 vs. Pcdha9 mutations in these brain abnormalities, we generated mice carrying mutations only in Sap130 or Pcdha9. Mice with forebrain targeted deletion of a floxed Sap130 allele mediated by Emx1Cre are without cardiac defects and adult viable. They have microcephaly with marked forebrain hypoplasia similar to that of the Ohia HLHS mutant fetuses. MRI volumetric analysis showed significant brain volume reduction in the cortex, hippocampus, and corpus callosum. DTI imaging and connectome analysis revealed marked alterations in neural connectivity in the amygdala. Similar MRI analysis of the Pcdha9 mutant mice, which are adult viable, revealed grossly normal brain structure, with few volumetric changes. However, DTI imaging and connectome analysis showed marked loss of neural connectivity with global reorganization of neural networks. Behavioral assessments revealed abnormalities in both the Emx1-cre deleted Sap130 and Pcdha9 mutants.

Conclusions: We showed mutations in Sap130 and Pcdha9 causing HLHS can each independently perturb brain development and cause behavioral deficits. This occurred in the absence of congenital heart defects, suggesting intrinsic defects of a genetic etiology may drive the poor neurodevelopmental outcome associated with HLHS.

Characterization of Aortic Smooth Muscle Cells in Regions of High and Low Longitudinal Tensile Stress in Ascending Thoracic Aortic Aneurysm: Implications for Risk Stratification

Lauren V. Huckaby (1), Ronald N. Fortunato (2), Leonid V. Emerel (1), Tara D. Richards (1), Jennifer C. Hill (1), Marie Billaud (1,2,3), Julie A. Phillippi (1,2,3), David Vorp (2), Spandan Maiti (2) and Thomas G. Gleason (1,2,3)

(1) Department of Cardiothoracic Surgery, (2) Department of Bioengineering, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Objective: Current diameter-based guidelines for elective aortic replacement fail to encompass the more than 60% of patients that experience aortic dissection at a lower diameter. Our prior work revealed overlap between the dissection initiation site and areas of high longitudinal tensile stress predicted based on CT angiography (CTA) scans obtained 1+ year prior to the dissection event. We sought to corroborate estimated areas of high longitudinal stress with aortic smooth muscle cell (SMC) function and extracellular matrix (ECM) properties in order to validate a computational modeling-based approach for risk prediction of aortic dissection.

Methods: A constitutive model was utilized to map patient-specific longitudinal and circumferential tensile stress using CTAs from patients scheduled for ascending aortic replacement. Aortic specimens corresponding to regions of high and low stress were collected during surgery, with informed patient consent and IRB approval. We compared ECM microarchitecture (using multiphoton microscopy), matrix metalloproteinase (MMP) activity (using gelatinase assays), and aortic SMC viability in regions of high and low predicted longitudinal tensile stress.

Results: Longitudinal tensile stress was obtained from CTAs of 13 patients (66.5±14.6 years, 76.9% male, mean diameter 47.7±4.2mm). This included 8 (61.5%) presenting with a congenitally anomalous bicuspid aortic valve. Histologic imaging demonstrated marked medial ECM degeneration localized to the high stress regions while low stress regions approximated healthy aortic tissue. Quantification of ECM microarchitecture revealed similarities in collagen fiber orientation but marked disorganization of elastin fibers in the high stress compared to the low stress region. Furthermore, activity of MMP-9 was increased in the high stress aortic specimens; there were no differences in MMP-2 activity. SMC viability under oxidative stress was correspondingly lower in SMCs from the high stress regions when compared with that of SMCs in the low stress regions ($p=0.016$), though there were no apparent differences in SMC contractility ($p=0.578$).

Conclusions: Non-invasive mapping of longitudinal tensile stress corresponds with ECM and cellular disruption thus improving our understanding of aortic wall biomechanics in ascending aortic disease. This, combined with interrogatories of dynamic imaging (i.e. echo and gated-CT), will contribute to tailored risk adjudication for thoracic aortic aneurysm.

Sustained Analgesic and Anti-inflammatory Efficacy of a Single Dose COX-2 Inhibiting Nanomedicine in a Mouse CFA-induced Inflammatory Model

Lu Liu (1,2), Michele Herneisey (1,2), Eric Lambert (1,2), Shannon Loftus (1,2), Takaaki Komatsu (3), Vijay S. Gorantla (4) and Jelena M. Janjic (1,2)

(1) Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, Pennsylvania, (2) Chronic Pain Research Consortium, Duquesne University, Pittsburgh, Pennsylvania, (3) Department of Pharmacology, Daiichi University of Pharmacy, Fukuoka, Japan, (4) Wake Forest Institute for Regenerative Medicine, Winston-Salem, North Carolina

Background: New and innovative concept of immune system targeted inflammatory pain treatment is presented. In this strategy non-opioid analgesics are delivered to immune cells infiltrating sites leading to overall drug dose decrease >2000 fold compared to systemic administration. The presence of COX-2 overexpression has been found to correlate the development of neuropathic pain in both animals and human. Based on these findings, we argued that macrophages are an attractive cellular target for further therapeutic development in both acute and chronic pain. Celecoxib, a COX-2 selective NSAID, is used to treat the signs and symptoms associated with inflammatory pain in chronic conditions. However, the in vivo efficacy of celecoxib is often poor and with adverse cardiovascular effects. To improve this, we successfully loaded celecoxib into near-infrared fluorescent (NIRF) dye labeled nanoemulsions (CXB-NE) to target macrophage-associated inflammation and to visualize infiltrating macrophage accumulation at the site of injury using in vivo NIRF imaging. Nanoemulsions, kinetically stable oil in water dispersions with a droplet size range of 100-150 nm, can provide a solution for the problems listed above. Theranostic nanoemulsions is an emerging technology that has the potential to personalize medical treatment by combining imaging and drug delivery properties into one nanosystem. In previous studies, we demonstrated that CXB-NE are internalized by macrophages and show greater accumulation in the inflamed footpad injected with Complete Freund's Adjuvant (CFA). We also showed that COX-2-inhibiting nanoparticles reduce macrophage infiltration in the CFA-treated mouse footpad. We hypothesized that nanoemulsions can lead to dramatically extended anti-inflammatory and anti-nociceptive effects in the CFA mouse models when formulated as extended release formulations with high drug levels and reduced droplet size.

Methods: Nanoemulsions are produced by microfluidization. Multiple-linear regression (MLR) model was used to study the relationship between process parameters/compositions and quality attributes during the design and development of theranostic nanosystem. Pearl® Trilogy Small Animal Imaging System was used to obtain whole body live imaging (WBI) and the organ distributions.

Results: In this study, we present novel high dose celecoxib theranostic nanoparticles designed with an extended drug release profile and tagged with a NIRF label to allow tracking of the nanoparticles. Mechanical allodynia studies in the CFA-induced mouse model, whole body live imaging confirming prolonged analgesic effects of single dose, targeted CXB-NE treatment are presented. We also show new design of experiments (DOE) computational approaches to theranostic nanomedicine design that utilize multidimensional data (behavior and imaging) for optimal inflammatory pain relief. We have employed multiple linear regression (MLR) to study the relationship between CPPs/compositions and particle size distribution in the theranostic nanosystem. To the best of our knowledge, this is the first study confirming the anti-inflammatory and analgesic efficacy of a single-dose nanomedicine platform in an experimental mouse CFA-induced inflammatory model.

Conclusions: Nanoemulsions obtain a long and robust shelf life, which can withstand different stress conditions. This study also shows an innovative application of MLR modeling to the development of pain nanotherapeutics. WBI and mechanical hypersensitivity data indicate i.v. administered COX-2 targeted nanoemulsion can provide pain relief in the mouse CFA-induced inflammatory model.

Does the Inclusion of Left Atrial Hemodynamic Analysis Improve Stroke Risk Prediction in Atrial Fibrillation?

Soroosh Sanatkhani (1), Sotirios Nedios (2), Sandeep K. Jain (3), Samir F. Saba (3), Prahlad G. Menon (1) and Sanjeev G. Shroff (1)

(1) Department of Bioengineering, University of Pittsburgh, (2) Massachusetts General Hospital, Boston, MA, (3) Department of Medicine, University of Pittsburgh

Background: Atrial fibrillation (AF) currently afflicts 3-6 million people in the USA and this number is expected to rise to 7.56 million by 2050. AF is the most common sustained arrhythmia, especially among elderly patients, costing the health care system ~$6 Billion/year. AF patients have 3-5-fold higher risk of stroke. All thromboprophylaxis decisions require physician assessment of the patient's stroke risk, which is currently determined by clinical data included in the CHA2DS2-VASc score. However, we remain very limited in predicting who will have a stroke in the setting of AF.

Hypothesis: The left atrial appendage (LAA) is a common location for formation of thrombi in non-valvular AF (91%) and in valvular AF (50%). This suggests a possible link between the LAA shape/hemodynamics and stroke risk. We hypothesize that adding a LAA hemodynamics index (scalar residence index, SRI) will significantly improve stroke risk stratification in AF patients as compared to the stratification based solely on the CHA2DS2-VASc score.

Methods: Cardiac computed tomography images of 40 patients with AF (6 of these patients had prior stroke) were acquired and patient-specific left atrial (LA) and LAA geometries were reconstructed in 3D and meshed for computational fluid dynamics (CFD) simulation. SRI for each patient was calculated using patient-specific left atrium/LAA geometry and a CFD-based hemodynamic analysis.

Results: Our analysis showed that the correlation between SRI and CHA2DS2-VASc score is low ($R2 = 0.28$, p-value = 0.03). The separation between patients with and without stroke was best accomplished by combining SRI and CHA2DS2-VASc score (17% increase in stroke prediction). Thus, it appears that the inclusion of SRI has a potential for improving stroke prediction in AF as compared to the stratification based solely on the CHA2DS2-VASc score. This conjecture is currently being tested in a larger cohort (157 AF patients) to provide a definitive conclusion.

Modeling Influenza Dynamics and the Innate Immune System

Jordan Weaver (1) and Jason Shoemaker (1,2)

(1) Department of Chemical and Petroleum Engineering, (2) Computational & Systems Biology, University of Pittsburgh

Innate immune response is the first line of defense against infection. Immune responses can help or hinder an organism's ability to overcome an infection; excessively inflammatory responses can cause greater tissue damage, higher mortality, and slow recovery, while proper response to a threat is a prerequisite for survival. Thus, tight regulation of the immune response is critical. The first link in the chain reaction is detection of the invaders, leading to the early, localized, innate immune response. Without initial sensing protein activity, no immune responses would be mounted. Understanding the dynamics of these sensors is vital to quantifying the innate immune response. Current models are unstable, which fail to capture the dynamics of shutdown and steady state transitions or represent sensing proteins as simple constant-action rather than a response to the viral invasion. We construct a new ODE model to quantify the innate immune response of human bronchial epithelial cells (HBECs) to influenza A infection. The model incorporates the classical viral dynamic model, Type I Interferon inhibition of viral growth, and the proportionality of sensor protein activity to vRNA levels in the cytoplasm, the first such integration of cell dynamics and viral replication. This model predicts cytokine signaling and cell death during infection and will become the basis of an agent-based model in future work.

Arterial Spin Labeling Neck Coil Array System for Ultra High Field MRI

Salem Alkhateeb (1), Tales Santini (1), Tiago Martins (1), Nadim Farhat (1) and Tamer S. Ibrahim (1)

(1) Bioengineering, Swanson School of Engineering, University of Pittsburgh

Functional MRI (fMRI) is one of the most reliable diagnostic imaging modality that is utilized to map brain activation sites during any cognitive or physical task. Arterial Spin Labeling (ASL) is an fMRI technique that is applied to measure the cerebral blood flow (CBF), ASL has gained more attention in the last decade for its measuring capability to absolutely quantify cerebral blood flow in physiological units (mL/g per min). Quantification of CBF as a physiological parameter can help better understand neurological disease such as dementia and Alzheimer's.

ASL neck coil array is a radiofrequency (RF) medical device that is used to magnetically tag the arterial blood flowing upwards into the brain, then a whole brain image (magnetized image) is acquired after 1-2 seconds to allow blood to reach regions of interest. A subtraction between this magnetized image and a normal brain image is the blood perfusion distribution in the brain.

ASL implementation at ultra-high field MRI (> 7 Tesla) has some technical challenges such as RF field-inhomogeneity and high specific absorption rate (SAR). Tic-Tac-Toe (TTT) innovative coil design has shown unprecedented results in overcoming those challenges on different parts of the human body, however, it hasn't been designed specifically for ASL. Therefore, a design of 4 TTT panels are composed of 16 transmit channels (4ch/each panel) are constructed and combined around the neck to provide the highest field coverage. Finite-Difference Time-Domain (FDTD) Simulations of this design have shown successful outcomes to reduce SAR in the neck to within FDA regulations, and increase the homogeneity in the tagging region of interest. This successful case is currently in the assembling phase for validation and verification.

Chemical Cross-Linked Peptidic Fibrils for Embedding Polymeric Particles and Cells

Jennifer M. Armen (1), Nathan R. Schueller (2), Ngoc B. Pham (2), Ketki Y. Velankar (2), Rachelle N. Palchesko (4,6), Yong Fan (3,5), Wilson S. Meng (2,7) and Ellen S. Gawalt (1,7)

(1) Department of Chemistry and Biochemistry and the (2) Graduate School of Pharmaceutical Sciences, Duquesne University, Pittsburgh, PA, (3) Institute of Cellular Therapeutics, Allegheny-Singer Research Institute, Allegheny Health Network, Pittsburgh, PA, Departments of (4) Biomedical Engineering and (5) Biological Sciences, Carnegie-Mellon University, Pittsburgh, PA, (6) Louis J. Fox Center for Vision Restoration and (7) McGowan Institute for Regenerative Medicine, University of Pittsburgh

EAK16-II (hereafter EAK) is a self-assembling peptide (SAP) that forms beta-sheets and beta-fibrils through ionic-complementary interactions at physiological ionic strengths. It has been postulated that the alanine residues between two EAK beta-sheets drive the formation of bilayer subunits; stacking of the subunits is induced by inorganic salts (e.g. NaCl) which serve to reduce the charge repulsion between lysines and glutamines on the outer leaf of the bilayer. At sufficient concentrations of EAK and salts, fibrils are generated from elongation of the bilayer subunits. We and others have generated EAK-derived fibrillar matrices for drug delivery and tissue engineering applications. In particular, the soft materials can be injected in vivo, creating depots of drugs and cells for rendering pharmacological and biological actions. One opportunity for optimization is to modulate the density of the fibrils in anatomical space in which the amount of extracellular fluid is limited. In such tissues the rate and extent of the fibrilization can be slow. In order to expand the scope of potential applications, we have generated a method by which EAK fibrils are pre-assembled yet remain injectable. We used the carbodiimide cross-linker, N-ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), to induce the formation of amide bonds between glutamate and lysine residues in the EAK peptides and beta-sheets. We hypothesized that the resulting de novo covalent linkages enhanced stacking of the beta-sheet bilayers, thereby increasing the lengths of the fibrils and the extent of their cross-linking, as evidenced in DRIFT spectroscopy, SEM, and AFM analyses. The cross-linked EAK (clEAK) was shown to trap polymeric microspheres with an average diameter of 1 μm. Macrophages admixed with clEAK were found to be viable. These results indicate that clEAK should be explored as a platform for delivering drugs and cells in vivo.

A High-Density Electrode Array for Mapping Corticospinal Recruitment of Upper-Limb Muscles After Stroke

Ernesto Bedoy (5,6), Efrain Diaz (4), Mike Urbin (2), George Wittenberg (3,5,6), Gaurav Sharma (7) and Douglas Weber (1,2,5)

(1) Department of Bioengineering, (2) Department of Physical Medicine and Rehabilitation, (3) Department of Neurology, (4) Department of Neuroscience and (5) Center for Neuroscience, University of Pittsburgh, (6) Center for the Neural Basis of Cognition, Carnegie Mellon University, Pittsburgh, (7) Battelle Memorial Institute, Columbus Ohio

Nearly 800,000 Americans per year experience a stroke and the majority of survivors are left with disability due to muscle weakness in the arm and hand. Stroke-induced damage to the corticospinal system can result in abnormal muscle synergies, reducing the ability to recruit agonist muscles and increasing co-contraction of agonist and antagonist muscles. The severity of impairments in humans with stroke is also associated with higher thresholds to elicit motor-evoked potentials (MEPs) from transcranial magnetic stimulation(TMS) of primary motor cortex. Previous work has shown that MEPs in paretic muscles are lower in amplitude, delayed in latency and prolonged in duration after stroke, but the spatial distribution of MEPs across different muscles of a limb has not been well characterized. Understanding patterns of corticospinal recruitment may provide unique insights into physiology and function after stroke. Therefore, our group has developed a high-density electromyographic (HD-EMG) array to record MEPs from the entire forearm. The array consists of 150 surface disc electrodes embedded in fabric spanning the length and circumference of the dominant (neurologically-intact) or paretic (stroke) forearm of human subjects. Single-pulse TMS was delivered at multiple increments of resting threshold over the optimal scalp location for eliciting MEPs in the extensor muscle of the resting forearm. Time delays between MEPs were calculated to identify more precise locations of activated motor entry points. Our results show that peak MEP amplitude may reflect the summation of propagation from multiple motor entry points. Preliminary findings also show that extensor muscle recruitment is impaired in stroke subjects. In summary, the HD-EMG array allows high-resolution mapping of corticospinal recruitment across the upper limb. The ability to map recruitment may provide a prognostic tool capable of evaluating impairments due to stroke and tracking progress in response to rehabilitation.

Open-Source, 3D-Printed Peristaltic Pumps for Small Volume Point-of-Care Liquid Handling

Michael R. Behrens (1), Haley C. Fuller (1), Emily R. Swist (1), Jingwen Wu (2), Md. Mydul Islam (2), Zhicheng Long (1), Warren C. Ruder (1) and Robert Steward Jr. (2)

(1) Department of Bioengineering, University of Pittsburgh, (2) Department of Mechanical and Aerospace Engineering, University of Central Florida, Orlando, FL

Microfluidic technologies are frequently employed as point-of-care diagnostic tools for improving time-to-diagnosis and improving patient outcomes in clinical settings. These microfluidic devices often are designed to operate with peripheral equipment for liquid handling that increases the cost and complexity of these systems and reduces their potential for widespread adoption in low resource healthcare applications. Here, we present a low-cost (~$120), open-source peristaltic pump constructed with a combination of three dimensional (3D)-printed parts and common hardware, which is amenable to deployment with microfluidic devices for point-of-care diagnostics. This pump accepts commonly available silicone rubber tubing in a range of sizes from 1.5 to 3 mm, and is capable of producing flow rates up to 1.6 mL per min. This device is programmed with an Arduino microcontroller, allowing for custom flow profiles to fit a wide range of low volume liquid handling applications including precision liquid aliquoting, flow control within microfluidics, and generation of physiologically relevant forces for studying cellular mechanobiology within microfluidic systems.

Detection of pump thrombosis in the ModELAS system using device vibrations

Vishaal Dhamotharan (1,2), Ryan A. Orizondo (2) and William Federspiel (1,2,3)

(1) Department of Bioengineering, University of Pittsburgh, (2) McGowan Institute for Regenerative Medicine, (3) Department of Critical Care Medicine, University of Pittsburgh Medical Center

Pump thrombosis and related thromboembolic events are common occurrences in circulatory support systems. In an ECMO setting, such events could lead to partial/ complete flow occlusion and an eventual reduction in the gas transfer efficiency. Thrombosis occurs primarily due to hemolysis by the pump impeller or via ingestion of thrombi from the patient. Pump thrombosis is currently monitored via elevated power consumption, impeller torque and hemolytic activity using high LDH or fHgB levels. Other indirect effects such as reduced pump flow and trans-bundle pressures are also selectively used to detect bundle thrombus. However, these parameters are very susceptible to system-related changes and hence, are not sensitive to detect the onset of thrombi.

The aim of this study was to develop a reliable and sensitive method for detection of thromboembolic events within the ModELAS device using device vibrations. The ModELAS is a custom pump-lung device being developed to support ECMO and ECCO2R. The data acquisition equipment consisted of a single axis accelerometer, a charge amplifier and exclusive hardware. A mock circuit was designed to investigate the baseline vibrations of the device under normal operating conditions. Device vibrations were also recorded intermittently on ModELAS devices subjected to 30-day ovine studies and data was retrospectively analyzed.

Power spectrum density analysis of the data was performed at their respective pump rotational speeds. Peak amplitude of first four harmonics, presence and number of non-harmonic peaks were used to distinguish studies with thrombus formation from those which had no such events. Retrospective analysis of animal studies shows that devices with thrombus formation were observed to have taller peaks at the 1st, 2nd and shorter peaks at the 3rd, 4th harmonic frequencies than a device with no thrombus. Also the devices with thrombus exhibited an increased number of peaks at non-harmonic frequencies.

The PERFUSE Dual Chamber Stent Improves Donor Organ Recovery in a Porcine Model

Brian Frenz (1), Catherine Go (2), Moataz Elsisy (3), Youngjae Chun (3) and Bryan Tillman (1,2)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Vascular Surgery, (3) Department of Industrial Engineering, University of Pittsburgh

Background: Amidst a critical shortage of organs for transplantation, ischemic injury from malperfusion during the agonal period remains a prohibitive barrier. We hypothesized that a dual chamber stent graft could isolate visceral perfusion from the agonal systemic circulation, while respecting the ethics of organ donation.

Methods: A retrievable dual chamber stent graft was welded from nitinol and covered with polytetrafluorethelene. A central lumen maintained aortic flow, with an outer visceral chamber perfused by an oxygenator. Anesthetized pigs were assigned to either agonal control or the dual chamber stent. A one hour agonal phase of hypoxia (saturations < 60-70%) and hypotension (MAP < 25) was simulated both medically and with partial balloon occlusion. The Perfuse stent visceral flow totaled 500 ml/min during the agonal phase followed by stent recapture and resuscitation to an endpoint of 2 days.

Results: Study groups had comparable agonal O2 saturations, HR and MAP. Cardiac output and right ventricular end diastolic volume did not change during stent graft deployment. Compared to the low pO2 of controls (48 mm Hg) and systemic stent animals (49 mm Hg), the visceral pO2 averaged 413 mm Hg and visceral flow was significantly higher in stent animals. 5/7 controls were euthanized from acute renal failure and volume overload while all stent animals survived without renal impairment. Transaminases were between 1.8 to 3 fold elevated in control as compared to stented animals.

Conclusions: During a simulation of the agonal period, a dual chamber stent provided endovascular separation and marked improvement in perfusion and organ outcome. This was accomplished without any increased impact on cardiac function, respecting current ethical considerations of the DCD donor. The ability to separate the perfusion of the abdominal organs from the agonal systemic circulation without the need for open surgery might significantly improve the availability of donor organs for transplant.

Geometry effect on targeted cell labeling using DNA origami

Ying Liu (1), Piyumi Wijesekara Kankanange (2), Charlie Ren (2) and Rebecca Taylor (1,2,3)

(1) Department of Mechanical engineering, (2) Department of Biomedical Engineering, (3) Department of Electrical and Computer Engineering, Carnegie Mellon University, Pittsburgh, PA

DNA origami is a promising technique on diagnosis and drug delivery due to its spatial programmability and bio-compatibility. Multiple bio-analytes and/or drug molecules can be assembled into a complex machine with nanometer spatial resolution using DNA origami. Which makes it possible to sense, target, and load drugs in bio-environment. It is important to study the mechanisms on how DNA origami interact with cells and how we can tune this interaction. In this study, we studied the targeted cell labeling with DNA origami via cholesterol anchors. We found that not only the number of anchors per origami structure matters but also by changing the geometry of the DNA origami shapes, we can turn on and off of the cell labeling using these DNA origami structures. Furthermore, the recovery of cell labeling by using a spacer indicates that this effect is likely to result from the glycocalyx on the cell surface hinder the cholesterol anchored inside the membrane.

Assessment of a Peripheral Nerve Extracellular Matrix Derived Hydrogel for Improving Functional Recovery Following Nerve Reconstruction

Tyler Meder (1), Travis Prest (1), Lucile Marchal (1,2), Valeria Yupanqui (1), Clint Skillen (3) and Bryan Brown (1,3)

(1) University of Pittsburgh, Department of Bioengineering, (2) University Nice Sophia Antipolis, (3) McGowan Institute of Regenerative Medicine

In the US, peripheral nerve injury (PNI) occurs in 3% of all trauma cases and affects an estimated 20 million people increasing by 250,000 annually with a cost of $150 billion1-4. Without intervention, peripheral nerves show a slow and lacking regenerative response following injury, making surgical intervention an imperative5. We developed a novel peripheral nerve-specific extracellular matrix (PNM) hydrogel and have shown that it increases constructive remodeling of injured peripheral nerves. The present study evaluates the PNM hydrogel's efficacy in 3 rat sciatic injury models. These include nerve crush, transection, and sub-critical (8mm) gap with appropriate standard of care comparisons. The crush model achieved full kinematic recovery at 4 weeks post-injury as opposed to 8 weeks when approached with clinical standard practice. PNM significantly increased number of axons and compound mean action potential (CMAP) over non-PNM groups, with 80% return to full function. The transection model remains in progress and has demonstrated superiority to neurorrhaphy without PNM at later time points (8 weeks) when comparing nerve conduction. Also, muscle function tested via peak tetanic force has shown equivalence at 8 weeks when compared with the standard of care. The gap model demonstrated overall recovery was enhanced with PNM and equivalent to autografting, the clinical standard of care, when comparing axon counts, CMAP, and kinematics at 24 weeks. These models demonstrate both a clear regenerative advantage for PNM application and equivalence to clinical standards. This strategy has demonstrated superior recovery compared to transection injuries repaired end-to-end. While we can conclude, overall, that our PNM gel increases nerve regeneration in most injury types, our next step will be to conduct a small gap model to demonstrate the PNM gel's efficacy in this commonly used surgical technique to overcome the difficulties present in a transection-type injury.

Contact Lens Delivery of Interleukin-4 for Treatment of Dry Eye Disease Promotes Anti-inflammatory Macrophage Populations

Alexis L Nolfi (1,2), Vishal Jhanji (3), Mangesh Kulkarni (1,2) and Bryan Brown (1,2,4)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Cornea, External Eye Diseases, and Refractive Surgery, University of Pittsburgh Medical Center Eye Institute, (4) Department of Obstetrics, Gynecology, and Reproductive Sciences, University of Pittsburgh

Dry eye disease is characterized by an inflammatory process that is mediated by macrophages, a multi-phenotype cell population, with the M1 pro-inflammatory phenotype being associated with perpetuation of the disease. Therefore, we argue that most, if not all, patients suffering from dry eye could benefit from an immunomodulatory strategy which targets M1 inflammatory macrophage populations. We hypothesize that a sustained release lens coating for the delivery of IL-4 will aid in “reprogramming” M1 macrophages to the anti-inflammatory M2 phenotype, thereby modifying downstream signaling processes and leading to the mitigation of the inflammation fueling dry eye.

A coating containing IL-4 was applied using a layer-by-layer technique. To confirm coating adherence, an alcian blue dye was used to stain glycosaminoglycan coating components. The ability of the lens coating to contain and release IL-4 was assessed with an in-vitro controlled release experiment in which lenses coated with IL-4-containing-coating were incubated in solution containing enzymes that mimic those produced by ocular macrophages in-vivo. Finally, macrophages were cultured and incubated with either coated/uncoated contact lenses or cytokines known to cause anti-/pro-inflammatory phenotypes. Ability to modify macrophage phenotype was assessed through staining for intracellular arginase (an M2 anti-inflammatory macrophage phenotype marker), through determination of arginase activity with a biochemical assay, and through determination of nitric oxide production (an M1 macrophage marker).

Alcian blue staining confirmed successful application of coating. In-vitro drug release assays show that our lens coating is capable of a sustained release of drug over the course of days and that release is mainly enzymatically driven. For in-vitro culture experiments, staining for intracellular arginase, as well as assessment of arginase activity with a biochemical assay, show that the IL-4 released from our lens device is capable of programming target cells to an anti-inflammatory phenotype. Importantly, IL-4 coated lenses did not cause any appreciable production of nitric oxide, which is a marker of the pro-inflammatory M1 macrophage phenotype that is responsible for perpetuation of dry eye disease.

IL-4 containing polymeric coatings can be applied successfully to soft contact lenses and release sufficient amounts of bioactive IL-4 to modify pro-inflammatory macrophages to the anti-inflammatory M2 population, with the ultimate goal of targeting the root cause of dry eye.

Effect of Hematocrit and Plasma Protein Concentration on CO2 Removal in Artificial Lungs

Alexandra G. May (1,2), Katelin S. Omecinski (1,3), Brian J. Frankowski (1) and William J. Federspiel (1,2,3,4)

(1) McGowan Institute for Regenerative Medicine, University of Pittsburgh, (2) Department of Chemical and Petroleum Engineering, University of Pittsburgh, (3) Department of Bioengineering, University of Pittsburgh, (4) University of Pittsburgh Medical Center

Study: Extracorporeal CO2 removal has the potential to benefit two important patient populations, patients with acute respiratory distress syndrome (ARDS) patients and those with acute exacerbations of COPD (aeCOPD). In ARDS patients ECCO2R may allow the use of lung protective mechanical ventilation and in the aeCOPD population it may prevent the need for invasive mechanical ventilation altogether. As the investigation of ECCO2R clinically rises, it is important for researchers and clinicians to understand all factors that influence the CO2 removal rate in ECCO2R in modeling and clinical applicationsshould be well understood. While many factors have been explored, some have not. In this study we explored in- vitro the effect of hematocrit (HCT) and presence of plasma proteins on CO2 removal in ECCO2R. The effects of blood and sweep gas flow rate, bundle surface area, and inlet PCO2 on CO2 removal (vCO2) are well understood. It has been observed in vivo that decreased vCO2 in hollow fiber membrane (HFM) devices occurs during periods of anemia but an in vitro study to evaluate only the effects of HCT and plasma proteins on vCO2 has not been conducted.

Methods: Bovine blood was diluted with saline or plasma to HCT levels between ranging from 33% to and 8%. Pump speed was adjusted to maintain a blood flow rate of 500 mL/min. In vitro gas transferCO2 removal was evaluated in our ambulatory artificial lunga HFM ECCO2R device according to ISO standards at a blood flowrate of 500 ml/min, a flowrate that provides therapeutic ECCO2R in our device 7199.

Results: vCO2 removal rate ($\dot{v}$vCO2) decreased linearly with reducing HCT for a reduction ofby 42% and 32% in saline and plasma respectively when diluted from a HCT of 33% to 8% with saline and plasma, respectively. The difference in $\dot{v}$vCO2 in plasma versus saline was not statistically significant. The effect of HCT on $\dot{v}$vCO2 is hypothesized, as is the case in the native lung, to be due The physiological reasons for this are likely analogous to those of the native lungs. The vCO2-HCT relationship within the native lungs has been attributed to the release of fewer Bohr protons, a decreased buffering capacity of blood due to lack of red blood cells (RBC), and a reduced flux of bicarbonate ion across the RBC membrane. The difference in vCO2 in plasma versus saline was not statistically significant. This study demonstrates that varying HCT has a significant effect on vCO2 in HFM ECCO2R devices. Thus, the HHCT of blood should be accounted for when assessing the CO2 removal rate in a variable when modelling ECCO2R. and should be normalized for when making device parameter changes based on in-vivo vCO2.

Hydrogel-enabled Intratumoral Co-Delivery of Anti-PD-1 Antibody and Adenosine Deaminase in a Mouse Model of Renal Cell Carcinoma

Ngoc B. Pham (1), Ketki Velankar (1), Nevil Abraham (1), Nathan R. Schueller (1), Ellen S. Gawalt (2,3) and Wilson S. Meng (1,3)

(1) Graduate School of Pharmaceutical Sciences, (2) Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, PA, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Background: It is hypothesized that tumor resistance to anti-PD-1 monoclonal antibodies is due in part to the accumulation of adenosine (ADO) generated in the tumor microenvironment (TME). ADO impairs the activation and proliferation of effector T cells while expanding regulatory T cell (Treg) population, which is inversely related to the overall survival of cancer patients, including those with renal cell carcinoma (RCC). We propose to develop an injectable system by which ADO are degraded in the TME in order to enhance the efficacy of anti-PD1 treatment. To this end, we have developed a hydrogel to co-deliver anti-PD-1 antibody with adenosine deaminase (ADA), which catabolizes ADO. The hydrogel contains a bioaffinity module (named “Z15_EAK”) to retain the anti-PD-1 antibody in tumors while limit the diffusion of ADA in TME for extended durations. We have previously shown that Z15_EAK hydrogel can retain IgG at subcutaneous injection site for at least two weeks [1]. The expectation is that persistent co-localization of anti-PD-1 and ADA in the TME will expand Th1 T cells and reduce Treg in draining lymph nodes (DLNs) and systemic lymphoid tissues. This postulation was tested in an immunocompetent mouse model of RCC.

Methods: A mouse RCC cell line (RENCA) was cultivated for in vitro assays and in vivo inoculation into BALB/c mice. Beginning three days after tumor inoculation, the hydrogel loaded with an anti-PD-1 IgG antibody and ADA was injected subcutaneously in the peri-tumoral region for three doses three days apart. DLNs, spleens, and tumors were collected for flow cytometric and RT-PCR analysis.

Results: DLNs in treated mice contained fewer CD4+CD25+FoxP3+ Treg cells compared to controls. In addition, the DLNs in mice received the hydrogel loaded with anti-PD-1 antibody and ADA were significantly larger than those in mice received saline control. PCR analysis on tumors of the treatment group exhibited a greater shift toward Th1 cytotoxic phenotype from Treg tumor accommodating phenotype compared to saline control.

Conclusion: The preliminary data indicate that the local delivery of anti-PD-1 and ADA with the hydrogel shifted the local T cell population toward an effector phenotype (Th1) while limiting the Treg expansion. An extended co-localization of anti-PD-1 and ADA in the TME not only modulates immune events in the local lymphoid tissues but can also enhance the anti-tumor response systemically. Furthermore, the localized delivery reduces off-target toxicities of anti-PD-1 antibody.

Effect of Viscosity on Mechanical Hemolysis within HeartWare HVAD in-Vitro

Shiv Rajesh (1,2), Greg W. Burgreen (3), James F. Antaki (4) and Marina V. Kameneva (1,2,4)

(1) Department of Bioengineering, University of Pittsburgh, (2) McGowan Institute of Regenerative Medicine, University of Pittsburgh, (3) CAVS, Mississippi State University, Starkville MS, (4) Meinig School of Biomedical Engineering, Cornell University NY, (5) Department of Surgery, University of Pittsburgh

The HeartWare HVAD is a centrifugal left ventricular assist device (LVAD) used clinically in patients with end-stage heart failure, however, this LVAD still presents adverse complications such as mechanical blood cell damage, leading to hemolysis and thrombosis. Previous experimental and numerical studies reported that the clearance gap between HVAD rotor and housing is altered by speed and blood viscosity. Consequently, both viscous and Reynolds shear damage environment within the pump is affected. The goal of this study is to experimentally isolate the influence of viscosity on the mechanical blood damage caused by the HVAD, independent of the hematocrit. A vertical reduced volume in vitro flow loop incorporating the HVAD operating was constructed to circulate bovine red blood cells (RBCs) resuspended in a fluid of varying viscosities for a total of 3 hours. Donor bovine blood was washed and resuspended in phosphate buffered saline (PBS) at hematocrit of 30 ± 1%. To increase the viscosity, PBS solution was mixed with dextran-40 to achieve a range of viscosities up to 10 cP, keeping the hematocrit consistent. This enabled experiments to be conducted with altered gap height and shear stress in HVAD while maintaining similar speed and hemodynamic conditions. Hemolysis (plasma free hemoglobin) measurements were recorded and calculated at 1-hour intervals as Normalized Index of Hemolysis (NIH). Early findings have demonstrated a positive correlation between hemolysis and blood viscosity at equivalent hematocrit and flow conditions. This confirms the complexity of mitigating hemolysis and subsequent thrombosis, in HVAD patients who present a wide range of blood viscosities. These results will be used to calibrate CFD models analyzing the hemolysis in LVADs.

Non-invasive optical imaging of vascular response to compression in healthy breast

Constance M. Robbins (1), Jason Yang (1), James F. Antaki (1,2) and Jana M. Kainerstorfer (1)

(1) Carnegie Mellon University, Department of Biomedical Engineering, Pittsburgh, PA, (2) Cornell University, School of Biomedical Engineering, Ithaca, NY

Neoadjuvant chemotherapy is often indicated for locally advanced breast cancer, shrinking the tumor before surgery and enabling breast conserving surgery in more cases. Complete pathologic response to neoadjuvant chemotherapy (assessed after surgery) is correlated with improved survival, but the prediction of this response early in the course of treatment remains a challenge. Tumor size (assessed via ultrasound or palpation) has shown to be a poor predictor of treatment outcome, so instead an imaging method is sought which provides information on breast tissue function. Breast tumors are often highly vascularized and thus contain high hemoglobin content which can be detected non-invasively with near-infrared optical imaging. Additionally, breast tumor vascular networks differ from those of healthy breast, which has previously been shown to lead to differences in the hemodynamic response to external perturbation. It has also been demonstrated that normalization of this response occurs in patients that achieve complete response to neoadjuvant chemotherapy. We have developed a handheld optical breast imaging device that is used to apply local pressure to the breast, inducing a hemodynamic response (blanching of tissue followed by re-perfusion) that has potential as a biomarker for tissue malignancy during treatment monitoring. The device utilizes spatial frequency domain imaging (SFDI), a wide-field diffuse optical imaging method that allows quantification of tissue optical properties. The design is portable and inexpensive and involves no ionizing radiation, making the technique well-suited and safe for frequent monitoring. Compression-induced hemodynamic response has been characterized in a group of healthy volunteers of various skin tones. Responses in total hemoglobin and tissue oxygen saturation percentage will be presented, and the use of a two-layer analytical model to account for differences in skin pigmentation will also be discussed.

Functionalized Out-of-Plane Graphene Microelectrode Arrays for Sensitive Biomolecule Sensing

Daniel San Roman (1), Raghav Garg (1), Bryan Brown (2,3) and Tzahi Cohen-Karni (1,4)

(1) Department of Material Science, Carnegie Mellon University, Pittsburgh, PA (2) Department of Bioengineering, University of Pittsburgh, (3) McGowan Institute for Regenerative Medicine, (4) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA

Bioelectrochemical sensing can provide real-time, continuous monitoring of physiological and pathological processes, such as neurotransmitter signaling and immune response. Nitric oxide (NO) is one such transcellular signaling molecule of interest. However, real-time detection of NO remains a challenge due to its low concentration, rapid diffusion, and short half-life under physiological conditions. Recently, nanocarbons have been investigated as an electrode material for biointerfaces due to their high specific surface areas and electrochemically active surface functionalities. In this work, we present a novel sensing platform based on high surface area three-dimensional (3D) out-of-plane fuzzy graphene microelectrode arrays (3DFG MEAs) functionalized with selective molecular catalysts to achieve in situ monitoring of NO released from living cells. 3DFG MEAs display excellent electrocatalytic activity towards NO with a sensitivity of 0.70 μA μM−1 cm−2 and a low detection limit of 200 nmol L−1. Further surface treatment will provide a protective coating and semi-permeable membrane for adequate durability and selectivity in challenging biological environments. Future applications may include in vivo monitoring of NO release from immune cells in small animal models to better understand wound healing processes.

SoliDrop for Extended/Controlled Release of Ophthalmic Medication

Parissa Ziaei (1) and Morgan Fedorchak (1,2,3,4,5)

(1) Department of Ophthalmology, (2) Bioengineering Department, (3) Chem/Petroleum Engineering Department, (4) McGowan Institute for Regenerative Medicine, (5)Clinical and Translational Science

Compliance with eye drop medications is a significant problem in ophthalmology, leading to poor outcomes in certain diseases. Even with perfect adherence to a given eye drop regimen, the bioavailability of medications can be low, and thus higher doses of medications are needed, potentially leading to increased levels of toxicity. New technology is introduced to confront these challenges. This technology can significantly increase compliance with ophthalmic medication by decreasing dosing frequency, thereby improving disease management. The necessary effective dose is also minimized using localized delivery, thus decreasing toxicity issues.

In this project, we will use our proprietary drug-loaded thermoresponsive gel eyedrop that controllably degrades to release the therapeutic agent to the ocular surface at a predetermined rate. This will include a short-acting drop that provides pre-intravitreal injection anesthesia combined with infection prophylaxis through lidocaine and betadine administration, respectively. The maximum release of lidocaine and betadine occurs in less than 20 minutes at body temperature, matching the design criteria for a short-acting, combined system. Most notably, the combination gel drop shows a significantly improved antiseptic effect compared with the traditional sequence of lidocaine gel/betadine solution. Future studies will evaluate the anesthetic effect of the gel drop using Cochet-Bonnet aesthesiometer and measuring the corneal touch threshold (CTT) in a healthy rabbit model. The amount of drug released will also be examined by sampling tear film over time and analyzed using high-performance liquid chromatography (HPLC).

Recapitulation of the Microenvironment of Patient-Specific Multiple Myeloma by 3D printed Organoids for Predicting Chemotherapy Response

Julio Aleman (1,2), Sunil George (1), Samuel Moss (1), Alexandra Maycock (1), Christopher Porada (1), Graca Almeida-Porada (1), Cesar Rodriguez (1) and Aleksander Skardal (1,3)

(1) Wake Forest Institute for Regenerative Medicine, (2) Department of Bioengineering University of Pittsburgh, (3) Department of Biomedical Engineering, The Ohio State University

Multiple myeloma (MM) is the second most common hematological malignancy, as such it needs the support of the bone marrow (BM) niche to advance. The stimulations the BM gives to the MM cells come from hormonal secretions, cell-cell and extracellular matrix interactions. The advances in genomic techniques has made it clear that each patient with MM is the result of a collection of mutations that share a common clinical outcome, exposing the need of high throughput platforms to screen patient specific combinational therapies. In order to recapitulate the environmental and cellular interaction of each patient, we have bioengineered a humanized ECM-based construct that support patient specific stromal and malignant cells. We have automatized a high-throughput bioprinting process to scale up the biofabrication of multiple myeloma organoids for personalized drug screening. We standardized a median of 122 organoids from trial sample. Cell viability was sustained above 70% for up to 7 days. Flow cytometry and immunohistochemistry at baseline and day 7 showed stable representation of CD138+ cells, CD3+ and CD11b+. Organoids screened in parallel with drug groups of Lenalidomide, Cyclophosphamide, and Pomalidomide with basal Bortezomib and Dexamethasone; showed differences in overall viability. We present here a system that provides in vitro mimicry of in vivo environmental and cellular composition of a patient-specific multiple myeloma bone marrow. Overall the demonstrated difference in metabolic and viability data has laid down the groundwork for the bioprinted myeloma platform to be adopted for future drug screening purposes. The automatized high throughput process to create these organoids facilitates consistent biofabrication of patient-derived models. In the future, all these features may help simplify the translation from bench to clinic by providing a tool for better understanding personalized response and therapy.

In vivo tracking of immune cell invasion in a stroke brain implanted with ECM hydrogel

Reem Azar (1), Harmanvir Ghuman (1), Ryan Krafty (5), Stephen Badylak (3,4) and Michel Modo (1,2,4)

(1) Department of Bioengineering, (2) Department of Radiology, (3) Department of Surgery, (4) McGowan Institute of Regenerative Medicine, (5) Department of Arts and Sciences, University of Pittsburgh

Stroke is the leading cause of serious long-term disability and the fifth leading cause of death in the United States. Cell therapy is an emerging treatment for patients suffering from stroke, but it fails to restore lost tissue. In peripheral organs, such as the skin, acellular biological scaffolds promote constructive remodeling of the tissue after incidences of volumetric loss (such as severe burns). These inductive acellular extracellular matrix (ECM)-derived bioscaffolds can provide structural support and a bioactive environment to promote host cell infiltration and tissue remodeling. Although ECM-derived bioscaffolds can provide both a structural and functional microenvironment, proper biodegradation of the material is necessary to facilitate this constructive remodeling. In vivo, macrophages are required for degradation of ECM-derived scaffolds in peripheral soft tissues. The purpose of this work is to determine the role of peripheral macrophage invasion in the constructive remodeling of de novo tissue. To accomplish this goal, rats with severe stroke damage (Middle Cerebral Artery occlusion) were implanted with porcine urinary bladder ECM-derived hydrogel to restore lost tissue in the brain. Using perfluorocarbon nanoemulsions taken up by circulating macrophages allowed the serial tracking of their trafficking by 19F-MRI. In vivo MRI, provided a 24-hour time course of peripheral macrophages' invasion into the implanted hydrogel indicating that an initial presence within the bioscaffold occurs by 9 hours post-implantation and peaks at 18 hours. To further evaluate the phenotypes of immune cells invading the hydrogel, a histological time course study was used to determine the presence, distribution and phenotypes of immune cells (neutrophils, macrophages, lymphocytes) at 9 hours, 1 day, and 3 days post-implantation. These studies will further our understanding of the immune system's role, specifically macrophages, in facilitating constructive remodeling of brain tissue.

Scaffold-Free Tissue Engineering for Full Tooth Root Regeneration

Tia Calabrese (1), Kristi Rothermund (2) and Fatima N. Syed-Picard (1,2,3)

(1) Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, (2) Department of Oral Biology and Center for Craniofacial Regeneration, School of Dental Medicine, University of Pittsburgh, (3) McGowan Institute for Regenerative Medicine

The current optimal treatment for tooth loss, the titanium implant, has a failure rate of 7% due to mismatches in properties between the implant and surrounding tissue. The development of a biological tooth root would allow for better tissue integration. We have previously observed scaffold-free constructs (SFCs) made from dental pulp cells (DPCs) self-assemble into a dentin-pulp complex, the central structure of the tooth. SFCs made from periodontal ligament cells (PDLCs) resulted in the formation of a self-assembled PDL-cementum complex, the external structure of the tooth root. The current study aims to combine these SFCs to now create a full tooth root-like structure containing dental pulp, dentin, cementum, and PDL. To achieve this, human DPCs and PDLCs were labelled fluorescent red and green, respectively. Dentin-pulp SFCs were formed with the DPCs and wrapped with PDLC sheets, and subsequently cultured for 7 and 14 days. Staining with hematoxylin and eosin showed formation of a solid, cellular structure. Alizarin red staining for mineral deposition showed a central soft tissue, surrounded by a mineralized structure, and another external soft tissue, mimicking the mineralization pattern of the natural tooth root. Fluorescent imaging showed that the spherical DPC construct maintained its position in the center of the construct, and the PDLC sheet maintained its externally wrapped position. Immunohistochemical staining for bone sialoprotein and dentin matrix protein 1 showed localization of these markers to the mineralized structure, emulating a dentino/cementogenic structure; asporin expression was localized to the peripheral soft tissue, similar to PDL. These results indicate that a tooth root-like construct was created, with a soft pulp-like tissue in the center, surrounded by a hard tissue with characteristics of dentin and cementum, enclosed in a soft PDL-like tissue. Scaffold-free engineered tooth roots could provide a natural method of tooth replacement, bypassing the limitations of metallic implants.

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

Ya-Wen Cheng (1), Utku Sonmez (2), William Okech (2), Beth L. Roman (2) and Lance A. Davidson (1)

(1) Departments of Bioengineering, (2) Department of Human Genetics, University of Pittsburgh

Vascular morphogenesis is driven by collective movements and proliferation of endothelial cells (ECs). A hierarchically organized vascular network emerges during angiogenesis, and matures as blood vessels remodel. Defects in angiogenesis and remodeling, via overgrowth or inadequate network elaboration, lead to various diseases. We are interested in ECs response to biomechanical and biochemical cues in situ microenvironment. We aim to recreate this complex microenvironment in a microfluidic device. We built an All-in-One microfluidic device capable of producing a range of laminar and gradient flow conditions and examined how the flow and ligand alter EC phenotype. Bone morphogenetic protein 9 (BMP9) has been shown to bind with ALK1 to modulate vascular morphogenesis. However, the molecular mechanism by which flow acts on these receptors to enhance BMP signaling is not clear. With the All-in-One device, we confirmed that flow and ligands together modulate SMAD signaling synergistically. Under conditions of reduced BMP9, nuclear translocation of pSMAD is strongly reduced. By comparing pSMAD translocation across many flow conditions, we find flow increased translocation but that the level of translocation was independent of the flow magnitude. All-in-One also allows quantification of cell polarity, defined as the vector from the center of the nucleus to the center of the Golgi apparatus simultaneously. Both high SS and serum stimulation reduced downstream-polarization and expanded the population of upstream-polarized ECs. Interestingly, the magnitude of SS has differing impact on SMAD signaling and flow-aligned EC polarity; while flow-alignment depends on SS-magnitude, SMAD signaling did not. To better understand the role SMAD signaling play in EC migration, we plan to extend our microfluidic study to include high-resolution imaging of live ECs under spatially varying SS and BMP9 cues.

De Novo Lung Biofabrication: Clinical Need, Design Strategy, and Novel Construction Methods

Erica Comber (1), Rachelle Palchesko (1), Xi Ren (1), Adam Feinberg (1), and Keith Cook (1)

(1) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh PA

Chronic lung disease is the 4th leading cause of death in the U.S. Nearly one million patients per year are either hospitalized or die due to exacerbations of their disease state, but there are only 2,600 lung transplants per year. Extracorporeal membrane oxygenation (ECMO) can support these patients for up to a few months, but the densely packed artificial surfaces of ECMO oxygenators lead to rapid clot formation and device failure. Systemic anticoagulation is used to slow this process, but oxygenators still fail every 1 to 4 weeks, and patients experience life-threatening bleeding complications. A means of respiratory support is thus needed for chronic lung disease patients that provides similar biocompatibility as a transplanted lung for years of uncomplicated use. Our objective, therefore, is to engineer a 100% tissue-based, biofabricated lung. This organ would be constructed from extracellular matrix proteins and lined on the blood side by endothelial cells and on the gas side by epithelial cells. Like artificial lungs, biofabricated lungs do not need to follow the shape and structure of a native lung, allowing for simpler manufacture. However, various functional requirements must still be met, including stable, efficient gas exchange for a period of years. Our initial goal in this research is to fabricate thin, endothelialized channels to act as the gas exchange membranes for these organs using collagen I as the main structural protein. Future work will determine cell seeding and culture conditions that optimize endothelial adhesion and phenotype and determine the gas, water, and large molecule permeability of the membranes. This work will complement computational modeling to inform the design of the full-scale support lung.

MBV-associated IL-33: A Mechanism by which Fibrosis and Tissue Restoration are Regulated

Madeline Cramer (1,2), Jenna Dziki (2,3), George Hussey (2,3), Tengfang Li (4,5), Heth R. Turnquist (2,3,4,5) and Stephen F. Badylak (1,2,3)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Surgery, (4) Department of Immunology, (5) Starzl Transplantation Institute, University of Pittsburgh

Chronic rejection following heart transplantation (HTx) is associated with cardiac allograft vasculopathy and myocardial fibrosis, which contribute to failure of more than 50% of transplants within 11 years. An excessive and chronic pro-inflammatory environment has been implicated in adverse cardiac remodeling and progression to HF. Numerous experimental studies have shown that a timely resolution of inflammation after MI or HTx may prevent development and progression of immune-driven fibrosis. The effects of the pro-inflammatory macrophage and T-cell phenotype can be minimized by anti-inflammatory and immunosuppressive drugs, but these drugs are ineffective in preventing pathogenic remodeling. An alternative immunomodulatory, but not immunosuppressive, approach involves signaling molecules contained within the extracellular matrix (ECM). Matrix bound nanovesicles (MBV) embedded within the extracellular matrix contain biologically active molecules that can rapidly activate macrophages to a pro-remodeling phenotype. MBV are a rich and stable source of extra-nuclear interleukin-33 (IL-33). IL-33 is an IL-1 family cytokine that is a transcription factor that is classically considered as an alarmin but has emerging reparative and immunoregulatory properties. We have found that the IL-33 encapsulated within the MBV can bypass the canonical IL-33/ST2 receptor signaling pathway and instead initiates a trafficking pathway required to activate macrophages toward a reparative M2-like phenotype. This non-canonical ST2-independent pathway results in mitigation of M1 activation even in the presence of potent pro-inflammatory stimuli (LPS/IFNg). The MBV-induced macrophage secretome also limits cardiac fibroblast to myofibroblast transition in vitro. In a mouse model of heart transplant, the absence of IL-33 in grafts isolated from il33-/- mice resulted in increased chronic rejection-associated fibrosis, vasculopathy and immune cell infiltration in both wild-type and il33-/- recipients. Administration of a collagen hydrogel loaded with IL-33+ MBV to IL-33 deficient grafts immediately following transplantation limited the infiltration of pro-inflammatory immune cells and prevented chronic rejection of the graft.

Therapeutic Ultrasound Triggered Silk Fibroin Scaffold Degradation

Megan DeBari (1), Xiaodan Niu (2), Mallory Griffin (2), Sean Pereira (2), Bin He (2) and Rosalyn Abbott (2)

(1) Department of Materials Science and Engineering, (2) Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, Pennsylvania

A patient's capacity for tissue regeneration varies based on age, nutritional status, disease state, lifestyle, and gender. However, there is currently no strategy to account for this variability in tissue regeneration. Because regeneration rate cannot be predicted prior to biomaterial implantation, there is a need for responsive biomaterials with adaptive, personalized degradation rates to improve regenerative outcomes. Silk fibroin is an ideal candidate for use in patient-specific tissue regeneration due to its tunable degradation rates, mechanical properties, and ability to be conjugated with growth factors and alternative therapeutics to enhance regeneration.

Clinically, ultrasound is mostly used for imaging and diagnostic applications, but it is rapidly gaining attention for therapeutic applications. It has also been shown that therapeutic levels of ultrasound decrease the molecular weight of silk. However, no studies have looked at using ultrasound to monitor and control silk scaffold degradation.

Our research demonstrates a new approach to use therapeutic ultrasound as a means of altering the degradation profile of silk fibroin biomaterials non-invasively post-implantation. By evaluating changes in weight, porosity, surface morphology, compressive modulus, and chemical structure, we conclude that therapeutic ultrasound increases the degradation rate of silk fibroin scaffolds. Specifically, sonicated scaffolds degraded more quickly in a protease solution than the control scaffolds. By removing microbubbles on the scaffold surface, we found that transient cavitation was the mechanism responsible for changing the degradation profile. Furthermore, the polymer structure and the mechanical properties of the scaffolds were not significantly altered during sonication. This method proved safe for human cells with no negative effects on cell viability or metabolism. Sonication through human skin also effectively triggered scaffold degradation, increasing the clinical relevance of these results. These findings suggest that silk is an ultrasound-responsive biomaterial, where degradation rates can be altered non-invasively to improve regenerative outcomes.

Scaffold-Free Nerve Conduit Engineered using Dental Pulp Cells

Michelle D. Drewry (1), Matthew T. Dailey (2), Kristi Rothermund (3) and Fatima N. Syed-Picard (1,3,4)

(1) Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, (2) Department of Oral and Maxillofacial Surgery, School of Dental Medicine, University of Pittsburgh, (3) Department of Oral Biology and Center for Craniofacial Regeneration, School of Dental Medicine, University of Pittsburgh, (4) McGowan Institute for Regenerative Medicine

Peripheral nerve injuries often have limited recovery and poor functional outcomes, necessitating interventions such as nerve conduits. An optimal conduit should provide both trophic cues to promote axonal growth and guidance cues to accurately direct axon extension, enabling regenerating axons to bridge a nerve defect. Dental pulp cells (DPCs) have previously been shown to produce high levels of endogenous neurotrophic factors (NTFs), capable of enhancing axonal regeneration and extension. Aligned extracellular matrix (ECM) is able to direct this extension by providing guidance cues to regenerating axons. The goal of this study is to develop a scaffold-free nerve conduit using DPCs and their endogenous aligned ECM to treat peripheral nerve defects. To accomplish this, DPCs were grown on a substrate comprising parallel micro-grooves to form a scaffold-free cell sheet which could then be rolled to form solid, cylindrical constructs. Analysis of NTFs, gene and protein expression, confirmed that DPCs grown on grooves expressed these factors at levels greater than those grown on flat surfaces. Phalloidin staining and immunohistochemistry against collagen I showed that the DPCs and ECM, respectively, aligned with the parallel patterned grooves. Quantification of cell alignment substantiated that DPCs had increased alignment when cultured on grooved substrates relative to flat substrates. To evaluate the capability of the aligned DPC constructs to direct axonal growth and extension, neuronal cells were plated on the aligned sheets and their neurite extension and alignment relative to the cell sheet ECM were assessed. Overall, this study shows that aligned DPC constructs could enhance nerve regeneration by providing a continuous supply of NTFs, to promote axon regeneration, and a guidance cues from an aligned ECM, to direct axon extension towards the end organ. This data demonstrates the potential of aligned DPC sheets to form effective, bioactive nerve conduits to enhance peripheral nerve regeneration.

A Flow Profile Generator for Elucidating Biological Biomechanics

Haley C. Fuller (1), Emily R. Swist (1) and Warren C. Ruder (1)

(1) Department of Bioengineering, University of Pittsburgh

The capillary frictional forces within the cardiovascular system are both essential for nutrient and oxygen delivery, and potentially detrimental for pathologically compromised cellular states. In cases of human disease, the experimental observation and understanding of these forces are stunted by an inability to recapitulate both key mechanics and mechanistic biological phenomena. State-of-the-art microfluidics, in combination with the field of synthetic biology, offer a set of tools that can be exploited for disease perturbation. Here, we present a tool for controlling fluid flow in an organ-on-a-chip system through the characterization and implementation of a bidirectional flow profile generator. Utilizing a programmable microcontroller, a 3D printed rocking platform is designed to adjust the hydrostatic pressure head differential between opposing media reservoirs, generating laminar flow profiles representative of physiological function. The incorporation of open-sourced electronic control modules with customizable 3D-printable components enables a design that is versatile, robust, and adaptable to a myriad of scientific inquisition. In the future, cells living within disease-specific devices can be monitored for phenotypic changes and with real-time fluid flow adjustment using an automated, feedback system. Ultimately, our goal is for this system to provide a path toward automating the discovery of the fundamental biomechanical ruleset governing tissue microenvironments, organ function, and would healing research.

Transforming Growth Factor Beta 2 Release from a Tissue-Engineered Graft in a Rat Model

Kenneth J. Furdella (1), Shinichi Higuchi (2), Kang Kim (1,3,4,5), William Wagner (1,2,3,5) and Jonathan Vande Geest (1,2,5)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Medicine, (4) Department of Cardiology, (5) Vascular Medicine Institute University of Pittsburgh

A functional tissue-engineered vascular graft (TEVG) will require the recruitment of smooth muscle cells (SMCs) to provide vascular tone and the production of extracellular matrix components, collagen for instance. SMC recruitment can be increased through the modification of the substrate or through environmental factors. Specifically, transforming growth factor-beta 2 (TGFβ2) has demonstrated the ability to promote collagen production or smooth muscle cell migration depending on extracellular concentration. Therefore, the goal of this study was to modulate the concentration of TGFβ2 in a compliance match and stiff TEVG to evaluate SMC proliferation and migration in a rat model.

Electrospun TEVGs were manufactured using an IME electrospinning device. The compliance of each graft was varied by modulating the graft's composition and the amount of TGFβ2 (0, 10, and 100 ng/mg). The compliance matched grafts (CM) had a similar compliance to rat aorta and the hypocompliant graft (Hypo) was about twice as stiff. The TGFβ2 concentrations were chosen from past in vitro work. Once manufactured, these grafts were implanted interpositionally into the abdominal aorta of a Sprague Dawley rat (n=3) for each group and TGFβ2 concentration for a total of 18 rats that were sacrificed at 5 days. After implantation, the rats were sacrificed and evaluated for SMC migration and macrophage markers.

Preliminary markers for SMCs and macrophages showed no significant differences between groups, but there were some trends. Specifically, we saw a decrease in SMCs between the CM vs. Hypo at 100 ng/mg suggesting that the CM group had a higher release of TGFβ2 which prevented SMC migration. There was also a decrease in M2 macrophages as the TGFβ2 concentration increased in the CM group. Currently, we are still investigating other markers, longer time points, and different locations in the implant.

Remote Non-Genetic Optical Modulation of Neuronal Activity using Fuzzy Graphene

Raghav Garg (1), Sahil Rastogi (2), Matteo Giuseppe Scopelliti (3), Bernardo I. Pinto (4), Jane E. Hartung (5), Seokhyoung Kim (6), Corban G.E. Murphey (6), Nicholas Johnson (1), Daniel San Roman (1), Francisco Bezanilla (4), James F. Cahoon (6), Michael Gold (5), Maysam Chamanzar (3) and Tzahi Cohen-Karni (1,2)

(1) Department of Materials Science and Engineering, (2) Department of Biomedical Engineering (3) Department of Electrical and Computer Engineering, Carnegie Mellon University, (4) Department of Biochemistry and Molecular Biology, University of Chicago, Chicago, IL, (5) Department of Neurobiology, University of Pittsburgh, (6) Department of Chemistry, University of North Carolina, Chapel Hill, NC

Electrical stimulation of tissue and ultimately individual cells has not only played an essential role in our understanding of the structure and function of excitable tissue but continues to serve as the basis for a variety of therapeutic interventions for the treatment of disorders ranging from cardiac arrhythmias to Parkinson's disease. Optogenetics has enabled selective optical stimulation of cellular activity. However, it requires genetic modification of the target cells. This presents challenges both in clinical translation to humans and regulatory approval. Au and Si-based nanomaterials have shown promise for non-genetic photostimulation of cells. However, these methods still suffer from key limitations: low thermal conversion efficiency, the need for high laser power, and unproven long-term stability. We report a breakthrough hybrid nanomaterial, composed of highly controlled three-dimensional (3D) arrangement of graphene flakes free-standing (fuzzy graphene) on a one-dimensional Si-nanowire (NW), for remote and non-genetic light-induced control of cell activity. The 3D arrangement of free-standing graphene flakes exhibits increased broadband photo-absorption and efficient photothermal energy conversion. We isolate these multidimensional graphene nanostructures for highly controlled photostimulation at subcellular precision with laser energies lower than hundred nanojoules. We demonstrate in-vitro integration of isolated NW-templated 3D fuzzy graphene (NT-3DFG) nanostructures into two-dimensional (2D) and 3D cellular arrangements. Furthermore, NT3DFG based photostimulation does not generate cellular stress. The proposed platform serves a novel powerful toolset for understanding the fundamental science of cell signaling within and between tissues and may enable new therapeutic interventions.

Characterization of Biological and Mechanical Properties of Fibrotic Adipose Tissue to Inform Better Regenerative Outcomes

Mallory D. Griffin (1), Megan K. DeBari (2) and Rosalyn D. Abbott (1,2)

(1) Department of Biomedical Engineering, (2) Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh PA

Adipose tissue defects result from traumatic wounds, congenital defects, and tumor resection, resulting in extensive disfigurement and dysfunction. Current clinical treatment for these wounds is autologous fat grafting. However, this technique relies on stem cells, endothelial cells, etc. in the stromal vascular fraction to facilitate angiogenesis and anti-inflammatory effects. Adipose tissue fibrosis is negatively correlated with stromal vascular fraction and stem cell concentration. Moreover, stem cells in fibrotic adipose tissue are more likely to differentiate to profibrotic cells over adipocytes. This results in an increase in ECM production, altering the mechanical and metabolic properties of the tissue. Thus, we hypothesize that adipose tissue fibrosis inhibits adipose tissue regeneration. The goal of this project is to correlate the material properties of adipose tissue with regenerative potential. Material properties of primary human adipose tissue were analyzed for mechanical properties (compressive strength and modulus of elasticity) and collagen content. Regenerative potential was analyzed by quantifying stem cell proliferation, metabolic activity, triglyceride content, gene expression and cell morphology.

Macrophage phenotype and function is dependent on both the composition and stiffness of the tissue microenvironment

Martin Haschak (1,2), Siddhartha Dash (3), Branimir Popovich (1) and Bryan Brown (1,2,3,5)

(1) Department of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Department of Biological Sciences, (4) Department of Obstetrics, Gynecology, and Reproductive Sciences, (5) Clinical and Translational Science Institute, University of Pittsburgh

In the cardiovascular system, tissue stiffness tends to increase with increasing age or pathophysiology development due to reactive fibrosis characterized by deposition and crosslinking of fibrillar collagen subtypes, degradation of elastin, disorganized matrix protein organization, and reactive hypertrophy of cardiomyocytes. While much work has been done to characterize the deleterious impacts of cardiac stiffening on cardiac function, the effects of microenvironmental mechanical changes on the numerous cell types present within the heart remain to be fully elucidated. Recent work has identified a previously unknown heterogeneity in cardiac tissue resident macrophages. In addition to unique cellular function, these macrophage subsets have been shown to undergo age-related changes in population size, with the aging process promoting an increase in pro-inflammatory macrophage subsets. However, the mechanisms governing this differential regulation are unknown. Thus, we sought to understand how age-specific, microenvironmental mechanical alterations between young and aged cardiac tissue differentially impact macrophage morphology, polarization, and functionality. To accomplish this, young (1-2mo) and aged (21-22mo) murine cardiac extracellular (cECM) matrix was isolated, decellularized, digested, and coated onto functionalized poly-dimethyl-siloxane hydrogels ranging in stiffness from 8kPA to 64kPA, encompassing reported ranges of young and aged cardiac tissue stiffness. Bone marrow-derived macrophages isolated from young (2mo) mice were then seeded onto the young and aged cECM-coated hydrogels and treated with growth media (M0), media supplemented with Th-1 cytokine IFN-g and LPS (M1) or media supplemented with Th-2 cytokine IL-4 (M2). Gel stiffness was found to have a substantial impact on cell morphology, with cells seeded onto softer gels exhibiting rounder morphologies with few to no filopodia. Conversely, cells seeded onto stiffer gels were observed to have spread morphologies often with several filipodia extensions. Gel stiffness was also found to have an impact on both pro- and anti-inflammatory macrophage function. Stiffer gels were found to promote enhanced secretion of radical oxidant nitric oxide. Macrophage culture on softer gels attenuated nitric oxide production and promoted enhanced arginase activity, a functional response associated with alternatively activated macrophage subsets. Finally, we observed an effect of matrix-coating age on macrophage function, with gels of equivalent stiffness coated with aged cECM promoting greater production of nitric oxide and reduced arginase activity as compared to young cECM coated gels, suggesting that macrophage polarization and functionality is a function of both tissue mechanical properties as well as age-related compositional alterations.

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

Chunrong He (1), Zhong Li (1), Hang Lin (1) and Peter G Alexander (1)

(1) Center for Cellular and Molecular Engineering, Department of Orthopaedic Surgery, University of Pittsburgh

Objective: Traumatic impacts on the articular joint surface in vitro 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 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 modelling. To overcome this insufficiency, we are developing hydrogel-based models using adult human cells to examine the effects of mechanical loading on human cell chondrogenesis.

Hypothesis: We hypothesize that cells encapsulated within biomimetic scaffolds will respond to mechanical loading in a manner congruent with early PTOA pathogenesis in animal models.

Study Design: In this study we first develop a system to traumatically load hydrogels, defined by strain rate and magnitude. We then encapsulate adult human mesenchymal stromal cells (hMSCs) in a photocrosslinkable, biomimetic hydrogel, chondrogenically differentiate them and subject them to traumatic impacts. Thereafter, we assess cell viability, metabolism, gene expression and stress response in comparison to low strain and unloaded conditions. Results will be compared to outcomes observed in explanted adult bovine articular cartilage.

Results: We demonstrate that traumatic impacts reduce cell viability in the constructs, increase cell stress markers prostaglandin-E type 2 and nitric oxide, and alter gene expression in a manner characteristic of PTOA: decreases in Collagen type II and Aggrecan and concomitant increases in MMP13 and Aggrecanase.

Conclusions: A single supraphysiologic impact negatively affects hMSC-based cartilage constructs in a manner similar to early post-traumatic changes in native tissues.

Future work: The traumatized cartilage model will be incorporated within an in vitro microJoint system to observe its effects on other engineered synovial tissues.

High throughput human pluripotent stem cell spheroid generation using 3D printed micorpillars

Ravikumar K. (1), Connor Wiegand (1), Kevin Pietz (2) and Ipsita Banerjee (1,2,3)

(1) Department of Chemical and Petroleum Engineering, University of Pittsburgh, (2) Department of Bioengineering, University of Pittsburgh, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh

Organoids are becoming a promising alternative to animal models because of the ability of some cells in 3D culture systems to self-organize into structures in vitro that closely resemble in vivo systems. This makes 3D culturing of organoids an important tool to study disease models and therapeutics more accurately than animal models. Since the concept of organoids and 3D culture in general has become more popular, high throughput generation of human pluripotent stem cell (hPSC) organoids with control on size and shape parameters has become an important aspect. One of efficient ways to produce large number of spheroids is by using the commercial 'v' bottomed plates and AggreWell plates. However, these platforms do not have room for modification of certain properties of the substrate (such as stiffness) on which aggregation takes place. In order to exercise a better control on the substrate properties, the current study describes the use of 3D printed micropillars as molds to fabricate agarose microwells and facilitate high throughput generation of hPSC spheroids with a well-defined size distribution. While agarose hydrogels have tunable mechanical properties and are known to support aggregation, they are bioinert, implying that they cannot efficiently facilitate maturation of aggregates formed in the microwells. This has been remedied in a recent study by our group which conclusively shows that amikacin-based hydrogels with a range of mechanical properties and essential biophysical cues support not only aggregation and co-aggregation of multiple cell types but also the differentiation of the aggregates. In this backdrop, this study shows the efficacy of using amikacin-based hydrogel microwells for high throughput aggregation and differentiation of hPSCs. The study also examines the potential of these microwells for use in hPSC-on-chip experiments to load single cells onto a microfluidic chip in order to drive in situ aggregation and differentiation under perfusion which better mimic the biophysical conditions in the human body.

Organ-on-e-chip: three-dimensional self-rolled biosensor array for electrical interrogations of electrogenic spheroids

Anna Kalmykov (1), Changjin Huang (2,3), Jacqueline Bliley (1), Daniel Shiwarski (1), Joshua Tashman (1), Arif Abdullah (4), Sahil Rastogi (1), Shivani Shukla (1,5), Elnatan Mataev (1), Adam W. Feinberg (1,5), K Jimmy Hsia (2,3) and Tzahi Cohen-Karni (1,5)

(1) Department of Biomedical Engineering, Carnegie Mellon University, (2) School of Mechanical & Aerospace Engineering, Nanyang Technological University, Singapore, (3) School of Chemical and Biomedical Engineering, Nanyang Technological University, Singapore, (4) Department of Mechanical Science and Engineering, University of Illinois at Urbana-Champaign, Urbana-Champaign, Illinois, (5) Department of Materials Science and Engineering, Carnegie Mellon University

Cell-cell communication plays a pivotal role in coordination and function of biological systems. Traditionally studies of cellular communication are performed in two-dimensional (2D) cell cultures, due to technological limitations. However, 2D cellular topology does not recapitulate the native cellular morphology, proliferation rates, migration, gene expression, differentiation, signaling, physiological function, and electrophysiological properties of both neurons and cardiomyocytes. Three-dimensional (3D) spheroids provide venues to explore cellular communication for tissue development and drug discovery, as their 3D architecture mimics native in vivo microenvironments. Cellular electrophysiology is a prevalent signaling paradigm for studying electroactive cells. In this talk, we will present a breakthrough bioelectrical interface, a 3D self-rolled biosensor arrays (3D-SR-BAs) of either active field-effect transistors or passive microelectrodes to measure electrophysiology of both cardiac and neural spheroids in 3D. The arrays provided continuous and stable multiplexed recordings of field potentials with high sensitivity and spatio-temporal resolution, supported with simultaneous calcium imaging. Our approach enables electrophysiological investigation and monitoring of the complex signal transduction in 3D cellular assemblies toward an organ-on-an-electronic-chip (organ-on-e-chip) platform for tissue maturation investigations and development of drugs for disease treatment.

Matrix-bound Nanovesicles as a Source of Lysyl Oxidase

Yoojin Lee (1,2), Jordan Birkhimer (2), George Hussey (1,3) and Stephen F. Badylak (1-3)

(1) McGowan Institute for Regenerative Medicine, (2) Department of Bioengineering, (3) Department of Surgery, University of Pittsburgh

Biologic scaffolds composed of extracellular matrix (ECM) have been extensively used as in situ implants to reinforce tissue strength and promote a variety of biologic effects such as angiogenesis, resident stem cell mobilization, proliferation and differentiation, and immunomodulation. Numerous studies have shown effective ECM-mediated repair and reconstruction of a variety of tissue types, including skeletal muscle, esophagus, dura mater, rotator cuff, and tendon, among others. Recently described matrix-bound nanovesicles (MBV), a component of ECM bioscaffolds, possess biologically active signaling molecules that can be isolated from the fibrillar network of the ECM. We have previously shown the influence of isolated MBV on cell behavior and macrophage phenotype. MBV effects are likely the result of not only the vesicle's internal cargo, but also a myriad of surface proteins. Herein, we show that lysyl oxidase (LOX), a protein responsible for collagen cross-linking, is one of the surface proteins associated with the outer membrane of MBV. Furthermore, we show that MBV-associated LOX is present in its enzymatically active 52 kDa pro-peptide form and that it retains its ability to promote collagen assembly. Specifically, in vitro studies showed that MBV-associated LOX enhanced the formation of cross-linked collagen in tendon constructs when compared to negative controls. MBV-associated LOX provides an attractive potential therapeutic alternative to recombinant LOX given that the latter requires extensive isolation and purification. MBV provide a feasible way to isolate enzymatically active LOX that can be utilized as a potential therapy for repairing and strengthening damaged tissues.

A Multi-tissue Chip for the Modeling of Osteoarthritis Pain

Zhong Li (1), Zixuan Lin (1), Monica Romero-Lopez (2), Benjamen O'Donnell (3), Peter G. Alexander (1,4), Stuart B. Goodman (2), Bruce A. Bunnell (3), Michael S. Gold (5), Hang Lin (1,4) and Rocky S. Tuan (1,4)

(1) Center for Cellular and Molecular Engineering, Department of Orthopedic Surgery, University of Pittsburgh, (2) Department of Orthopedic Surgery, Stanford University, (3) Center for Stem Cell Research and Regenerative Medicine, Tulane University, (4) McGowan Institute for Regenerative Medicine, (5) Department of Neuroscience, University of Pittsburgh

Pain is the most prominent symptom of Osteoarthritis (OA), and pain caused by lower extremity OA is the leading reason for impaired mobility in older adults. Therefore, pain relief remains the primary focus of current treatments. Unfortunately, there are no consistent, effective approaches to retard or reverse disease progression or treat OA pain. Thus, there exists a critical need for understanding the mechanisms of OA pain in human and screening/developing more efficacious pain relievers. Herein we propose the development of a novel three dimensional (3D), human cell-based, multi-tissue chip that contains major joint elements and a neural component. We first established a human stem cell-based microphysiological joint (microJoint) chip. The 3D hydrogel-based osteochondral complex, synovium, and adipose tissue were integrated into a 3D printed bioreactor to enable their crosstalk. Subsequently, we generated “OA” microJoints by challenging synovium with the pro-inflammatory cytokine interleukin (IL)-1β. To generate the pain-enabled microJoint (Neu-microJoint) chip, a new chamber was 3D printed and incorporated into the microJoint chip, in which human sensory neurons were cultured. Neural activities in Neu-microJoint chips under normal and “OA” conditions were assessed with electrophysiology and imaging. We have also introduced potential pain management drugs in the Neu-microJoint chip and examined their effects on pain level and tissue health. The real-time PCR, histology and immunohistochemistry results confirmed the phenotypes of normal and “OA” Neu-microJoint. Under the OA-mimicking conditions, the exposure of neurons to “synovial” eluate markedly changed their excitability, suggesting the generation of pain-associated changes in the tissue chip. Interestingly, we also found that the introduction of the anti-inflammatory cytokine IL-4 may alleviate cartilage degeneration in the Neu-microJoint. The pain-enabled microphysiological joint chip developed in this study promises to serve as a robust system to study the mechanisms of OA pain and develop effective pharmaceutic interventions.

How Adherent Cells Reorient in Response to Cyclic Stretching

Jui-Chien Lien (1) and Yu-Li Wang (1)

(1) Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA

Cells often respond to non-cyclic mechanical cues along the direction of stimulation; adherent cells often reorient perpendicularly to the direction of uniaxial cyclic stretching. While this phenomenon has been documented for decades, the underlying control mechanism remains poorly understood. To address this question, we used a simple on-stage stretching approach that allows programmable stretching-relaxation and synchronized imaging of cells cultured on polyacrylamide substrates. We observed that the reorientation of NRK epithelial cells involves rapid shortening along the direction of stretching (referred to as longitudinal) in the early stage and delayed but steady extension perpendicularly to the direction stretching (referred to as lateral). By analyzing differences of images collected at the beginning and end of stretching or relaxation phases, we demonstrated that cell retraction predominantly occurs during the stretching phase, while protrusion primarily takes place during the relaxation phase. By increasing the stretching cycles and measuring the protrusions change in two directions, we showed that, in relaxation phase, longitudinal protrusions continuously decreased, which cause a progressive decrease in longitudinal length. On the other hand, lateral protrusions were consistently maintained. Additionally, cells performed a similar extent of retractions in both directions. Together, these results suggest that stretching-induced retraction may represent a fast, direct response to stretching, whereas protrusion may represent a slow, persistent feedback response to stretching-induced retraction. In conclusion, the net effect of reorientation is determined by the interplay of longitudinal shortening and lateral extension, in conjunction with cell retractions in stretching phase and differential dynamics of cell extensions in relaxation phase.

Novel Model of Bile Duct Paucity Demonstrates the Critical Role of Yap1 in Biliary Morphogenesis in Development and Regeneration

Laura Molina (1,2), Alan Watson (3,4), Qin Li (5), Tirthadipa Pradhan-Sundd (6,7), Minakshi Poddar (2), Sucha Singh (2), Drew Feranchak (5,7), Simon Watkins (3,4), Kari Nejak-Bowen (2,7) and Satdarshan P. Monga (2,7,8)

(1) Medical Scientist Training Program, School of Medicine, (2) Division of Experimental Pathology, Department of Pathology, (3) Center for Biologic Imaging, (4) Department of Cell Biology, (5) Division of Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, (6) Division of Hematology/Oncology, Department of Medicine, (7) Pittsburgh Liver Research Center, (8) Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Pittsburgh, and University of Pittsburgh Medical Center

Background: Yes-associated protein 1 (Yap1) is a critical regulator of liver embryonic development, regeneration and tumorigenesis. Previous studies have shown that Yap1 regulates bile duct formation and hepatocyte transdifferentiation to cholangiocytes, but the mechanisms by which Yap1 regulates biliary morphogenesis as well as the functions of Yap1 in early embryonic liver development remain unknown.

Methods: To elucidate the role of Yap1 in early liver development, we developed a novel mouse model (Yap1 KO) in which Yap1 is deleted from the foregut endoderm progenitors by E12.5 using the Foxa3 promoter to drive Cre recombinase expression. Here, we comprehensively characterize the Yap1 KO mice through histology, whole organ tissue clearing and confocal microscopy and additional biochemical and functional techniques.

Results: Yap1 KO mice lack Yap1 in the liver from early development. Strong nuclear expression of Yap1 that was seen in hepatoblasts in WT livers at E14.5 was clearly absent in the KO mice. Although Yap1 KO mice were viable and have fully formed liver and digestive organs, they exhibited jaundice, and stunted growth. Histological analysis identified a failure of intrahepatic bile duct formation. Yap1 deletion did not block Notch activation as evidenced by the appearance of Hes1-positive and Sox9-positive ductal plate cells in both WT and KO mice. Rather, Yap1 deletion prevented the morphological assembly of a two-cell layer with a lumen from an asymmetrical single-layered ductal plate. Surprisingly, KO mice survive long-term with normalization of serum liver enzymes despite persistent cholestatic injury, suggesting hepatocyte adaptation to injury. While a recent study of Notch-deficient livers (a model of Alagille syndrome) described complete de novo hepatocyte-derived generation of bile ducts by 4 months of age, Yap1-deficient livers fail to regenerate bile ducts by 8 months of age. Liver tissue clearing and 3D whole liver immunofluorescence imaging showed severely abbreviated biliary tree in KO mice relative to WT. Notably, we observe the appearance of a ductular reaction in the hilar region of the liver consisting of Yap1-positive ducts surrounded by fibrosis and inflammation. Although all hepatocytes remain Yap1-negative, the extrahepatic biliary tree remains unaffected by the Cre and is Yap1-positive, suggesting a potential source of Yap1-positive ductular cells near the hilum of the liver.

Conclusions: Our results demonstrate that Yap1 is dispensable for early development of the liver and foregut but is critical for biliary morphogenesis. We further show absence of hepatocyte-to-cholangiocyte transdifferentiation in Yap1 KO. Thus, Yap1 is essential for biliary morphogenesis regardless of the cellular origin, whether from hepatoblasts in development or from hepatocytes in liver regeneration.

Small Molecule-driven Simultaneous Cardiopulmonary Co-Differentiation from hPSCs

Wai Hoe Ng (1), Elizabeth Johnston (1), Jun Jie Tan (2) and Xi Ren (1)

(1) Biomedical Engineering Department, Carnegie Mellon University, Pittsburgh, PA, (2) Advanced Medical and Dental Institute, Universiti Sains Malaysia, Penang, Malaysia

Introduction. The mesoderm-derived heart and endoderm-derived lung are intricately connected during human fetal development. However, investigation of this critical mutual interaction during embryogenesis has been challenging due to limited availability of proper in vivo and in vitro models. To bridge this gap, we developed a novel mesodermal-endodermal co-differentiation platform to enable simultaneous induction of both the cardiac and pulmonary lineages within a single differentiation of human pluripotent stem cells (hPSCs).

Methods. A stepwise differentiation strategy was designed and optimized for sequential induction of (1) mesoderm & definitive endoderm, then (2) cardiac mesoderm & anterior foregut endoderm, and finally (3) cardiac & pulmonary progenitors. Subsequently, 3D suspension culture and air-liquid interface culture was performed for cardio-pulmonary tissue maturation.

Results. Fine-tuned activation of Wnt, BMP and TFG-ß signaling enabled balanced induction of mesoderm (Brachyury+) and definitive endoderm (FoxA2+Sox17+). Subsequently, inhibition of Wnt signaling promoted both cardiac mesoderm (NKX2.5+) and anterior foregut (Sox2+) induction, and suppressed the hindgut fate. Further, re-activation of Wnt signaling promoted cardiac expansion and lung progenitor (NKX2.1+) specification. Following 3D culture, the cardio-pulmonary lineages matured into contractile cardiac tissues and surfactant-producing alveolar epithelium.

Conclusion. Our study demonstrated the striking convergence in signaling required for cardiac and pulmonary induction, echoing their intricate connection during human fetal development. Our simultaneous multilineage co-differentiation platform provides an experimentally tractable system for investigating heart-lung crosstalk during embryogenesis and development of congenital cardio-pulmonary dysfunctions.

Development of an iPSC-Islet-on-a-Chip with Microfluidics Device

Kevin Pietz (1), Connor Wiegand (2), Ravikumar K (2) and Ipsita Banerjee (1,2,3)

(1) Department of Bioengineering, (2) Department of Chemical Engineering, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh

The Islets of Langerhans are clusters of cells housed within the pancreas that are responsible for blood glucose homeostasis, in part by insulin secretion. Diabetes results from the dysfunction of these islets and loss in ability to secrete insulin. Current research on this disease is restricted due to limited islet availability and a short window for in vitro culture. Generating islet-mimetic organoids derived from induced Pluripotent Stem Cells (iPSCs) increases access to islets and broadens the pool of experiments that are possible. In this project, we incorporate iPSC-islets into a microfluidics device for an islet-on-a-chip model that remains viable and functional for weeks of in vitro culture.

Our differentiation protocol utilizes both chemical and biophysical cues from growth factors and alginate encapsulation, respectively, to convert iPSCs into glucose-sensitive insulin-producing cells. This 3D environment created by the alginate provides improved differentiation over traditional 3D and 2D culture. Upon maturation, we expose the iPSC-islets to dynamic perfusion to recreate the interstitial flow experienced by the islets in vivo. To accomplish this, we have set up a perfusion culture in a two-chamber microfluidics device. In this system, we see high viability for over two weeks, comparable to that of traditional static cultures. ELISA's show our iPSC-islets are responsive to glucose while cultured in the device, suggesting improved functionality over static culture. In addition, we are developing a COMSOL Multiphysics fluid flow simulation to characterize flow conditions within the chamber, such as determining shear stress and molecular diffusion rates. Based off of this, we are developing techniques to 3D print the islets precisely within the device to reproduce simulated flow conditions. Our iPSC-islet on chip provides an accurate islet model that remains viable and functional for extended in vitro culture, providing a platform for future diabetes research to be carried out on.

Controlled Fabrication of Extracellular Matrix Threads for Fiber-reinforced Bioprinting

Wenhuan Sun (1), Victoria Webster-Wood (1,2,3) and Adam Feinberg (2,4)

(1) Department of Mechanical Engineering, Carnegie Mellon University, (2) Department of Biomedical Engineering, Carnegie Mellon University, (3) McGowan Institute for Regenerative Medicine, University of Pittsburgh, (4) Department of Materials Science & Engineering, Carnegie Mellon University, Pittsburgh, PA

Electrochemically aligned collagen (ELAC) threads fabricated by the isoelectric focusing of dialyzed collagen exhibit robust mechanical properties as well as the potential to induce tenogenic differentiation. However, the effects of isoelectric focusing parameters on the mechanical and geometric properties of resulting threads have not been well characterized. In this study, the single-factor effects of collagen solution temperature, collagen concentration, isoelectric focusing duration, and isoelectric focusing voltage on the tensile strength, tensile modulus, failure strain and cross-sectional area of ELAC threads were investigated. Tuning fabrication parameters enables production of high strength threads suitable for suspended printing in gelatin support baths. The four isoelectric focusing parameters studied showed statistically significant influence on the properties of ELAC threads. Longer focusing duration (120 s) increased tensile strength, failure strain, and cross-sectional area. Higher collagen concentration increased cross-sectional area but had no significant effect on mechanical properties. Higher focusing voltage (20 V) significantly elevated cross-sectional area and tensile strength, and significant differences in tensile modulus were observed between 10 V and 15 V groups. Collagen solution temperature had significant effects on all properties except failure strain. ELAC threads made here were amongst the strongest collagen-based threads previously reported. By tuning fabrication parameters, while using the same crosslinking treatment, the tensile modulus could be varied across a range from 242 ± 86 MPa to 475 ± 241 MPa and the tensile strength from 20 ± 7.2 MPa to 68 ± 6.1 MPa. Differences in ELAC mechanical properties may be attributable to variations in packing density of collagen molecules induced by different isoelectric focusing parameters, as preliminary studies using scanning electron microscopy revealed differences in cross-sectional porosity between ELAC made with short (30 s) and long (120 s) duration isoelectric focusing. The findings in the study reported here enabled the production of ELAC threads with tunable and application-specific properties, which may be used for fiber-reinforced bioprinting.

A Novel Tissue Organ Culture System Supported Monkey and Mouse Spermatogenesis

Kien Tran (1), Wenbo Li (2), Yi Sheng (1), Sarah Steimer (1), Kwon Sung Cho (2) and Kyle E. Orwig (1)

(1) Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, (2) Department of Mechanical Engineering and Materials Science, University of Pittsburgh

Spermatogonial stem cells (SSCs) have a tremendous capacity to regenerate spermatogenesis under proper conditions. Testicular tissue organ culture (TTOC), utilizing the potency of SSCs to differentiate them into sperm outside of the body, can become a potential therapy to restore fertility in the future for pre-pubertal male patients, who encounter a permanent infertility risk as a side effect of gonadotoxic treatments. In previous literature, mouse sperm produced in vitro using the TTOC technology could derive to healthy offspring. This study aims to translate this technology to non-human primate and human pre-pubertal testicular tissues. We have invented a novel PDMS-roof transwell (PRT) culture system, comprised of a polycarbonate-membrane-transwell and a polydimethylsiloxane roof that could support the development of fresh and cryopreserved immature testicular tissues. After 1 month culturing fresh neonatal mouse testes in the PRT system, more than 70% tubules showed active spermatogenesis. Our immunofluorescence analysis confirmed VASA-positive germ cells in 81.6±6.63% of tubules, SALL4-positive undifferentiated spermatogonia in 70.1±10.21%, STRA8-positive differentiating spermatogonia in 50.0±14.01%, SYCP3-positive spermatocytes in 75.9±9.2%, and SOX9-positive Sertoli cells in 99.08±0.9% of tubules. A similar experiment using cryopreserved neonatal mouse testes is underway. The PRT system was also tested on fresh and cryopreserved pre-pubertal Rhesus macaque testicular tissues, from which we observed not only the survived VASA-positive germ cells and SOX9-positive Sertoli cells, but the culture system also successfully initiated meiosis in undifferentiated spermatogonia to produce spermatocytes. The goal of this study is to develop a simplified, efficient culture system to induce in vitro spermatogenesis from fresh and cryopreserved immature testicular tissues, and translate this technology from mouse to monkeys to humans to test safety and feasibility for future clinical applications.

Characterization of cardiac matrix-bound nanovesicles in failing and ischemic heart tissue identifies protein targets for corrective tissue engineering

Madeline Cramer (4), Tengfang Li (1,2), Joseph Bartolacci (4), George Hussey (4), Lisa Mathews (1,2), John Sembrat (6), Mauricio Rojas (6), Charlie McTiernan (6), Stephen F. Badylak (4) and Heth R. Turnquist (1,2,3)

(1) Department of Surgery, (2) Thomas E. Starzl Transplantation Institute, (3) Department of Immunology, (4) McGowan Institute for Regenerative Medicine, (5) Department of Bioengineering, (6) Department of Medicine, University of Pittsburgh School of Medicine, University of Pittsburgh Medical Center

Precisely regulated remodeling of the extracellular matrix (ECM) is critical for effective wound healing and the maintenance of organ homeostasis. We have recently established that the ECM isolated from healthy tissues, including cardiac, contains small vesicles, or matrix-bound nanovesicles (MBVs). MBV contain functional miRNA and proteins that are able to modulate the function of both immune and stromal cells. It is well appreciated that the ECM remodeling is extensive during heart disease and pathological ECM remodeling contributes to heart stiffness leading to heart failure. It is unknown, however, if MBV concentration or content is modified during the development of heart disease. In this HLBVMI enabled Human Cardiac Tissue Bank bio-specimen study (IRB# 0404033), we compared de-identified human left ventricle (LV) samples from: 1) non-ischemic failing hearts (n=4) and non-failing and non-ischemic hearts (n=4). The tissue samples were digested using enzymes and detergents to dislodge and isolate nanovesicles contained in the extracellular matrix (ECM) of tissues, and protein content was assessed using Proteome Profiler Human XL Cytokine Array. Arrays were quantitated using ImageJ and the data normalized and examined using Partek. Failing and non-failing heart tissue had similar concentrations of MBV, however, their cargo differed. MBV from failing tissue had increased levels of Apolipoprotein A-1 (ApoE) and decreased levels of adiponectin (APN). The protein hormone APN has been shown to limit profibrotic ECM by regulating matrix metalloproteinase expression in cardiac and immune cells. Our data suggest that disease progression may be perpetuated when cardiac MBV become skewed towards those unable to limit fibrosis. We will next use of MBV isolated from the cardiac tissue of homeostatic wild type or transgenic mice lacking APN to back-translate the impact of this transition of MBV cargo on heart disease.

Generation of computationally guided, genetically supervised human liver organoids

Jeremy Velazquez (1,2)*, Ryan LeGraw (1,2)*, Farzaneh Moghadam (3), Jacquelyn Kilbourne (3), Christopher Paisier (4), Silvia Liu (1,2), Patrick Cahan (5,6,7), Samira Kiani (1,8) and Mo Ebrahimkhani (1,2,8); * Equal contribution

(1) Division of Experimental Pathology, Department of Pathology, University of Pittsburgh School of Medicine, (2) Pittsburgh Liver Research Center, (3) Biodesign Institute, Arizona State University, Tempe, AZ, (4) School of Biological and Health Systems Engineering, Arizona State University, Tempe, AZ, (5) Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, (6) Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, (7) Department of Molecular Biology and Genetics, Johns Hopkins University School of Medicine, Baltimore, MD, (8) McGowan Institute for Regenerative Medicine, University of Pittsburgh School of Medicine

Introduction: Human organoid technologies show great promise as novel tools for understanding human development and disease with patient specificity. However, they fall short of recapitulating primary tissues in gene expression, vascularity, functionality, and multicellular composition that would poise them to be ideal ex vivo human models.

Hypothesis: By combining approaches from synthetic, computational, and stem cell biology, we show that our multicellular, self-vascularizing, human induced pluripotent stem cell (hiPSC) derived Designer Liver Organoids (DesLO) make significant headway on these challenges.

Methods and Results: DesLO were generated from a hiPSC line with incorporation of a doxycycline (dox)-inducible GATA6 expression vector. After induction of GATA6 for 5 days, we observed consistent differentiation of endothelial cells, stellate-like cells, and hepatocyte-like cells in culture on basal medium (APEL™). We analyzed DesLO with the CellNet algorithm, which evaluates sample tissue classification and gene regulatory network alignment with primary tissue libraries. By expressing transcription factors AFT5 and PROX1 identified by CellNet, and CRISPR activation (CRISPRa) mediated upregulation of the cytochrome P450 3A4 (CYP3A4) enzyme after GATA6 induction, we observed significant increases in liver functionality, vascular network stability, bile acid synthesis regulation, and ability to express fibrotic phenotype coupled with therapeutic prevention. We used single cell RNAseq to confirm the presence the endothelial, hepatic-like, and stellate-like populations in DesLO, and further showed increase in hepatic gene expression in the hepatic compartment. Finally, we showed the ability of DesLO to engraft and produce human proteins in an in vivo immune-compromised mouse transplantation model, and prolong survival upon chronic liver injury.

Discussion: This novel, multidisciplinary approach shows significant improvement of liver organoid modeling and functional capabilities and opens the doors for development of more engineerable, patient-specific modeling of liver development and disease using renewable cell sources (hiPSC) without the need for expensive, arduous, and variable media coaxing steps.

Toward high-resolution three-dimensional reconstruction of optic nerve head vasculature via optical clearing

Susannah Waxman (1), Ralitsa Loewen (2), Nils Loewen (2) and Ian Sigal (1,3,4)

(1) Department of Ophthalmology, University of Pittsburgh School of Medicine, (2) Department of Ophthalmology, University of Würzburg, Würzburg, Germany, (3) Department of Bioengineering, Swanson School of Engineering, University of Pittsburgh, (4) McGowan Institute for Regenerative Medicine, University of Pittsburgh School of Medicine and University of Pittsburgh

Purpose: To develop a method to visualize the vascular network of the intact optic nerve head in fixed samples.

Introduction: Glaucoma is a progressive optic neuropathy and a leading cause of irreversible blindness worldwide. Mechanical distortion and dysregulation of the vasculature in the optic nerve head region have been heavily implicated in pathogenesis. Existing methods for vessel visualization have insufficient depth penetration (confocal microscopy or optical coherence tomography) or may suffer distortion (serial histology). Optical clearing of intact tissue may provide novel benefits for visualization.

Methods: Paraformaldehyde-fixed porcine optic nerve heads were bleached in 3%, 10%, or 15% H202 for 48 hours and washed in PBS for 5 days, exchanging PBS daily. Tissue was blocked and permeabilized with 10% goat serum and 0.2% Triton X-100 for 3 days. DyLight 649-conjugated tomato lectin (1:50) was incubated with the tissue for 7 days. Samples were washed, dehydrated via an ascending ethanol gradient, and optically cleared via benzyl alcohol benzyl benzoate (BABB) for imaging via confocal microscopy. Control samples received no lectin label.

Results: Preliminary results demonstrated the highest degree of melanin bleaching via 15% H202. Samples bleached in this manner retained epitopes for the tomato lectin vascular label. Samples required a full 7 days of ethanol dehydration for adequate optical clearance via BABB. Confocal microscopy revealed complete penetration of the lectin, which labelled the vasculature and putative microglia.

Conclusions: We were able to generate high-resolution three-dimensional reconstructions of optic nerve head vasculature in fixed samples via optical clearing. These results open doors for a variety of flexible and highly informative experiments. Better understanding of the effects of intraocular pressure, pathology and therapeutics on the vascular network that supplies the nerve fibers in the back of the eye will help prevent and treat disease

Forming a Vascular Network in iPSC-derived Islet-mimetic Organoids

Connor Wiegand (1), Ravi Krishnamurthy (1), Kevin Pietz (2), Joseph Candiello (2), Prashant N. Kumta (1,2,3,4,5), Jay Hoying (6) and Ipsita Banerjee (1,2,5)

(1) Department of Chemical and Petroleum Engineering, University of Pittsburgh, (2) Department of Bioengineering, University of Pittsburgh, (3) Department of Mechanical Engineering and Material Science, University of Pittsburgh, (4) Center for Complex Engineered Multifunctional Materials, University of Pittsburgh, (5) McGowan Institute for Regenerative Medicine, University of Pittsburgh, (6) Advanced Solutions Life Sciences, Manchester, NH

Islets are vital for the regulation of blood glucose levels through the function of their glucagon secreting alpha cells and insulin secreting beta cells. Diabetes develops when these beta cells fail to function properly. Islet transplantation has shown to be a long-term solution for treating diabetes but is limited by a lack of viable donors. Induced pluripotent stem cells (iPSC) derived islet organoids are a promising alternative. The aim of this project is to form an islet-mimetic organoid that maintains islet functionality with glucagon and insulin responsiveness with the inclusion of an intra-islet vascular network. Since beta cells reside near vasculature in vivo, we hypothesize that the resulting organoid will replicate the structure and functionality of primary human islets.

A critical step for islet organoid engineering is the controlled aggregation of the varying cell types into a 3-D spheroid morphology. Our lab has developed methods for controlling the co-aggregation of iPSC -derived islet endocrine cells and endothelial cells. Additionally, we have had promising results in forming an intra-islet vascular network in the iPSC-derived cells by aggregating iPSC-derived islet endocrine cells, adipose-derived microvascular fragments, and stromal cells. The resulting gene expression of maturing pancreatic beta cell markers (NKX, PDX1, and Insulin), an islet specific endothelial gene (APl), and an endothelial diaphragm fenestration indicator (PLVAP) increased compared to homotypic aggregates of iPSC-derived islets and the initial microvascular fragments. With enhanced pancreatic phenotype and vascular network, we sought to improve angiogenesis through the incorporation of the organoids in a microfluidic device. By configuring the system to flow across the organoids, while supporting the spherical structure, the growth of the vessel network was improved. With these findings, the formed vascularized organoids are highly applicable as a regenerative therapy for diabetes and for the implementation into a microphysiological system for disease modeling.

Anchor-Dependent DNA Origami Accessibility to Cell Surface as a Functional Measure of Glycocalyx Integrity in Vascular Diseases and Regeneration

Piyumi Wijesekara (1), Ying Liu (2), Rebecca Taylor (1,2) and Xi (Charlie) Ren (1,2)

(1) Department of Biomedical Engineering, Carnegie Mellon University, (2) Department of Mechanical Engineering, Carnegie Mellon University

The endothelial glycocalyx is a proteoglycan-rich protective coating on the luminal surface of blood vessels. The glycocalyx serves as an essential barrier that prevents aberrant contact of macromolecules and blood cells to the endothelium, thus its degradation leads to compromised vascular homeostasis and barrier function, as is seen in diabetes, sepsis, and ischemia/reperfusion injury. Despite the importance, conventional ways for glycocalyx characterization have largely been morphological rather than functional. Here, we report a novel method of using DNA origami nano tiles as a functional measure of cell surface glycocalyx integrity. DNA nano tiles were targeted to the cell surface by hybridization with single-stranded DNA (ssDNA) initiators anchored onto the phospholipid bilayer via cholesterol or onto the glycocalyx via biorthogonal glycan labeling. We demonstrated that the accessibility of DNA nano tiles to cell surface initiators is regulated by anchor location and glycocalyx thickness. DNA nano tiles effectively bound to glycocalyx-anchored initiators regardless of spacing in-between. While their access to phospholipid bilayer-anchored initiators can only be permitted by extended nano tile-to-initiator spacing using DNA duplex, or by enzymatic degradation of the glycocalyx. Thus, the DNA nano tiles but not DNA duplex can be effectively expelled by physiologic glycocalyx on the endothelial surface, and serve as an effective functional readout of glycocalyx integrity. We expect that our findings will open the door to developing DNA nanostructure-based tools for assessing glycocalyx function in vascular diseases and during vascular regeneration.