Poster Session
Tuesday, March 9, 2021
4:15 – 5:15 pm

https://pitt.zoom.us/j/99350476551
Meeting ID: 993 5047 6551 • Passcode: 375075

Cellular and Gene Therapy

1. **Dasia Aldarondo** and Elizabeth Wayne. *Investigating the effects of nanoparticle phagocytosis on monocyte activation in diabetic hypertension*

2. **Abigail Allen***, David Gau*, Paul Francoeur, Jordan Sturm, Yue Wang, Ryan Martin, Jodi Maranchie, Anette Duensing, Adam Kaczorowski Stefan Duensing, Lily Wu Michael T. Lotze, David Koes, Walter J. Storkus, and Partha Roy. *Actin-Binding Protein Profilin1 Promotes Aggressiveness of Clear-Cell Renal Cell Carcinoma Cells*

3. **Amin Cheikhi**, Bing Han, Maria Giovanna Francipane, and Eric Lagasse. *In vivo Transcriptional Reprogramming of Hepatic Metabolism through Lymphatic-based Ectopic Liver Organoids*

4. **Hannah Fox** and Dr. Elizabeth Wayne. *Developing methods to assess the exosome cross-talk between maternal and fetal cells in normal pregnancy and preeclampsia*

5. **Meghan Mooring**, K Yao, S Liu, Y Liu, and D Yimlamai. *Cyr61 coordinates liver fibrosis through monocyte and macrophage recruitment and polarization*

6. **Kien Tran**, Wenbo Li, Tianjiao Chu, Kwon Sung Cho, and Kyle E. Orwig. *A Novel Organotypic Culture System Supported Germ Cell Development of Immature Rhesus macaque Testicular Tissues*

7. **Yingqiao Wang**, Raghav Garg, Jane E. Hartung, Adam Z. Goad, Dipna Patel, Kyoungin Kang, Flavia Vitale, Michael S. Gold, Yury Gogotsi, and Tzahi Cohen-Karni. *Remote Nongenetic Optical Modulation of Cellular Electrical Activity Using Two-dimensional Ti3C2 MXene*

8. **Shiyuan Zheng**, Kirill Lavrenyuk, Katherine Fein, Nicholas Lamson, Katheryn Whitehead, and Kris N. Dahl. *Multiscale Structural Characterization of Epithelial Cell Monolayers Associated with the Addition of Permeability Enhancers for Enhancing Drug Delivery*

Poster Session
Wednesday, March 10, 2021
4:30 – 5:30 pm

Tissue Engineering and Biomaterials (parallel sessions)

Session A

https://pitt.zoom.us/j/92396990300
Meeting ID: 923 9699 0300
Passcode: 891966

9. **Arianna Adamo**, Joseph G. Bartolacci, Marco Traina, William R. Wagner, Stephen F. Badylak, and Antonio D'Amore. *A Continuous Microfiber Wire Mandrel-Less Biofabrication For Soft Tissue Engineering Applications*

10. **Madeline Cramer**, William D'Angelo, and Stephen F. Badylak. *Matrix Bound Nanovesicles Represent a Distinct Subset of Extracellular Vesicle*

11. **Kenneth J. Furdella**, Shinichi Higuchi, Kang Kim, Tom Doetschman, William R. Wagner, and Jonathan P. Vande Geest. *Transforming Growth Factor Beta 2 Elution From A Tissue Engineered Vascular Graft Influences In Vivo Smooth Muscle Cell Activity Over An Acute Time Point*

12. **Raghav Garg**, Reem B. Rashid, Daniel San Roman, Yingqiao Wang, Samuel A. Gershanok, Maria Stang, Stephen F. Badylak, Adam W. Feinberg, Douglas J. Weber, Jonathan Rivnay, and Tzahi Cohen-Karni. *Multi-Dimensional Fuzzy Graphene Bioelectronic Actuators*

13. **Andrew Hudson**, Daniel Shiwarski, Joshua Tashman, and Adam Feinberg. *Engineering In Vitro Vascularized Tissues Using FRESH 3D Bioprinted Collagen Scaffolds*

14. **Dorota Jazwinska** and Ioannis Zervantonakis. *Tumor-Mesothelial Assay to Study Ovarian Cancer Clearance Dynamics*

15. **Elizabeth K. Johnston**, Megan K. DeBari, Mallory D. Griffin, and Rosalyn D. Abbott. *Characterization of Biological and Mechanical Properties of Fibrotic Adipose Tissue to Inform Better Regenerative Outcomes*

16. **Tyler Meder**, Travis Prest, Clint Skillen, Lucile Marchal, Valeria Tupac Yupanqui, Lorenzo Soletti, Paul Gardner, Jonathan Cheetham, and Bryan Brown. *Nerve specific extracellular matrix hydrogel promotes functional regeneration following nerve crush and gap injury*

Session B

https://pitt.zoom.us/j/91710243288
Meeting ID: 917 1024 3288
Passcode: 757872

17. **Ravikumar K**, Kevin Pietz, Connor Wiegand, Anne Zeleniak, Wen Liu, Catherine McCormick, Haonan Guan, Yong Fan, and Ipsita Banerjee. *Investigating the scaffold architecture of thymus to inform its synthetic reconstruction*

18. **Miranda Poklar**, Ravi Krishnamurthy, Prashant N. Kumta, and Ipsita Banerjee. *Increasing bioink printability for future tissue fabrication*

19. **Abhijit Roy**, Mubin Ali Aral, Matthew Criado, John Ohodnicki, Vijay Gorantla, MaCalus V. Hogan, and Prashant N. Kumta. *Novel Biodegradable Porous Mg Alloy Scaffolds for Critical Sized Cranial Bone Defect Repair and Regeneration*

20. **Marrisa Therriault**, Aimon Iftikhar, Branimir Popovic, Clint D. Skillen, McKenzie Sicke, Meegan Ambrose, Pamela A. Moalli, and Bryan N. Brown. *Evaluating Immunomodulatory Biomaterials in a Rabbit Model of Lumbar Colpoplexy*

21. **Weitao Wang**, Rebecca Taylor, and Charlie Xi. *Building a DNA Nano-shell with DNA origami tubes*

22. **Connor Wiegand**, Ravi Krishnamurthy, Kevin Pietz, Xiang Li, Lans Taylor, and Ipsita Banerjee. *Developing Islet-on-Chip Model towards T2D disease Modeling*

23. **Piyumi Wijesekara**, Ying Liu, Weitao Wang, Elizabeth K. Johnston, Rebecca E. Taylor, and Xi Ren. *Accessing and Assessing the Cell-Surface Glycocalyx Using DNA Origami*

24. **Kelsey Hall**, Arthi Shridhar, Alvin Liu, and Stephen Badylak. *Effects of Anti-Bacterial Coated Extracellular Matrix Bioscaffolds on Immunomodulation and Mobilization of Progenitor Cells for Volumetric Muscle Loss Treatment*

Poster Session
Thursday, March 11, 2021
1:00 – 1:45 pm

https://pitt.zoom.us/j/93386020742
Meeting ID: 933 8602 0742 • Passcode: 616774

Medical Devices and Computational Modeling

25. **Sommer Anjum** and Lance Davidson. *Understanding the mechanics of passive cellular responses during epithelial convergent extension*

26. **Shaniel Bowen** and Steven Abramowitch. *Characterization of Pelvic Floor Muscle Fiber Architecture for Computational Modeling*

27. **Ronald Fortunato**, Juan Cebral, Anne Robertson, and Spandan Maiti. *Using In-Vivo Morphological Measurements of Cerebral Aneurysm Blebs to Predict Aneurysm Rupture Risk*

28. **Matthew D. Poskus**, Thomas O. McDonald, Alexis L. Scott, Lia Franco, and Ioannis K. Zervantonakis. *A Predictive Model of Stromal Fibroblast-Mediated Drug Resistancein HER2+ Breast Cancer*

29. **Constance M. Robbins**, Kuanren Qian, Yongjie Jessica Zhang, and Jana M. Kainerstorfer. *Combined mechanical and optical simulation of the effect of compression on breast-tumor mimicking software phantoms*

30. **Daniel San Roman**, Yingqiao Wang, Raghav Garg, Marissa Behun, Bryan Brown, Stephen Badylak, and Tzahi Cohen-Karni. *Three-Dimensional Graphene Microelectrode Arrays for Detection of Wound Healing Biomarkers*

Investigating the effects of nanoparticle phagocytosis on monocyte activation in diabetic hypertension

Dasia Aldarondo (1) and Elizabeth Wayne (1,2)

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

Patients suffering from diabetes are at an increased risk of vascular injury due to the chronic inflammation associated with the disease. A common comorbidity of diabetes is hypertension or increased blood pressure, commonly referred to as diabetic hypertension. Monocyte recruitment and activation plays a decisive role during the pathogenesis of diabetic hypertension. As such, monocytes have the potential to be targeted carriers for therapeutic nanoparticles. Monocyte activation status provides information on the intracellular trafficking of nanoparticles during phagocytosis. However, there is little known about the effect of activation on intracellular trafficking of nanoparticles in diabetic hypertensive monocytes. Angiotensin Converting Enzyme 2 (ACE2) dysregulation is common on the surface of monocytes in diabetic hypertensive patients, and there is a lack of studies to understand the role this plays in monocyte phagocytosis and activation. The goal of this research is to develop a monocyte model similar to diabetic hypertension and characterize the impact of a variety of nanoparticles on monocyte activation. To achieve this model monocyte cell lines which mimics ACE 2 dysregulation were created using lentiviral transduction. After completing a phagocytosis assays in which cells are co-cultured with nanoparticles both under stagnate and shear conditions fluorescence microscopy is used to observe changes in nanoparticle uptake. Common markers of monocyte activation are also measured post assay by qPCR. This research will better characterize how nanotherapeutics impact monocyte activation to better inform drug efficacy in diabetic hypertension.

Actin-Binding Protein Profilin1 Promotes Aggressiveness of Clear-Cell Renal Cell Carcinoma Cells

Abigail Allen (1)*, David Gau (1)*, Paul Francoeur (2), Jordan Sturm (1), Yue Wang (3), Ryan Martin (4), Jodi Maranchie (5), Anette Duensing (6), Adam Kaczorowski (7), Stefan Duensing (7), Lily Wu (8), Michael T. Lotze (1,3,9), David Koes (2), Walter J. Storkus (1,5,9,10), and Partha Roy (1,6)

(1) Bioengineering, (2) Computational and Systems Biology, (3) Surgery, (4) Biology, (5) Urology, (6) Pathology, (9) Immunology, and (10) Dermatology (University of Pittsburgh), (7) Department of Urology (Heidelberg School of Medicine), and (8) Department of Urology (University of California, Los Angeles)

Clear cell renal cell carcinoma (ccRCC), the most common subtype of renal cancer, has a poor clinical outcome. A hallmark of ccRCC is genetic loss-of-function of Von-Hippel Lindau (VHL) that leads to a highly vascularized tumor microenvironment. While many ccRCC patients initially respond to anti-angiogenic therapies, virtually all develop progressive, drug-refractory disease. Given the role of dysregulated expressions of cytoskeletal and cytoskeleton-regulatory proteins in tumor progression, we performed analyses of The Cancer Genome Atlas (TCGA) transcriptome data for different classes of actin-binding proteins to demonstrate that increased mRNA expression of profilin1 (Pfn1), Arp3, cofilin1, Ena/VASP and CapZ, are indicators of poor prognosis in ccRCC. Focusing further on Pfn1, we performed immunohistochemistry-based classification of Pfn1 staining in tissue microarrays, which indicated Pfn1-positivity in both tumor and stromal cells; however, the vast majority of ccRCC tumors tend to be Pfn1-positive selectively in stromal cells only. This finding is further supported by evidence for dramatic transcriptional upregulation of Pfn1 in tumor-associated vascular endothelial cells (VEC) in the clinical specimens of ccRCC. In vitro studies support the importance of Pfn1 in proliferation and migration of RCC cells, and in soluble Pfn1's involvement in VEC-tumor cell crosstalk. Furthermore, proof-of-concept studies demonstrate that treatment with a novel computationally designed Pfn1-actin interaction inhibitor identified herein reduces proliferation and migration of RCC cells in vitro and RCC tumor growth in vivo. Based on these findings, we propose a potentiating role for Pfn1 in promoting tumor cell aggressiveness in the setting of ccRCC.

In vivo Transcriptional Reprogramming of Hepatic Metabolism through Lymphatic-based Ectopic Liver Organoids

Amin Cheikhi (1), Bing Han (1), Maria Giovanna Francipane (1), and Eric Lagasse (1)

(1) Department of Pathology, McGowan Insitute for Regenerative Medicine, Univ Pittsburgh

Liver transplantation is a life-saving therapy for end-stage liver disease. However, its therapeutic potential is constrained by organ shortage which remains one of the biggest challenges facing liver transplantation. Hepatocyte transplantation represents an alternative approach to liver transplantation. We previously demonstrated that hepatocytes transplantation into the lymphatic sites (Lymph nodes and/or Fat Associated Lymphoid Tissues) are able to generate functional ectopic liver that would rescue animals with fatal liver disease. These in vivo lymphatic-based ectopic liver organoids (LB- ELO) have unparalleled potential to create a paradigm shift for regenerating liver functions. Specifically, the current standard of whole liver transplantation strategies inflicts substantial collateral metabolic damage to the liver tissue notably due the mitochondrial bioenergetic derangement of the liver graft under transplant surgery conditions, which in turn could lead to progressive deterioration of graft quality and performance outcome. In stark contrast, LB-ELO is a non-invasive procedure that stimulates the transcriptional control of metabolic processes, thus boosting the efficiency of donor pool for transplantation. Our transcriptomic analyses of ectopic and native livers revealed a selective ectopic compensatory gene expression of hepatic functions-controlling genes in native livers, implying a regulated functional integration between the native diseased liver and its LB-ELO counterpart. Importantly, these data suggest that metabolic reprogramming plays a critical role in their physiological coupling. We here describe transcriptomic signatures of LB-ELO and suggest how they may promote anti-aging effects on the hepatic function. Through omics analysis, we seek to define the most efficacious and ideal parameters application of LB-ELO to liver transplants. These studies also provide a wealth of data to inform future empirical designs to identify multi-parametric discriminators of organ suitability for transplantation as a prelude to clinical trials.

Developing methods to assess the exosome cross-talk between maternal and fetal cells in normal pregnancy and preeclampsia

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

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

Exosomes are cellular vesicles that serve as a means of intercellular communication. Originating via an endosomal pathway, exosome content is cell-specific and reflects the cell's biological state. Preeclampsia is a maternal health condition that remains difficult to diagnose, due to its unknown etiology and complex pathophysiology. Consequently, there is a lack of available treatments for this disease. While exosomes play a significant role in the progression of preeclampsia, the exact mechanisms are still unknown. Because of this, further analysis of exosomes from preeclampsia models is needed to elucidate biomarkers of disease and potential therapeutic targets. Moreover, research has shown that exosome cross-talk exists between the maternal and fetal cells during normal and preeclamptic pregnancy. As such, when investigating preeclampsia it is necessary to examine the pathways of intercellular communication occurring at the maternal-fetal interface. To accomplish this aim, exosomes will be harvested from THP-1 monocytes and isolated via the method of polymer precipitation using polyethylene glycol. The exosomes will then be cocultured with BeWo cells, and changes in cellular activity will be monitored via cell proliferation and cell migration assays. This investigation will provide insight into potential diagnostic markers and therapeutic targets for preeclampsia, as well as the mechanisms of disease progression.

Cyr61 coordinates liver fibrosis through monocyte and macrophage recruitment and polarization

Meghan Mooring (1,2), K Yao (2), S Liu (3), Y Liu (1), and D Yimlamai (1,2,3,4)

(1) Cellular and Molecular Pathology, University of Pittsburgh School of Medicine,, (2) Department of Pediatrics, Yale University, (3) Pittsburgh Liver Research Center, University of Pittsburgh, (4) The Yale Liver Center, Yale University

Obesity is increasing worldwide and can lead to a multitude of GI complications. Major complications of obesity include non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatosis (NASH). Aside from dietary intervention, there are few treatments available. The Hippo signaling pathway and its effector transcriptional coactivator YAP are responsible for fibrotic and inflammatory responses in injured hepatocytes. Our previous work demonstrates that YAP-target Cyr61 is the main mediator of these effects. The fibrotic activity of YAP/Cyr61 is only seen in the presence of circulating and liver-resident monocytes and macrophages. Using a mouse model of NASH, we have determined that livers lacking hepatocytic Cyr61 (Cyr61ΔHep) showed less fibrosis than control. RNA sequencing shows decreased inflammatory (TNFa, CCL2, Ly6C, TLR1/2) and fibrotic (Col1a1, Acta2, TIMP1, CTGF) gene expression in Cyr61ΔHep NASH livers compared to control, and increased expression of the pro-resolution macrophage marker CD163. Cyr61ΔHep NASH livers have fewer infiltrating monocytes (CD11b+) that express high levels of inflammatory and fibrotic markers (TNFa, TGFb) and more pro-resolution macrophages (CD11blo, F4/80+) that express low levels of inflammatory and fibrotic markers. Activation of Cyr61 expression in hepatocytes (AAV-Cyr61Hep) resulted in increased fibrosis compared to control. AAV- Cyr61Hep livers have more pro-inflammatory macrophages and fewer pro-resolution macrophages. Furthermore, treatment with Cyr61 protein increases expression of pro-inflammatory genes (iNOS, IL-6) in bone marrow-derived macrophages. Conclusions: Cyr61 from hepatocytes attracts and polarizes macrophages towards an inflammatory state upon liver injury, leading to fibrosis. Lack of Cyr61 during injury reduces fibrosis and inflammation, indicating that targeting Cyr61 during liver injury could be an effective therapeutic avenue.

A Novel Organotypic Culture System Supported Germ Cell Development of Immature Rhesus macaque Testicular Tissues

Kien Tran (1,2), Wenbo Li (3), Tianjiao Chu (2), Kwon Sung Cho (3), and Kyle E. Orwig (2)

(1) Department of Molecular Genetics and Developmental Biology, (2) Department of Obstetrics, Gynecology and Reproductive Sciences, Magee-Womens Research Institute, University of Pittsburgh School of Medicine, (3) Department of Mechanical Engineering and Materials Science, University of Pittsburgh

Pre-pubertal male patients who undergo gonadotoxic treatments face a high risk of permanent infertility. Since these patients are not yet producing sperm, they are often advised to cryopreserve testicular tissues, which home spermatogonial stem cells (SSCs), if they wish to preserve their fertility. Next-generation technologies are being developed to restore fertility by utilizing SSC potential to regenerate spermatogenesis. Testicular tissue culture (TTC), taking advantage of SSC potential and small tissue amounts to produce sperm in vitro, may become a potential therapy to restore fertility for these patients.

Previous literature reported fertilization-competent spermatozoa/spermatids produced from cultures of immature mouse and human testicular tissues. However, they either used complicated culture systems or obtained a very low spermatogenesis efficiency. These are the barriers that hinder the translational applications of this technology. The goal of this study is to develop a simplified, efficient culture system to induce in vitro spermatogenesis using immature mouse and non-human primate testicular tissues. We invented a novel (polydimethylsiloxane = PDMS) PDMS-roof transwell (PRT) culture system that overcame the limitations of previous culture systems, including laborious device production, difficult tissue loading, complicated culture maintenance, while yielding high culture efficiency. Neonatal mouse testes cultured in the PRTs showed active spermatogenesis in >70% of tissue areas with presence of VASA+ germ cells, SALL4 + undifferentiated spermatogonia, STRA8+ differentiating spermatogonia, and SYCP3+ spermatocytes. In 2-month PRT cultures of cryopreserved pre-pubertal monkey tissues, we observed not only an increased Ki67+ proliferative activity of VASA+ germ cells, but also the number cells expressing post-meiotic CREM+ marker, while the number of SYCP3+ cells did not change. The seminiferous tubule cross-section diameter of the 2-month cultured monkey tissues also grew significantly larger in most of the medium conditions. Moreover, the basal medium condition significantly enhanced survival, proliferation, and differentiation of germ cells compared to any other supplements, while retinoic acid was the most damaging factor to tissue viability compared to other tested supplements. This multi-species study may validate safety and feasibility of the TTC technology for future clinical applications to give our patients a chance of fatherhood in the future.

Remote Nongenetic Optical Modulation of Cellular Electrical Activity Using Two-dimensional Ti3C2 MXene

Yingqiao Wang (1), Raghav Garg (1), Jane E. Hartung (4), Adam Z. Goad (5), Dipna Patel (5), Kyoungin Kang (2), Flavia Vitale (6), Michael S. Gold (4), Yury Gogotsi (5), and Tzahi Cohen-Karni (1,2,3)

(1) Dept of Materials Science and Engineering, Carnegie Mellon Univ, (2) Dept of Biomedical Engineering, Carnegie Mellon Univ, (3) McGowan Institute for Regenerative Medicine, Univ of Pittsburgh, (4) Dept of Neurobiology, University of Pittsburgh , (5) A.J. Drexel Nanomaterials Institute and Dept of Materials Science and Engineering, Drexel University, (6) Dept of Bioengineering, Center for Neuroengineering and Therapeutics, Dept of Neurosurgery, Dept of Neurology, and Dept of Physical Medicine & Rehabilitation, University of Pennsylvania

The ability to probe and manipulate electrophysiology at the cellular level is crucial for understanding cellular communications and enabling new therapeutics for neurological disorders. Nanomaterial-facilitated photothermal stimulation is a non-invasive technique to manipulate cellular electrophysiology with high spatiotemporal resolution without genetic modifications. Although recently reported Si-, Au-, and C-based nanomaterials demonstrated their photothermal stimulation capability, they exhibit limited photothermal conversion efficiency in the near-infrared (NIR) window or have complicated synthesis protocols that prevents direct scale-up.

Two-dimensional (2D) Ti3C2 MXene has high NIR absorption and enables largescale production. Here, we demonstrated that Ti3C2 MXene enables remote nongenetic optical modulation of cellular electrical activity. Illumination of isolated Ti3C2 MXene flakes with either 1 ms pulses of 10 mW red (635 nm) or NIR laser (808 nm), resulted with a measured local temperature rise of 2.3 ± 0.7 K and 3.1 ± 0.7 K, respectively. Dorsal root ganglion (DRG) neurons incubated with MXene film (25 μg/cm2) and MXene dispersion (100 μg/mL) for 24 h did not show significant change in viability compared to the control samples, indicating MXene is non-cytotoxic. Scanning electrons microscopy and scanning laser confocal imaging were performed to reveal that the MXene flakes externally adhered to the cell membrane. Photothermal stimulation of DRG neural networks was achieved by illuminating the interface between MXene films and flakes and DRG neuron with incident energies with 4 µJ and 18 µJ per pulse, respectively. Optical stimulation of DRG neurons using Ti3C2 MXene flakes is safe and does not generate cellular stress. Due to its straightforward and large-scale synthesis, MXene can enable neuronal modulation at various scales and dimensions, thus it is a powerful tool for future remote, non-genetic biological interface, such as stimulation of engineered tissues.

Multiscale Structural Characterization of Epithelial Cell Monolayers Associated with the Addition of Permeability Enhancers for Enhancing Drug Delivery

Shiyuan Zheng (1), Kirill Lavrenyuk (2), Katherine Fein (2), Nicholas Lamson (2), Katheryn Whitehead (2), and Kris N. Dahl (2)

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

A major obstacle for topical and enteral drug delivery is the poor transport of macromolecular drugs through the epithelium. One potential solution to resolve this problem is the use of permeation enhancers that alter epithelial structures. Specifically, piperazine derivatives are permeation enhancers that can modulate epithelial structures and augment the absorption of macromolecular drugs. We are investigating the mechanism by which the piperazine derivatives disrupt the structures of epithelial monolayers. In this project, the model cell line NRK-52E was used as the subject to measure the function and cytotoxicity of 1-phenylpiperiazine (1-ppz) and 1-Methal-4-phenylpiperiazine (1-M-4-ppz). Live cell imaging reveals a dose-dependent gross reorganization of monolayers at high concentrations, but reorganization differs based on the piperazine type. We also examine subcellular effects on NRK-52E monolayers including cytoskeletal reorganization force generation within cells using confocal widefield imaging and biophysical techniques. Once the mechanisms of epithelial permeability can be quantified it will be possible to develop better delivery systems as well as new permeability enhancers.

A Continuous Microfiber Wire Mandrel-Less Biofabrication For Soft Tissue Engineering Applications
TISSUE ENGINEERING APPLICATIONS

Arianna Adamo (1,2), Joseph G. Bartolacci (1), Marco Traina (1), William R. Wagner (2), Stephen F. Badylak (2), and Antonio D'Amore (1,2)

(1) RiMED Foundation, (2) McGowan Institute for Regenerative Medicine

Soft tissue injuries are common in daily clinical and surgical practice. Outcomes of degradable and non degradable suture materials are often affected by mechanical mismatch, excessive fibrosis and inflammation. This study introduces a mandrel-less electrodeposition method able to fabricate continuous microfiber wires, with controlled micro-architecture and tunable mechanics. Poly(ester urethane) urea (PEUU) microfiber wire morphology and mechanical properties have been characterized by scanning electron microscopy uniaxial tensile test respectively Furthermore, the in vitro response of mouse bone marrow-derived macrophages to PEUU degradation products, PEUU electrospun and casted scaffold, PEUU electrospun wires was evaluated by immunoblotting and immunolabeling. Moreover, the host response to electrospun wires in vivo was tested: twenty rats, randomized in 5 groups, received a 2cm infra-scapular incision and the skin was closed using PEUU microfiber and cast wires and the most common suture materials used (polyglycolic acid, polydioxanone and polypropylene). After one month, mechanical and histological evaluation of explants and suture wires was performed. In vitro results have shown an anti-inflammatory macrophage response associated to PEUU microfiber scaffold and wires. In vivo, PEUU electrospun wires group showed better mechanical performance compared to the other groups, a favorable collagen remodeling comparable to the healthy group and a mild host response reaction. These results suggest that microfiber wires reduce macrophage pro-inflammatory response and improve collagen deposition, which make it an ideal candidate for soft tissue suture applications.

Matrix Bound Nanovesicles Represent a Distinct Subset of Extracellular Vesicle

Madeline Cramer (1,2), William D'Angelo (2,3), and Stephen F. Badylak (1,2,3)

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

The term “extracellular vesicle” is broadly used to describe naturally released cellular vesicles with a lipid bilayer and without a nucleus. Extracellular vesicles represent a heterogeneous population that is generally categorized into exosomes, calcifying matrix vesicles, microvesicles, and apoptotic bodies based on characteristics such as size, biogenesis, function and cargo. Exosomes are 30-250nm vesicles that are exclusively described within the liquid-phase, including all bodily fluids in vivo and the conditioned media of cells in vitro. Calcifying matrix vesicles are 20-200nm in size and are described within the matrix of mineralizing tissue where their specific function is to serve as a nucleation site for hydroxyapatite crystal formation. Recently, extracellular vesicles termed matrix bound nanovesicles (MBV) have been identified within the extracellular matrix of non-mineralizing soft tissues. MBV are similar in size (50-250nm) to exosomes and calcifying matrix vesicles and are also known to contain protein and microRNA cargo with diverse bioactivities. However, an in depth characterization of the defining compositional characteristics of MBV has yet to be completed.

In this study, we compared the size, protein composition, and immunomodulatory activity of murine plasma exosomes, calcifying matrix vesicles, and skeletal muscle MBV. MBV had low expression levels of proteins commonly associated with both exosomes and calcifying matrix vesicles such as CD63, CD81, Annexin V and alkaline phosphatase. Each vesicle subtype also had a distinct profile of cytokine cargo. Evaluation of macrophage activation in vitro revealed proinflammatory effects of calcifying matrix vesicles, while exposure of macrophages to exosomes and MBV induced a more anti-inflammatory phenotype. These results show that MBV represent a distinct population of extracellular vesicle that is distinguishable from exosomes and calcifying matrix vesicles. Future work will characterize the lipid and miRNA cargo of these vesicle populations.

Transforming Growth Factor Beta 2 Elution From A Tissue Engineered Vascular Graft Influences In Vivo Smooth Muscle Cell Activity Over An Acute Time Point

Kenneth J. Furdella (1), Shinichi Higuchi (2), Kang Kim (1,3,4,5), Tom Doetschman (6), William R. Wagner (1,2,3,5), and Jonathan P. Vande Geest (1,2,4,5)

(1) Dept. of Bioengineering, (2) McGowan Institute for Regenerative Medicine, (3) Dept. of Medicine, (4) Dept. of Mechanical Engineering and Material Science, (5) Vascular Medicine Institute, University of Pittsburgh, Pittsburgh, (6) Cellular and Molecular Medicine, University of Arizona

Transforming growth factor beta 2 (TGFB2) is a cytokine that plays a vital role in vascular remodeling and cell cycle regulation. One of the primary components of the vascular system is the smooth muscle cell (SMC) which has been shown to modulate phenotype depending on the TGFB2 concentration. Specifically, the phenotype of the SMC (synthetic or contractile) population can be determined by evaluating the viability of the cells (increase or decrease) and extracellular matrix deposition, collagen. In this work, electrospun compliance matched TGFB2 eluting TEVGs were implanted into Sprague Dawley rats for 5 day to observe SMC infiltration and ECM production. TEVGs were fabricated using a computational/experimental approach to match the compliance of rat aorta. TGFB2 concentrations of 0, 10, 100 ng/mg were added to the grafts, the TEVGs (n=3) were implanted into the abdominal aorta of a Sprague Dawley rats for 5 days, imaged in vivo using ultrasound, and evaluated using histological markers (SMC, macrophage, collagen, and elastin). In vivo ultrasound showed that the implant became stiffer as TGFB2 increased, 100 ng/mg to rat aorta ($p < 0.01$). In vivo velocity and diameter were found to be similar between all groups and rat aorta. For histology, the 10 ng/mg group had an elevated SMC signal (myosin heavy chain) in reference to the 0 and 100 ng/mg, $p < 0.001$. The 100 ng/mg TGFB2 group had an increase in collagen production ($p < 0.01$), general immune response ($p < 0.05$), and a decrease in SMCs in relation to the other groups. These results suggest that TGFB2 modulates in vivo SMC phenotype over an acute time point and confirms prior in vitro work.

Multi-Dimensional Fuzzy Graphene Bioelectronic Actuators

Raghav Garg (1), Reem B. Rashid (2,3), Daniel San Roman (1), Yingqiao Wang (1), Samuel A. Gershanok (1), Maria Stang (1), Stephen F. Badylak (4,5,6), Adam W. Feinberg (1,6,7), Douglas J. Weber (6,7,8,9), Jonathan Rivnay (2,3), and Tzahi Cohen-Karni (1,6,7)

(1) Dept of Materials Science and Engineering, Carnegie Mellon Univ, (2) Dept of Biomedical Engineering, Northwestern Univ, (3) Simpson Querrey Institute, Northwestern Univ, (4) Dept of Surgery, Univ of Pittsburgh, (5) Dept of Bioengineering, Univ of Pittsburgh, (6) McGowan Institute for Regenerative Medicine, Univ of Pittsburgh, (7) Dept of Biomedical Engineering, Carnegie Mellon Univ, (8) Dept of Mechanical Engineering, Carnegie Mellon Univ, (9) Neuroscience Institute, Carnegie Mellon Univ

The ability to manipulate the electrophysiology of electrically active cells and tissues has enabled a deeper understanding of healthy and diseased states. This has primarily been achieved via bioelectronic actuators that interface engineered materials with biological entities. Graphene has gained recent interest as a building-block for bioelectronic actuators due to its advantageous electrochemical properties and biocompatibility. However, functional graphene bioelectronics exhibit a two-dimensional (2D) topology. This leads to inherent performance limitations due to the limited exposed surface-area and poor interactions with interfaced cells and tissues. Ideal geometry of graphene-based actuators needs to leverage the material's high surface-area-to-volume ratio to facilitate maximum interaction with the electrode.

Here we report a breakthrough three-dimensional (3D) topology of graphene: nanowire templated 3D fuzzy graphene (NT-3DFG), for actuation of electrically active cells and tissues. Using a bottom-up approach, we synthesize an interconnected network of free-standing graphene flakes on Si nanowires (SiNWs). The 3D topology of the free-standing graphene flakes leads to enhanced surface-area compared to planar surfaces, such as conventional Pt microelectrodes. This allows NT-3DFG microelectrodes to exhibit lower electrode impedance than planar structures. The increased surface area also allows NT-3DFG MEAs to hold higher electrochemical charge via capacitive charging, thus resulting in up to 5-fold increase in the CSCC compared to Pt microelectrodes. Addition of PEDOT:PSS onto the 3D template of NT-3DFG further enhances the exhibited CSCC. We observe that both NT-3DFG and NT-3DFG with PEDOT:PSS microelectrodes exhibit up to 8- fold and 30-fold increase in CIC compared to Pt microelectrodes. This can enable further miniaturization of graphene-based microelectrodes to ultra-microelectrodes for functional bioelectronics. Our results demonstrate the importance of extending the topology of nanomaterials to 3D to push the physical and functional limits of conventional bioelectronics.

Engineering In Vitro Vascularized Tissues Using FRESH 3D Bioprinted Collagen Scaffolds

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Heart failure is a major cause of death worldwide, with a wide range of causes from congenital birth defects to myocardial infarction. While organ transplantation is successful, it is an imperfect solution as demand has steadily increased while supply has not, resulting in a growing unmet need for organ repair and replacement. Tissue engineering is a possible solution, with the goal of eventually creating full-scale organs. Unfortunately, organ fabrication remains difficult to achieve, with a key roadblock being building tissues greater than one cubic centimeter and the microvascular and capillary networks required for tissue viability. The challenge is that microvasculature is a complex 3D-branching cellularized structure at micron scale built from soft (E < 150 kPa) extracellular matrix (ECM) proteins such as collagen. To address this we previously developed Freeform Reversible Embedding of Suspended Hydrogels (FRESH) to 3D bioprint complex structures from collagen. However, directly 3D bioprinting functional microvasculature has remained beyond our capabilities. Here, we aim to FRESH 3D bioprint multiscale (>100 micron) vascular scaffolds, which are combined with a porous tissue microstructure to maximize the viability and angiogenic potential of endothelial cells. Generating microvascularized tissues in vitro would overcome a major barrier in tissue engineering and represent a path forward to engineering tissues and possibly organs for future transplantation. Here, we demonstrate vascular scaffolds can be FRESH printed from collagen and seeded with endothelial cells which then form a coherent endothelium. Cell viability significantly increases by increasing scaffold porosity. Future work will seek to overcome the major tissue engineering challenge of microvascularizing large tissues by casting highly porous cellular gels around endothelialized vessels in a bioreactor system to maximize angiogenesis into the tissue.

Tumor-Mesothelial Assay to Study Ovarian Cancer Clearance Dynamics

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Ovarian cancer is the leading cause of death among all gynecological cancers; the 5-year survival is poor (~50%), and distant metastases occur in 60% of patients. Our current understanding of mechanisms driving ovarian cancer dissemination is lacking due to the limitations of available models to recapitulate the metastatic peritoneal environment. Metastasis occurs when cancer cells attach and invade through the mesothelial monolayer lining peritoneal organs. Here, we utilize 2D models to identify strategies that block ovarian cancer spheroid invasion through the mesothelial monolayer defined as clearance.

Mesothelial monolayers were formed (ZTGFP) in 96 well plates and high grade serous ovarian cancer spheroids (OVCAR8, OVCAR3, OV90 and OVCA432) were seeded on top. To modulate the mesothelial barrier function and examine effects on clearance rates, the monolayers were treated with forskolin and time-lapse live cell imaging was performed to assess clearance dynamics. Ovarian cancer cell clearance was quantified utilizing Nikon NIS Elements pipeline.

Treatment with forskolin reduced the clearance rate compared to control conditions. In addition, we found that forskolin reduced the total number of spheroids clearing through the mesothelial monolayer. Currently we are developing a novel microfluidic 3D culture assay, in order to evaluate the role of the mesothelial barrier in ovarian cancer metastasis, in a more physiologically relevant setting.

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

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The appearance and function of subcutaneous adipose tissue can be compromised by traumatic injury, ablative procedures, or congenital malformations. Defects in adipose tissue are currently treated with autologous fat grafts whereby the stromal vascular fraction (SVF) assists by promoting an angiogenic and anti-inflammatory environment for tissue restoration. It has been shown that obesity and adipose tissue fibrosis are negatively correlated with SVF and stem cell concentrations. Not only are stem cells less abundant in fibrotic adipose tissue, but they are also predisposed to become profibrotic cells increasing extracellular matrix (ECM) production, changing the biomechanics of the tissue, and causing metabolic dysfunction. Thus, it is hypothesized that adipose tissue fibrosis is negatively correlated with the regenerative potential of the tissue. The goal of this project is to correlate the regenerative capacity to structural and functional components of the adipose samples. 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, gene expression and cell morphology.

Nerve specific extracellular matrix hydrogel promotes functional regeneration following nerve crush and gap injury

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Peripheral nerve injuries (PNI) are a frequent result of traumatic injury and can cause deficits in sensory or motor function including paralysis. Acute nerve crush injuries are the most prevalent type of PNI and can spontaneously recover functional losses, however recovery is not guaranteed and treatment of crush injuries is largely understudied. Acute nerve gap injuries (when the nerve is severed) very rarely recover spontaneously and require surgical intervention to restore function. Despite advances in PNI treatment, recovery remains largely unsatisfactory and functional deficits become permanent.

This study investigates the use of a decellularized nerve hydrogel (peripheral nerve matrix; PNM) as a therapeutic for both crush and gap injury. Nerve recovery was assessed in a rat sciatic nerve injury model via gait analysis in the left hind limb, motor action potential electrophysiological analysis at the downstream muscle group, and axon quantification/histological analysis at the injury site. The nerve crush injury was repaired via subepineurial injection of PNM over 12 weeks and compared with an untreated nerve injury as the clinical standard of care. Simultaneously, the nerve gap injury repaired via silicone conduit spanning an 8mm gap filled with PNM over 24 weeks and compared to autologous grafting as the clinical standard of care and a saline filled conduit as a negative control. Deployment of PNM as a therapeutic demonstrated superior axon regrowth in both models, with significantly more axons reaching the distal injury site. Furthermore, PNM treatment increased nerve conduction and improved gait in the crush model, and PNM treatment demonstrated equivalent results to autografting. These results indicate that PNM has the capacity to restore function following traumatic PNI and has an effect greater than or equal to current standards of care.

Investigating the scaffold architecture of thymus to inform its synthetic reconstruction

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The adaptive immune response of the body is mainly due to the action of T-cells that enable the host's defense against foreign objects such as pathogens that cause infections (negative selection) and recognizing cells of the host, thereby protecting the body from autoimmune responses (positive selection). These T-cells are generated by the Thymus, a gland that is located in the thoracic cavity and is responsible for 'training' (maturing) the T-cells to elicit appropriate immune responses. Thymus has two lobes each with two morphologically distinct regions known as the medulla and the cortex. These two regions have their own unique cell populations: medullary Thymic Epithelial cells (mTECs) and cortical Thymic Epithelial cells (cTECs), along with its unique function in reprogramming the T cells for positive selection in the cortex (cTECs) and negative selection in the medulla (mTECs). In this regard, recent attempts with bioengineered thymus organoids with primary TEC cultured in a decellularized scaffold have been shown to be successful in mouse models. While the TEC organization and phenotype in the designed thymus is vital, the scaffold architecture appears to be of equal importance.

In the current work, the architecture of the decellularized mouse thymus is captured using multiphoton microscopy which can image the collagen due to the Second Harmonic Generation with advantages such as high penetration depth and virtually no tissue damage. The architecture of the scaffold reveals marked differences in morphology of the cortex and medulla regions in terms of the orientation of the fibers quantified using fiber reconstruction analysis. Importantly, respective regional architecture characteristics of the native organ is preserved upon decellularization. Subsequent repopulation of the scaffold with primary TECs and continued perfusion culture results in scaffold modification, which was also characterized in the study. Interestingly, the architecture of reconstructed thymus scaffolds under perfusion compared better with naïve mouse thymus based on the quantitative analysis of fiber parameters. The architecture of the native scaffold and its dynamic evolution upon organoid engineering offer a unique perspective, likely to be valuable in future design of synthetic thymus scaffold.

Increasing bioink printability for future tissue fabrication

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One of the largest medical problems in the world today is the lack of organs available for transplantation. In the US alone, as of February 2021, over 107,000 people were waiting for an transplant, and the need for organs increases while the supply remains stagnant (1). 3D bioprinting is designed to fight this issue by printing layers of biomaterials with embedded cells that can synergistically recreate a tissue construct. There are several challenges however, when working with 3D bioprinting, one of them being the need for extensive trial and error testing to determine the optimal bioink and structure for a specific tissue application. This need for comprehensive testing is often made more difficult when working with human cells, which are sensitive to the biophysical properties of the environment as well. The current aim of this project is designed to reduce the amount of testing needed, by utilizing a biomechanics-based analytical model to predict the printability and resolution of the structure based on bioink factors, printing settings, and post-extrusion environment properties. The initial analytical model combines the bioink properties of concentration and viscosity, the controllable printer settings of pressure, speed, and nozzle diameter, and the post extrusion properties such as type of substrate and surface spreading. These factors together can predict a final resolution and printability of a specific bioink structure. Current work is being done to collect printing resolution measurements in order to validate the initial predictive model, with future modeling aimed to address the addition of pre and post crosslinking, surface coating, and the inclusion of cells in the ink. This project will allow for a reduction in initial bioink testing by increasing the reproducibility of a printed structure and providing a firm basis for selection of a bioink based on the target of the application.

(1) “Organ Donation Statistics.” OrganDonor.gov, Health Resources and Services Administration, 25 Feb. 2021, www.organdonor.gov/statistics-stories/statistics.html.

Novel Biodegradable Porous Mg Alloy Scaffolds for Critical Sized Cranial Bone Defect Repair and Regeneration

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Bone reconstruction of critical-sized craniomaxillofacial bone defects due to tumor excisions, injuries, and congenital disorders is a major challenge for orthopedic surgeons due to lack of suitable synthetic bone grafts. Bone resorption, infections, inflammation and suboptimal osseointegration are also common with existing metal, polymer and ceramic based cranial implants. The present study investigates a proprietary and patented novel biodegradable magnesium alloy (WJ11) as bone regenerative scaffolds using the rat critical size calvarial defect (RCSD) model. Bone regeneration efficacy and toxicity of fluoride coated WJ11 alloy (FWJ11) was assessed against clinically used non-degradable poly-ether-etherketone (PEEK) polymer control, in 8 mm RCSD model. Machined porous cranial grafts of 8 mm diameter and 0.8 mm thickness were implanted and then retrieved at 4, 8, 16, and 26 weeks, followed by microcomputed tomography (micro-CT) and histology assessments. Local and systemic toxicity of the FWJ11 and PEEK scaffolds were studied by blood analysis and examination of brain, kidney, liver and spleen tissues harvested at various endpoints. The FWJ11 scaffolds demonstrate absence of any apparent local and systemic toxicities indicating safety and efficacy comparable to clinically used non-degradable PEEK scaffolds. No hydrogen gas pocket was observed in implanted FWJ11 scaffolds. Micro-CT results also show new bone formation on FWJ11 scaffolds and the new bone formation volume increased with implantation time. Further, new bone formation volume for the FWJ11 scaffolds exceeds that of PEEK scaffolds at a given implantation time-point. Most of the scaffolds at end of 26 weeks show bony bridging over partial length of defect. The histology results of both scaffolds further confirm new bone formation in the defect area and at the interface. Micro-CT results also demonstrate progressive degradation of FWJ11 scaffolds with time which is clearly visible at end of 16 and 26 weeks. Results of these studies will be presented and discussed.

Evaluating Immunomodulatory Biomaterials in a Rabbit Model of Lumbar Colpoplexy

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Objective: A chronically activated immune response has been correlated with urogynecologic mesh complications. The early host macrophage response to implanted materials is now widely accepted as an indicator of downstream implant integration and successful surgical outcomes. This study focuses on modulating the early host macrophage response with interleukin-4 (IL-4) coated polypropylene mesh to drive a pro-healing, anti-inflammatory M2 environment and lessen the M1 pro-inflammatory response to promote tissue integration.

Methods: Nulliparous New Zealand rabbits were implanted with IL-4 coated Gynemesh PS (Ethicon) by a modified colpopexy after hysterectomy, and the local host immune response was defined at 14 days (N =7/group) and mesh tissue integration at 90 days (N =5/group). Uncoated mesh implanted in animals by the same method served as controls. The host immune response was evaluated histologically, using immunofluorescent labeling of macrophages, and gene expression for pro- and anti-inflammatory macrophage subsets by RT-PCR. The integration of the mesh materials was then evaluated histologically and biomechanically at 90 days.

Results: In vitro testing demonstrated that the IL-4 coating was bioactive following terminal sterilization of the coated mesh, promoting macrophage transition to an anti-inflammatory phenotype. When implanted, IL-4 coated mesh materials were shown to shift the host macrophage response towards a more anti-inflammatory phenotype at 14 days as compared to uncoated polypropylene mesh

Conclusions: Modulation of the early host response to polypropylene mesh toward an M2 phenotype has the potential to result in significant improvements in mesh integration, reduction in tissue degradation and associated complications, and overall clinical success. Future studies will inform the long-term potential of this promising modification of urogynecologic polypropylene mesh.

Building a DNA Nano-shell with DNA origami tubes

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Structural DNA nanotechnology offers opportunities to build functional nanostructures due to its precise manufacturing in size, shape, and diverse motifs conjugations at well-defined positions. Targeted binding of functional DNA nanostructures to cell membranes has the potential to deliver membrane-targeting drugs or sensing probes to understand the biological and biomechanical properties of the membrane. To accomplish a long-term functionality of the drugs and probes, it is imperative to build a platform that minimizes nanostructure internalization by the cell and detachment due to extracellular environment disturbance to “hold” the drugs and probes on the membrane for an extended period of time. However, it is particularly challenging because the membranes are dynamic and vibrant, involving multiscale deformations, for example cellular uptake. This work investigates the manufacturing of a two-layer interconnected mesh network using DNA origami tubes, and its ability to enable long-term retention on the exterior surface of the cell membrane. The first layer of DNA origami tubes bound to the membrane by hybridizing with membrane-immobilized initiators. The interconnections between tubes were accomplished by adding a second layer of tubes that hybridized with the first layer. Preliminary results showed the binding of two layers of DNA origami tubes were successful and the mesh network was formed on the surface of human umbilical vein endothelial cells (HUVECs) and human T-lymphocyte cells (Jurkat). Fluorescence intensity data revealed that the mesh network maintained a high level of signal intensity for at least 3 hours as compared to its initial intensity, whereas the intensity of a single layer tube without interconnections decreased rapidly. For future studies, membrane targeting drugs and peptides will be conjugated to the mesh and tested for their duration of functionality. This work will lay the foundation for long-term investigations of extracellular membrane environment, and extend the stability and therefore therapeutic efficacy of membrane-targeting drugs and peptides.

Developing Islet-on-Chip Model towards T2D disease Modeling

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Diabetes is a widespread disease that develops after the decreased function of pancreatic islets, which produce insulin for blood glucose regulation. The decreased function of islets can stem from an autoimmune elimination of the insulin producing beta cells (Type 1) or a toxic environment damaging the beta cells (Type 2). This project aims to use a fluidic device and human induced pluripotent stem cells (hiPSCs) to mimic the biochemical and mechanical environment of in vivo islets to develop a type 2 diabetic islet-on-a-chip model.

The islet-on-chip platform was developed using a modification of a commercially available Micronit fluidic device, which utilizes three glass slides to form a 2-chamber system partitioned by a middle membrane layer. The parent design allows for culturing adherent cells on the middle membrane layer, with the flexibility of cell seeding through perfusion flow or before chip assembly. Pancreatic islets lose their function and phenotype upon adherence and require retaining 3D conformation. Alternate seeding strategies were investigated to culture primary human islets in the Micronit device, leading to the development of a novel hydrogel-supported islet micropatterning technique on the membrane. Our islet-on-chip system could retain high viability and glucose stimulated insulin secretion (GSIS) of primary human islets over 4 weeks of perfusion culture under normal ‘fasting’ condition. Towards modeling of Type 2 Diabetes, islet glucotoxicity was simulated by long term exposure to pathological glucose levels, lipotoxocity by elevated free fatty acids and glucolipotoxocity with combinations of the two. In parallel, islet organoids were derived from hiPSCs to replace the primary human islet, and the fluidic culture system could maintain survival and function of the hiPSC-derived islets over extended culture period. These models can be further used to test T2D treatments to determine the reversibility of the toxic states and ability to simulate treating the disease in patients.

Accessing and Assessing the Cell-Surface Glycocalyx Using DNA Origami

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The glycocalyx is a physiologic biopolymer coating on the outer cell surface, serving as a critical barrier regulating cell-surface accessibility to macromolecules and other cells. Despite its pivotal pathophysiological roles, conventional glycocalyx characterization has largely been morphological rather than functional. Here, we report the use of DNA origami nanotiles as a functional measure of the glycocalyx barrier integrity. DNA nanotiles effectively accessed single-stranded DNA initiators anchored on the glycocalyx, but they failed to reach initiators associated with the underlying phospholipid bilayer (PLB). PLB accessibility was only permitted by extended nanotile-to-initiator spacing using DNA duplex bridges or by enzymatic glycocalyx degradation. Thus, DNA nanotiles but not DNA duplexes can be effectively expelled by the physiologic cell-surface glycocalyx, providing an effective functional readout of the glycocalyx barrier function. Furthermore, the nanotile-to-cell recruitment faithfully predicts cell-to-cell accessibility during DNA-guided multicellular assembly. Lastly, glycocalyx-anchored nanotiles exhibited enhanced stability and cellular uptake compared to PLB-associated nanotiles. We expect our findings to open possibilities of developing DNA nanostructure-based tools for assessing and manipulating the glycocalyx to modulate cellular interaction with its environment.

Effects of Anti-Bacterial Coated Extracellular Matrix Bioscaffolds on Immunomodulation and Mobilization of Progenitor Cells for Volumetric Muscle Loss Treatment

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Background: Porcine dermal Extracellular Matrix derived Bioscaffolds such as XenMatrix™ have been shown to promote constructive tissue remodeling and effective muscle regeneration in volumetric muscle loss models in both pre-clinical and clinical studies. The incorporation of anti-bacterial coatings within these bioscaffolds has been shown to inhibit the colonization of several strains of bacteria and is also associated with a decrease in post-operative complications.

Objective: The current study evaluated the effects of coating XenMatrix™ with two different anti-bacterial coatings, (i) minocycline and (ii) doxycycline, in combination with rifampin on the remodeling response in a murine volumetric muscle loss model.

Methods: XenMatrix™, XenMatrix™AB (rifampin and minocycline), and XenMatrix™AB-2 (rifampin and doxycycline) were provided by BD-BARD (Providence, RI). Scaffolds were implanted into the quadriceps of C57BL6/J mice (N=6 mice per treatment group) using an established model of volumetric muscle loss (Sicari et al., 2012). Animals with no scaffold implanted were used as a negative control. Animals were survived for 14 and 28 days after which they were sacrificed, quadriceps were explanted and sent for histomorphological processing. Explants were characterized for cell infiltration, vascularity, and multinucleated giant cells using histomorphological scoring by three independent scorers. Pro inflammatory (M1-like) and anti-inflammatory (M2-like) macrophage phenotype as well as perivascular stem cell (PVSC) mobilization were evaluated using immunohistochemical staining for CD11b/iNOS, CD11b/Fizz, and CD31/CD146 respectively.

Results: An increase in cell infiltration was observed in all scaffold treatment groups between 14 and 28 days. XenMatrix™AB-2 showed an increase in vascularity between 14 and 28 days and was significantly higher than the uncoated XenMatrix™ group at 28 days. All scaffold treatment groups expressed higher levels of M2-like macrophages (Cd11b+/Fizz+) than the untreated controls at 28 days. XenMatrix™ coated with doxycyline expressed higher level of M2-like macrophages than the XenMatrix™ coated with minocycline at 28 days. All groups showed basal levels of PVSC mobilization away from vasculature. At 14 days, all scaffold groups showed greater levels of PVSC mobilization in the center of the scaffold. At 28 days, anti-bacterial coated scaffolds showed greater levels of PBSC mobilization at the periphery of the scaffold.

Conclusion: Overall, the results from the present study support previous findings that ECM scaffold treatment promotes an M2-like phenotype in macrophages. While all ECM scaffold treatment groups promoted increased PVSC mobilization, anti-bacterial coated scaffolds showed significantly higher levels than uncoated scaffolds. Overall, the incorporation of anti-bacterial coating on ECM bioscaffolds may serve to not only reduce bacterial colonization, but also contribute to a constructive downstream healing response.

Understanding the mechanics of passive cellular responses during epithelial convergent extension

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Convergent extension (CE) shapes embryos and organs by narrowing a tissue in one direction and lengthening it in the orthogonal direction. Failure of CE leads to a variety of structural birth defects. Anisotropic cell behaviors are proposed to provide the motive forces for this process. However, a challenge arises from the heterogeneous nature of the tissues where these behaviors occur; for instance, prospective endoderm cells are considered largely passive participants in CE. Force-generating cell behaviors have been identified and characterized in several cell types, including prospective mesoderm and prospective neural deep cells. A broad array of computational models have focused on the role of anisotropic force production in CE, but none have sought to understand passive remodeling of cell sheets to externally applied anisotropic force. Here, we focus on this topic and have adopted a computational modeling approach to isolate the passive responses to tissue field elongation from contributions from active cellular processes. In the model, cells are represented as interacting particles in a rectangular bounding box. The incorporation of an attractive potential enhances tissue cohesivity and improves representation of long-range force transmission. Boundaries are moved such that the field of cells undergoes area-conserving CE. We present a new method of tessellation of cell centroids to allow for the heterogeneity needed to represent radial cell intercalation and extrusion. Time series of tessellated polygonal cell networks are analyzed to quantify cell neighbor exchanges and cell area fluctuations. Deformation and strain are calculated both globally at the tissue scale and locally by considering the domain of a cell and its immediate neighbors. We compare our model with in vivo observations of Xenopus neural CE. Extending our existing simulations by incorporating forces from active cell contractions will enable quantitative assessment of specific biochemical and mechanical drivers of tissue shape change.

Characterization of Pelvic Floor Muscle Fiber Architecture for Computational Modeling

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Objective: Current computational models of pelvic floor disorders (PFDs) lack functional physiologic representation of the fiber organization and mechanical behavior of the pelvic floor muscle (PFM). Knowledge of the PFM fascicle configuration is lacking, and is necessary to more accurately simulate PFDs. Therefore, the aim of this study was to establish an imaging analysis approach to determine muscle fiber orientation of the female pelvic floor for computational applications using images of cross-sectional anatomy from the Visible Korean (VK) dataset.

Methods: High-resolution colored images of pelvic anatomy from two female cadaveric subjects were used to segment the PFM. After processing the segmentations to normalize and reduce the file size of the VK images, a custom Mathematica code was used to calculate the fiber direction via gradient filter algorithms and principal component analysis. Houdini FX was then used to visualize and qualitatively assess the fiber orientation data represented by a 3D vector field.

Results and Conclusion: The image analysis method developed was able to quantify and show the fascicle architecture of the PFM. Future work will implement the fiber direction data into finite element models of the PFM to more appropriately simulate its mechanical behavior and to investigate the relationship between PFM morphology and function in patients with PFDs.

Using In-Vivo Morphological Measurements of Cerebral Aneurysm Blebs to Predict Aneurysm Rupture Risk

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Introduction: Cerebral aneurysm rupture is an extremely deadly but sporadic pathological condition of cerebral aneurysm. Thus, physicians must balance the risk of rupture during under close clinical observation and the risks associated with brain surgery. Currently published prediction models do not include morphological information besides aneurysm size. We hypothesize incorporation of bleb specific morphological information can better stratify aneurysms at risk of rupture.

Methods: A total of 35 patients with three-dimensional rotational angiography or computed tomographic angiography that had a patient record of rupture status were included in this study. From those images' patient-specific cerebral vasculature surfaces were segmented. We calculated surface curvature using a least-squares quadratic patch method using a 3-ring vertex neighborhood to construct the patch. We used an inter-observant agreed method to identify which elements on the vasculature surface belonged to the bleb, then calculated surface curvature both Gaussian and Mean at each vertex on the bleb surface. Finally, we calculated two global surface curvature metrics: the L2-norm of the Gaussian curvature (GLN) and L2-norm of the mean curvature (MLN) of blebs. A receiver operator curve and thereby area under curve (AUC) was calculated using global metrics for each patient in either the rupture or unruptured cohorts.

Results: We calculated a significant difference in MLN ($p=0.0003$) and GLN ($p=0.0007$) between ruptured and unruptured cohorts. This contributed to a high AUC for the MLN (AUC=0.8013) and GLN (AUC=0.8147) in rupture and unruptured cohorts.

Conclusions: The significant difference in MLN and GLN in blebs that have records of rupture status provides a good non-invasive discriminating factor and predictor of aneurysm rupture. In future studies, we would like to incorporate these and other strongly discriminating morphological factors in a statistical model that can be used in clinical decision making. Additionally, we are investigating the relationship between morphological measurements and their effects on biomechanics of the aneurysm wall.

A Predictive Model of Stromal Fibroblast-Mediated Drug Resistance in HER2+ Breast Cancer

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The tumor microenvironment can mediate tumor development and drug resistance through a myriad of growth factor/cytokine signals that can activate multiple pathways in tumor cells. Drug resistance is a major challenge in patients with HER2 overexpressing (HER2+) breast cancer, which accounts for ~20% of all breast cancer cases. Many of these patients (38-75%) do not respond to HER2 targeted therapies. Fibroblasts are a prominent cell type found in the tumor microenvironment that are linked to poor patient prognosis and drug resistance. Recent studies have found that fibroblasts co-cultured with HER2+ tumor cells prevent tumor cell death and increase tumor cell proliferation in the presence of a HER2-kinase inhibitor (Lapatinib). Fibroblasts confer lapatinib resistance in part through increased anti-apoptotic protein expression and PI3K/Akt/mTOR pathway activation in tumor cells; however, this resistance can be modulated by altering the number of tumor cells, number of fibroblasts, and drug concentration in vitro. An ordinary differential equation (ODE) model is used to identify factors that influence fibroblast-mediated protection (time, drug concentration, cell density) and predict conditions that yield tumor cell growth through tumor-fibroblast signaling despite HER2-signaling blockade.

Combined mechanical and optical simulation of the effect of compression on breast-tumor mimicking software phantoms

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Neoadjuvant chemotherapy (NAC) is used to shrink breast tumors prior to surgery and improve surgical outcomes. Pathologic complete response (assessed after surgery) is associated with patient survival, but no imaging method is approved for predicting this response early in therapy. Clinical palpation and structural imaging can track tumor size reduction but can sometimes fail to distinguish fibrosis from residual cancer. Near-infrared optical imaging is attractive for this application because of its sensitivity to endogenous contrast from hemoglobin, allowing non-invasive sensing of highly vascularized breast tumors. Spatial frequency domain imaging is a wide-field diffuse optical imaging method that uses frequency dependent blurring of patterned illumination to map tissue absorption and scattering properties in two dimensions. When used to image a non-homogeneous medium, the resulting image reflects a depth-averaged measurement with the highest sensitivity to superficial tissue. Embedded heterogeneities can be detected with SFDI, but contrast is lost with depth. For this reason, we have developed a hand-held breast imaging device in which with which localized compression is to be used to decrease the thickness of healthy tissue covering a breast tumor and enhance image contrast. In addition, the application of compression induces a hemodynamic response with the potential to serve as a biomarker for tumor vascular function. In response to NAC, breast tumors typically shrink, become less stiff, and exhibit reduced vascularity (lower light absorption by hemoglobin.) To better predict how these changes during therapy would affect the measured optical contrast from SFDI (as well as predict the contrast gained from compression) finite element analysis was used to model compression of a soft material with a stiffer embedded inclusion, followed by optical simulation to obtain a virtual SFDI result. The dependence of optical contrast and compression-induced contrast gain on tumor properties is discussed for its potential value in NAC outcome prediction.

Three-Dimensional Graphene Microelectrode Arrays for Detection of Wound Healing Biomarkers

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Volumetric muscle loss (VML) injuries from high-energy trauma or tumor ablation often result in scar tissue formation and permanent loss of skeletomuscular function. Following VML injury formation, neutrophils and macrophages are recruited to the wound site to release cytokines and growth factors to allow proliferation and innervation. Monitoring the wound healing process by spatially mapping key regulatory wound biomarkers, such as nitric oxide (NO), can elucidate the state of the wound and thus inform critical clinical interventions. While single-point NO probes with sufficient performance for short-term measurements have been produced previously, improvements in device sensitivity, stability, and the number of sensing nodes are needed for in vivo applications. Here we leverage novel nanomaterials and their exceptional electrochemical properties to construct three-dimensional fuzzy graphene (3DFG) microelectrode arrays (MEAs) for the multiplexed electrochemical sensing of NO. We have developed selectivity polymer coatings for 3DFG MEAs capable of selectively detecting NO (versus known interferents such as nitrite, ascorbic acid, and uric acid) at physiological concentrations in the nanomolar regime (limit of detection < 10 nM). Future work will entail in vivo NO detection to aid in monitoring wound states in animal models.