UNIVERSITY of WASHINGTON

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Online Proceedings

2E

Stem Cells and Regenerative Medicine

Session Moderator: Benjamin Freedman, Medicine/Nephrology

MGH 238

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Decellularized Cardiac Matrix Promotes Cardiomyocyte Maturation in Patient Derived iPSCs

Jessica Suzanne Johnson, Junior, Pre-Sciences
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

Patient specific individualized pluripotent (iPS) cells can be differentiated into cardiomyocytes that beat spontaneously, express sarcomeric proteins, and contain ion channels. However, these differentiated cardiomyocytes express a "fetal" like phenotype in that they are smaller, irregularly shaped, have a shortened sarcomere length, and lowered excitation-contraction rates that prevents their clinical applicability. In addition to executing their intrinsic genetic program, functional cardiomyocytes also receive inductive cues from their microenvironment. In the intact heart, those cues are supplied by the extra cellular matrix (ECM). The marriage of iPSC-derived cardiomyocyte technology with ECM differentiation cues will result in more mature cardiomyocytes that better represent natural human cardiomyocytes. Therefore, we are using a "native" cardiac matrix derived from a decellularized pig heart to try and promote maturation in cardiomyocytes using no mechanical inductive cues. We are particularly interested in the fibronectin to laminin ratio because the work of Wang, Lei, and Weighton (2015) suggests a proper ratio of these ECM components promotes maturation. Additionally, we are interested in the stiffness of the ECM coating as the cardiac environment has an optimal stiffness level. The iPSC will be differentiated into cardiomyocytes by using chemicals that alter the WNT signaling pathway. Some cells were exposed to the ECM during differentiation to measure the extent of cardiomyocyte maturation in the presence of ECM. Other cells were transferred to the ECM after the differentiation process. The extent of maturation will be measured by gene expression profiling and RNA sequencing. We hypothesize that the presence of cECM will enhance maturation based on the work of Oberwallner et al. (2014). If successful, this work will lead to improved drug screening and allow for tissue engineering for cell replacement therapies.

Development of an *in vitro* Disease Model of X-Linked Myotubular Myopathy from Patient-Specific Induced Pluripotent Stem Cells

Jonathan James Paul (Jonathan) Lawson, Junior, Biochemistry
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

X-Link Myotubular Myopathy (XLMTM) is a progressive congenital pediatric disease that affects one in every 50,000 male births. XLMTM is caused by a mutation of the MTM1 gene. MTM1 codes for myotubularin, an enzyme that is involved in the excitation-contraction coupling of muscle cells. XLMTM is characterized by a severe phenotype marked with muscle weakness and hypotonia that can result in many life-threatening complications, including diaphragmatic weakness leading to respiratory failure. Adeno-associated virus (AAV)-mediated gene therapy has reversed muscle pathology in murine and canine models. Treatment of the canine models with an AAV8-MTM1 vector indicated improved muscle strength as well as prolonged life. The field of muscle disease research would benefit greatly if an in vitro patient-specific induced pluripotent stem cell (iPSC) model were utilized to generate XLMTM-null muscle myotubes. It is necessary to develop an in vitro model of the disease using specific patient-derived cells because the patient-specific disease model has the predictive utility to determine if there are aspects of the disease not mitigated by gene therapy. This will help pinpoint the areas the disease affects in order to come up with a cure. To do this, MTM1-mutant iPSCs were differentiated stepwise to myogenic precursors and then to multinucleated myotubes using a small molecule approach that has been shown to mimic embryonic muscle specification. Extent of myotube differentiation was qualified with immunochemistry, using muscle-specific markers such as Myogenin, MyoD, and MF20. Experiments are currently underway to

identify the disease phenotype in vitro and correlate with known pathology observed in the patients with XLMTM.

Design of a Tissue-Engineered, Small-Diameter Arteriole for Vascular Disease Modeling

Dom (Dominic) Min Tran, Senior, Bioengineering
Mary Gates Scholar, UW Honors Program
Mentor: Ying Zheng, Bioengineering

Thrombotic microangiopathy is characterized by endothelial activation and microvascular thrombosis, and is associated with a group of life-threatening disorders, such arteriothrombosis, sepsis, and other small vascular diseases. With systemic inflammation, the arteriolar and capillary vessels are often found to be obstructed by platelet rich thrombi, leading to tissue ischemia and organ failure. Understanding the mechanism behind endothelial activation and induced thrombi formation during inflammation and vessel injury has important clinical implications that can lead to therapeutic development. Here, I developed a novel *in vitro* microvascular platform that mimics the *in vivo* arteriole architecture and function. This platform is generated based on a biocompatible polymer, polydimethylsiloxane, using injection molding. The device is lined with an extracellular matrix (ECM) using type I collagen for structural support. Human coronary artery smooth muscle cells (hCASMCs) are seeded within the bulk collagen to allow spontaneous reorganization and migration. A channel is created within the ECM using needle-based subtraction molding and is then seeded with human umbilical vein endothelial cells (HUVECs) to form an endothelium. Optimization was performed to provide consistent cell seeding of HUVECs within the channel, to allow cellular reorganization and alignment, and to form a functional small-diameter arteriole. We observed reorganization and alignment of both HUVECs and hCASMCs after 3 days of culture under continual flow. In addition, luminal patency was maintained with proper hCASMC contractile function. On-going studies include development of functional assays to recapitulate the inflammatory response and thrombi formation in arterioles. The study is expected to not only further elucidate the fundamental mechanism of arteriothrombosis, but to also provide a base platform to develop preventative or therapeutic medicine for those affected by these pathological phenomena.

Engineering an *in vitro* 3D Glomeruli-on-a-Chip in Native Matrices

Kiet T. (Kiet) Phong, Senior, Bioengineering
CoMotion Mary Gates Innovation Scholar, Mary Gates Scholar
Mentor: Ying Zheng, Bioengineering

The primary function of the kidneys is to filter waste from blood, and the kidney glomeruli are the major site for filtration. The glomerulus is a blood filtration barrier with low resistance to small molecules, but is relatively impermeable to macromolecules. Each glomerulus consists of a blood capillaries network with endothelial cells on a basement membrane, the other side of which is lined with specialized epithelial cells known as podocytes. The integrity and function of the glomerular filtration barrier is critical for kidney function, and disruption of this barrier correlates with kidney disease progression, and eventually kidney failure. Current research models for kidney disease include animal models, 2D, and 3D kidney epithelial cell cultures. However, animal models suffer from high cost and are too complex for studying detailed cellular interactions, while existing *in vitro* models lack the architecture and components to fully reproduce *in vivo* conditions. We propose a 3D microphysiological system that recapitulates the endothelial-epithelial interface found in the kidney glomerulus. We have developed a method to form independent microscale channels within a collagen I substrate, separated by a very thin layer of collagen. Collagen I is a native matrix protein that supports cell growth, invasion, and exchange of cellular signals. Primary endothelial and epithelial cells has been seeded into the channels to form closed lumina, whose overlaps form the endothelial-epithelial interface. However, culture conditions remain to be optimized to support both types of cells. Structure, morphology, and function of the interface are monitored by confocal microscopy, biochemical assays, and electron microscopy. When fully developed and validated, this culture system can be used to study the transport properties and injury responses of glomerular filtration. It can also provide a platform for drug screening to identify compounds toxic to the kidney, or therapeutic compounds for kidney diseases.

Automated Rehabilitative Training and Epidural Stimulation Following Spinal Cord Injury

Alice Catherine Bosma Moody, Senior, Neurobiology, Bioengineering
Goldwater Scholar, UW Honors Program
Mentor: Michael Sunshine, Rehabilitation Medicine
Mentor: Chet Moritz, Physiology & Biophysics

Over a quarter of a million individuals live with Spinal Cord Injury (SCI) in the United States and experience debilitating chronic conditions, reduction of quality of life, and an accumulation of high healthcare costs. Recent clinical studies have shown that epidural spinal stimulation can lead to improvements in motor function, weight bearing, and autonomic dysreflexia among patients with SCI. It is thought that epidural stimulation creates an excitatory environment in the spinal cord and allows descending signals that were previously too weak to effect a motor output to reach a suprathreshold level. While the results are promising, both the mechanism of action and optimal parameters for stimulation and rehabilitation remain unknown. Therefore, in order to further develop this potential therapy, it is necessary to cre-

ate a clinically relevant research model for the study of the treatment in question. This project addresses this through the development of an automated rehabilitative device for animal subjects following spinal cord injury, designed to mimic the extensive rehabilitation received by human patients with SCI and measure functional recovery following injury and during epidural stimulation treatment. Initial results show that subjects interact with the automated device, indicating a potential for more widespread implementation. Specifically, this high-throughput and automated system will be initially utilized for the study of the effects of epidural spinal stimulation in the rat cervical cord following contusion injury in order to quantify both transient and lasting motor improvements.

Assessment of Recovery from Spinal Cord Injury using a Haptic Interface Device

Oliver Riley (Oliver) Stanley, Senior, Bioengineering, Neurobiology

Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program

Mentor: Chet Moritz, Physiology & Biophysics

Spinal cord injury (SCI) can severely impair quality of life. While there are a variety of accommodations for individuals with SCI, there are no clinically available treatments which restore pre-injury levels of function to these patients. The optimization of emerging treatments for SCI will require a large amount of detailed experimental trial data in animal models. Assessments of trained motor behavior in animal models of SCI recovery typically fail to capture information about fine gradations in the course of recovery, which interferes with the development of new restorative treatments. To better characterize these gradations during the study of an emerging method for cervical SCI rehabilitation, I monitored forelimb movements of a cohort of injured rats during a motor behavior task using a high-precision haptic interface device - a machine which simulates physical interactions with virtual objects or events by applying forces to its user. I used the motion information gathered by this device to trigger electrical stimulation of the spinal cord to enhance attempted movements and encourage Hebbian plasticity within the spinal cord. Functional recovery of strength and range of motion was assessed relative to pre-injury baseline, demonstrating the utility of 3D haptic interfaces for the assessment of promising SCI treatments. Our goal is to help individuals with disabilities due to spinal cord injury regain function and independence by quantifying, optimizing, and the accelerating the translation of potential new treatments for SCI using these precise and clinically relevant recovery measures.