A hiPSC Model of Cardiomyopathies
Lauren D’amico, Senior, Public Health-Global Health
Levinson Emerging Scholar, Mary Gates Scholar
Mentor: Farid Moussavi-Harami, Internal Medicine
Mentor: Abigail Nagle

Cardiomyopathies are diseases of the heart characterized by structurally and functionally abnormal cardiac tissue and can be caused by non-genetic or genetic causes. Genetic cardiomyopathies are the most common genetic cardiac condition, affecting 1 in 250 to 500. The two most common types of genetic cardiomyopathies are hypertrophic (HCM) and dilated (DCM). HCM is characterized by a thickening of the heart muscle. This thickening can lead to a blockage in the blood flow and cardiac relaxation abnormalities. DCM is pathologized by a weakening in the cardiac muscle, leading to a lengthening and thinning in the muscle. My research focuses on determining human specific mechanisms of DCM and HCM, specifically on determining the early developmental phenotypes of the cells that lead to downstream pathologies. I particularly emphasize how changes in sarcomere function lead to HCM and DCM using human induced pluripotent stem cell cardiomyocytes (hiPSC-CM). We have shown that sarcomeric mutations alter the amount of tension integrated over time (TTI) and those variations in TTI are predictive of HCM and DCM. I am generating two mutant hiPSC-CM lines, L48Q and I61Q, using CRISPR/Cas9. These mutations are both in the sarcomere, more specifically in cardiac troponin C (cTnC). They alter the calcium binding properties of cTnC. I have optimized the polymerase chain reactions in order to make the sequencing data clean for validation. I have generated the I61Q line and am working on the L48Q line. After validating the lines, I will differentiate them to cardiomyocytes in order to study the cellular mechanisms involved. I will then use IonOptix to test early vs. late calcium transience, cell contractility, and cell size. There are currently no treatments that address the contractile abnormalities present in HCM or DCM. My research will allow for greater understanding of these mechanisms which will inform potential therapies.

Exploring Calcium Flux-Correcting Drugs to Alleviate Duchenne Muscular Dystrophy (DMD) Cardiomyopathy
Naveen Arunachalam Sakthiyendran, Senior, Biology (Physiology)
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

Cardiomyopathy is currently the leading cause of death for patients with Duchenne muscular dystrophy (DMD), a severe neuromuscular disease affecting young boys. With no current cure, gene therapy is a promising solution, but supplementation with drug therapies is likely inevitable to fully address the pathology seen in older patients. The use of human-induced pluripotent stem cell (hiPSC) models for drug studies is beneficial due to the direct relevance to human physiology and the potential development of personalized care. Dystrophic hiPSC cardiomyocytes have been shown to exhibit calcium reuptake delays, higher resting calcium levels, and frequent arrhythmias. The Mack Lab previously conducted a preliminary drug screen on healthy and DMD-affected cardiomyocytes and found that certain L-type calcium channel blockers (CCBs) indicated a cardioprotective effect. These drug compounds (namely Nitrendipine and Nimodipine) have been shown to alleviate cardiac fibrosis in patients through vasodilation. In this project, I am validating the beneficial aspects of the drug compounds. I initially hypothesized that treatment of DMD hiPSC cardiomyocytes with L-type CCBs will rescue resting calcium levels and normalize relaxation kinetics. To assess this, I cultured mature hiPSC cardiomyocytes on a Microelectrode Array (MEA) system capable of maintaining physiological conditions while measuring properties of cardiac electrophysiology. To enhance cellular maturity, I treated hiPSC cardiomyocytes with MicroRNA Matura-
tion Cocktail (MiMaC) and cultured long-term prior to MEA assessment. Using the MEA, I have found that the QT interval for DMD hiPSC cardiomyocytes was significantly longer than isogenic controls. In current experiments, I am using this platform to validate the effect of L-type CCB compounds of interest in relation to DMD cardiomyopathy. The development of this novel platform may not only have broader implications for DMD drug discoveries and targeted therapies, but it can potentially serve as a powerful preclinical model for other neuromuscular disorders.

Elucidating the Mechanisms of Desmoplakin Premature Truncating Variants Leading to Arrhythmogenic Cardiomyopathy
Leslie Sy Ling Chao, Senior; Microbiology
Washington Research Foundation Fellow
Mentor: Daniel Yang, Medicine

Arrhythmogenic cardiomyopathy is a life-threatening inherited disease that can result in sudden cardiac death or heart failure. One cause of this heart disease is a pathogenic mutation in the desmosomal protein named desmoplakin (DSP), which is a critical component in humans for maintaining the structural integrity of adjacent cells. Since over 70% of pathogenic DSP variants have been found to be premature truncating variants (ptvs), we hypothesize that DSP haploinsufficiency leads to arrhythmogenic cardiomyopathy. Engineered heart tissues (EHTs) from patient specific induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) were previously generated from two unrelated patients with different DSP-pty mutations. Max EHT twitch force measurements suggested that the DSPptvs were weaker compared to wild type. To control for genetic differences in patient derived iPSC-CMs, the DSPptv mutations of these two patients were created in an unrelated normal patient WTC iPSC line using CRISPR/Cas9 in order to study these mutations in a genetically isogenic background. iPSC-CMs from these two isogenic lines are currently being used to generate EHTs to determine the impact these DSPptvs have on cardiac tissue function. DSP protein levels of these isogenic iPSC-CMs are also currently being analyzed to determine the effect that these mutations have on DSP protein abundance. We expect the max EHT twitch force measurements to be weaker and lower levels of DSP protein found in the DSP isoforms EHTs and iPSC-CMs compared to wild type. If these experiments suggest haploinsufficiency, we will overexpress DSP in the mutant lines to determine if restoring normal DSP protein levels can rescue contractile function. Overall, this study will help us better understand the mechanisms of DSPptv-mediated arrhythmogenic cardiomyopathy, in hopes to potentially identify a novel therapeutic treatment for these patients.

Engineered Heart Tissue Image Processing Suite
Alan Reuben Levinson, Senior; Bioengineering
Mary Gates Scholar
Mentor: Nathan Snidecki, Mechanical Engineering
Mentor: Samantha Bremner, Bioengineering

Induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) that have been engineered into three-dimensional heart tissues (EHTs) are valuable research tools for investigating debilitating genetic diseases that affect the heart, such as Duchenne muscular dystrophy (DMD). Ensuring iPSC-CMs can be sufficiently matured to model such diseases remains a hurdle in current research, and maturational analysis techniques for iPSC-CMs are either qualitative, manual, or primarily based in two dimensions, leaving much to be desired. This poster details the creation of a suite of MATLAB image-processing scripts that can quantify the effect of three-dimensional culture and disease-causing DMD mutations on cardiomyocyte structure and maturation state. The iPSC-CMs were differentiated from stem cells, cast into EHTs, stained using immunofluorescence, and imaged using confocal microscopy. Using the scripts to analyze these 3D images of iPSC-CM stains, key maturational features of the cells can be quantified such as nuclei count; cardiomyocyte area; and sarcomere length, orientation, and z-disk width. Analyzing cardiomyocyte area can give key information on cardiomyocyte hypertrophy while examining sarcomere length, orientation, and Z-disk width can provide information on myofibril structure and organization. The suite allows analysis of these maturational features in both 2D and 3D cultures and offers a method for quantitatively assessing maturation in an automated manner. Measuring iPSC-CM maturation will also allow better comparison of existing maturational methods, such as mechanical loading, electrical stimulation, and small molecule treatment. The suite can also create graphical outputs to elegantly display data. Recent progress also includes a script that can count cell nuclei and quantify cell area. Overall, the suite will help improve maturational analysis of EHTs, and hopefully contribute to the discovery of new treatments for diseases that affect the heart.

Modeling Structure and Mechanical Changes for Nemaline Myopathy-Inducing Mutation H40Y in ACTA1 Simulated in the Presence of Designed Therapeutic Small Molecule
Joanne Boysen, Senior; Bioengineering
Mary Gates Scholar
Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine
Mentor: Matthew Childers, Bioengineering

Nemaline Myopathy (NM) is a severe genetic muscle disorder defined by muscle weakness and the presence of nemaline rods (rod-shaped intracellular aggregates). This dis-
ease is associated with multiple clinical subtypes that result from pathogenic genetic variants across 12 different genes, including skeletal α-actin (ACTA1). NM-associate mutation H40Y impacts the ACTA1 monomer structure such that it disrupts polymerization. Without efficient and accurate polymerization, ACTA1 monomers form altered protofilaments which do not properly support the cross-bridge cycle and result in contractile dysfunction. The variation and complexity of NM pathology coupled with the rarity of this disease have served as significant barriers to the development of any treatments for NM. Here we show the effects of top performing therapeutic small molecules simulated in the presence of NM-associated mutation H40Y on the structural and mechanical properties of ACTA1. Using Molecular Dynamics simulation data, we have quantified differences between H40Y and wildtype ACTA1. Furthermore, we searched for and designed a target small molecule to fix mutant actin polymerization and mechanical instability. Our results demonstrate how our lead designed small molecule alters the dynamics of the H40Y ACTA1 pentamer when simulated docked in its intended binding pocket. We anticipate that our best small molecule candidate will be tested in vitro for its ability to impact actin polymerization in polymerization assays produced from induced pluripotent stem cells bearing the H40Y mutation. Furthermore, following successful in vitro validation the small molecule may be extensively studied as a potential novel therapy for NM.

**Three-Dimensional (3D) Engineered Bone Tissue to Investigate the Effects of Dynamic and Static Loading on Bone Development In Vitro**

*Karen Sugimoto Gaffney, Senior, Bioengineering: Data Science*

Mary Gates Scholar

Mentor: David Mack, Rehabilitation Medicine & Bioengineering, Institute for Stem Cell and Regenerative Medicine

Mentor: Samantha Bremner, Bioengineering

In the United States, 1.5 million individuals suffer a fracture due to bone disease each year. It is well documented that mechanical load affects bone development, but our understanding of the cellular mechanisms behind bone development under load is limited. Current human induced pluripotent stem cell (hiPSC) derived bone tissue models have more relevant human physiology compared to traditional animal models. However, there is a lack of dynamically loaded hiPSC bone tissue and diseased hiPSC bone tissue models in vitro. We propose a novel, three-dimensional bone tissue model as a platform for musculoskeletal disease modeling that allows for compressive loading that will enhance maturity as well as induce diseased bone phenotypes. We improved upon existing poly-L-lactide solvent cast scaffold techniques by incorporating a polyvinyl alcohol mold and an annealing step that increases the uniformity of the scaffolds and allows for higher throughput fabrication. Osteoblasts were derived from hiPSCs using established differentiation protocols and seeded into the 3D, porous, poly-L-lactide scaffold to generate in vitro bone tissue that generates significant extracellular calcium. We propose an arduino-powered, 3D-printed loading device that can apply physiologically relevant dynamic loads to the scaffold and hypothesize improved bone tissue maturity in comparison to 2D cultures and unloaded 3D scaffolds. By screening for markers of early bone development such as type I collagen, markers of later development such as osteocalcin, and assays for extracellular calcium, we can track the maturity and development of bone tissue. We expect that 3D bone growth with static loading will reveal diseased bone phenotypes such as decreased calcium deposition and immature bone, whereas dynamic loading will promote bone growth and lead to mature bone. Ultimately, this model will improve our ability to investigate the effects of mechanical loading in developing and diseased bone.

A Novel Approach to Segment Specialized Annotations in Electron Microscopy Images of Glomerular Podocytes

*Andre Ye, Freshman, Center for Study of Capable Youth*

*David Dmitrievich Smerkous, Senior, Applied & Computational Mathematical Sciences (Scientific Computing & Numerical Algorithms)*

Mentor: Behzad Najafian, Laboratory Medicine & Pathology

Podocytes reside in the glomerulus of the kidney and play a key role in the glomerular filtration barrier. Most diseases causing end-stage kidney disease are linked to podocyte injury. Moreover, these cells do not regenerate. Thus, detecting podocyte injury is critical. Adjacent podocytes are connected by foot processes, cellular extension structures that can be viewed by electron microscopy. Increased foot process width (FPW) is a key feature of podocyte injury and correlates with impaired glomerular filtration and kidney disease progression. The current gold standard for measuring FPW is unbiased stereology involving human measurements, which takes 6-8 hours per biopsy. Deep convolutional neural networks (DCNNs) can be used to significantly decrease the time and labor required by identifying cell features in electron microscopy images. However, when annotations are locality-specific and physically small, traditional DCNN approaches perform poorly. We present a novel approach for the segmentation of locality-specific annotations in cellular images and demonstrate its superior performance in identifying cell features on podocyte images. Firstly, we show that the problem of modeling small annotations consistently in proximity to a cross-image cell feature can be simplified into a two-step modeling process: one model segments the cross-image cell feature; another dependent model segments on smaller windows along the predicted feature segmentation. Secondly,
we show that dynamically dilating the size of small annotations from an inflated representation down to its original size over the duration of training improves model generalization. This approach yields a validation DC (dice coefficient) of 0.80 compared to a baseline of 0.64 (range: 0-1) on a podocyte segmentation dataset. These findings demonstrate general techniques for robust modeling of locality-specific and small cell segmentation tasks beyond just podocyte cell segmentation. I was involved with designing, implementing, and experimenting with the development of the deep learning approaches and their evaluation on the dataset.