Engineering High-Performance Cardiac Tissue by Simulating the Mechanics of the Heart
Shiv Bhandari, Senior, Bioengineering, Biochemistry
Mary Gates Scholar, UW Honors Program, Washington Research Foundation Fellow
Mentor: Charles Murry, Pathology
Mentor: Jialing Ruan, Bioengineering

Affecting millions of people across the globe, the WHO deems myocardial infarctions (heart attacks) as the largest cause of death worldwide. The heart’s weak regenerative capacity leads to scarring of the dead tissue and a reduction in cardiac output that presents numerous health consequences. Tissue engineering is a promising outlook in restoring the structural integrity and contractile force of the damaged area. Recent findings have demonstrated that engineered heart tissues grown from stem cells can display significantly improved characteristics when subjected to mechanical stimuli. Inspired by these results, this project seeks to improve tissue performance even further by investigating a novel concept where stem cell-derived cardiac tissues are treated with the same mechanical profile experienced by native heart tissue. In order to simulate and implement these forces, tissues are grown and then anchored onto two posts that can vary in distance and stiffness. Upon contracting, post stiffness is increased providing a resistance to each beat. This resistance simulates the afterload stress that the ventricular wall contracts against to eject blood into the aorta. Next, the tissues are stretched by increasing the distance between each post. The stress produced by elongating the tissues simulates the preload stress exhibited by the ventricular wall as blood is pumped into the chamber prior to the next contraction. Finally, combining the stiffness (afterload) and stretching (preload) schemes allows the tissues to experience the entire force profile exhibited by the native heart. Simulating the cardiac mechanical environment gives the engineered tissues the stimulus to yield contractile forces and a cellular structure at par with their native counterparts. This investigation thus aims to advance regenerative medicine by bringing stem-cell derived cardiac tissues one step closer towards curing myocardial infarctions.

AAV6-Mediated Overexpression of Ribonucleotide Reductase (R1R2) Enhances 2-Deoxy-ATP Concentration In Vivo and Improves Cardiac Function
Elizabeth A (Beth) Gay, Senior, Biochemistry, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program, Undergraduate Research Conference Travel Awardee
Mentor: Michael Regnier, Bioengineering
Mentor: Sarah Nowakowski, Bioengineering

Impaired systolic function resulting from acute injury, congenital defect, or aging can lead to cardiac complications and heart failure. Current therapies are unable to rescue cardiac function, but merely slow disease progression. We have demonstrated that when myosin uses 2-deoxy-ATP (dATP) as an energy substrate for contraction instead of ATP, cardiac contractility is enhanced by 20% or more. Viral-vector mediated overexpression of R1R2, the enzyme that catalyzes production of deoxy-nucleotides, increases intracellular dATP. In this study we determined the expression pattern of R1R2 in mice following systemic delivery of recombinant adenovirus pseudotyped with the type 6 capsid (rAAV6) carrying R1 and R2 subunits, and a cardiac specific gene regulatory cassette (cTnT455). Cardiac function was assessed in vivo via echocardiography, and ex vivo using Langendorf perfusion to test the hypothesis that elevating myocardial dATP by a gene therapy approach enhances cardiac function. We also performed metabolomics evaluation of both skeletal and cardiac muscle to correlate with changes in cardiac function. Our current data suggest that systemic delivery of this rAAV6 system results in myocardial specific overexpression of R1R2 and improves basal cardiac performance, which supports our previously published data for transgenic animals with long-term overexpression of R1R2. Metabolic analysis suggests that R1R2 overexpression...
Cardiomyocytes are the optimum cell source to model and study heart diseases and to screen drugs for cardiotoxicity in vitro. Cardiomyocytes differentiated in vitro from human pluripotent stem cells, however, are structurally and functionally immature. It is necessary to accurately recapitulate the behaviors of cells in the native myocardium for pathogenesis and drug-screening assays. Therefore, before the application of human pluripotent stem cells-derived cardiomyocytes (hPSC-CMs), we must address their maturation state. Physical cues presented to neonatal rat cardiomyocytes in a specific scale matching their native microenvironmental niche have been shown to profoundly regulate their structure and function, as characterized by elongated anisotropic morphology, well-organized contractile machinery, and faster action potential propagation. It is unknown, however, whether the same nanotopographical cues would have a similar positive influence on the maturation of hPSC-CMs. In this study, we tested the hypothesis that anisotropic nanopatterned substrates would enhance the maturation of hPSC-CMs, resulting in cardiomyocytes more suitable for in vitro cardiac tissue engineering applications. To test this hypothesis, we seeded hPSC-CMs differentiated from the matrix sandwich method on nanopatterns of various dimensions and compared their maturation with hPSC-CMs cultured on traditional flat substrates. To characterize maturation, the structural and functional phenotypes of hPSC-CMs were analyzed from their morphology/alignment, calcium handling properties, and gene expression. It was anticipated that, similar to neonatal rat ventricular cardiomyocytes, hPSC-CMs would exhibit a size-dependent change in their structure and function and that there would be an optimal substrate dimension size resulting in the most mature hPSC-CMs. The experimental result could yield a hPSC-CMs culturing protocol for deriving mature human cardiomyocytes for future drug screening assays and heart disease modeling. After optimizing substrate dimensionality, other culturing aspects such as chemical and mechanical cues and their temporal introduction to cells can be tested to further enhance the maturation of hPSC-CMs.

Cardiovascular disease remains the leading cause of death worldwide. The heart is one of the most resistant organs to self-repair from injury, e.g. myocardial infarction. Stem cell therapies, and tissue engineering have had limited success in inducing heart tissue to regenerate owing to the failing incorporating morphological, electrical, mechanical cues that are important for physiological functionality of transplanted heart tissues. Pre-engineered functional tissue on scaffold prior to myocardial transplantation enables recapitulating topological, mechanical, electrical and biological cues of healthy myocardium extracellular matrix (ECM), while providing cites for adhesion and migration of cells. In this study, we investigated one of the potential novel materials, Graphene Oxide, for pre-engineered cardiac tissue maturation. We performed various analyses to confirm maturation of cardiac tissue on graphene oxide physiologically, morphologically, and functionally. Graphene Oxide showed positive effects on maturation of cardiac tissue. Neonatal Rat Ventricular Myocyte tissue showed anisotropic cooperation with contraction when it was on the material. Interestingly, Graphene Oxide substrates were reduced during cell culture, which was confirmed by Raman spectroscopy. However, mechanisms for the reduction are still need to be explored. We would like to continue our study on finding underlying mechanisms and role of Graphene Oxide on cellular redox reaction. We are cooperating this material with nanogrooved substrates to build a platform for cardiac tissue maturation for tissue implantation.
human pluripotent stem cell-derived cardiomyocytes. Made possible by well-established nanopatterning techniques, cardiomyocytes are cultured on a poly(dimethylsiloxane) thin film with topographical features layered with multiple functional substrates using spin coating and soft molding. The thin film with tunable dimensions is employed as a substrate upon which force generation of cardiomyocytes can be characterized. Under the poly(dimethylsiloxane) layer, poly(N-isopropylacrylamide), a thermal responsive polymer that transforms from a hydrophobic form to a hydrophilic form, is spin coated and utilized as a sacrificial layer that will release the thin film when the temperature is lowered. After the release of the thin film, measurement of the deformation of the thin film allows for the calculation of force generation and gives a direct representation of the effects of cardioxicity on the overall cardiovascular system functionality. Optimization of the thin film fabrication was completed and upon scanning electron microscopy observation, high fidelity of the nanopattern was observed. This biomaterial platform better mimics the native human heart tissue and has the ability to characterize the force generation of cardiomyocytes. Furthermore, this platform could be utilized as a drug-induced cardiotoxicity assay which could potentially benefit the pharmaceutical drug development.

Characterization of Cell Viability and Proliferation of Chitosan-Alginate Scaffolds for Tissue Engineering
Samara Kate (Sam) Sytsma, Senior, Materials Science & Engineering

UW Honors Program, Undergraduate Research Conference Travel Awardee
Mentor: Miqin Zhang, Materials Science & Engineering
Mentor: Sheeny Lan Levengood, Materials Science and Engineering

Tissue engineering is a field of study where porous scaffolds made of materials such as polymers and/or ceramics are used to promote tissue regeneration. This is important for treatment of tissue defects resulting from trauma or disease where repair does not occur via normal, physiological processes. In this work we fabricated 3D, porous scaffolds made of naturally-derived polymers, chitosan and alginate, using a phase separation and lyophilization technique. This chitosan-alginate (CA) scaffold is promising for tissue engineering because it is made of sustainable materials and is biocompatible, biodegradable, and cost-effective. We characterized the porosity of the scaffolds using scanning electron microscopy and observed interconnected pores on the order of 100 microns in diameter. As a first step in determining the potential of this CA scaffold for muscle tissue engineering, we cultured C2C12 mouse myoblasts and evaluated cell viability and proliferation using the Alamar Blue assay. The scaffolds support cell attachment and viability and we expect to observe cell proliferation.

Effects of Mechanical Stretch on AAV Mediated Gene Therapy in Cultured Skeletal Muscle
Hannah Maricia (Hannah) Wear, Senior, Aquatic & Fishery Sciences

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

Gene therapy for monogenic diseases is undergoing an exciting resurgence in the last few years. Patients with hereditary blindness, blood cancers and hemophilia have been cured with minimal to non-existent long-term side effects. Our lab has been studying X-linked myotubular myopathy—a rare disease of young boys that causes profound weakness in all skeletal muscles including the diaphragm. An adeno-associated viral (AAV) vector gene therapy has been proposed to treat the X-linked muscle degenerative disease. However, the viral dose required is in excess of 10^{13} viral particles per kilogram. The goal of this proposed project is to find ways to manipulate muscle cells to increase virus uptake and enhance activity of the muscle protein expression. Artificially induced mechanical stretch and contraction have been shown to stimulate intracellular signaling and gene expression. Thus, we hypothesize that preconditioning muscle cells by in vitro stretching will increase both virus uptake and gene expression. This study aims to test the influence of mechanical stretch on muscles cells treated with the AAV mediated gene therapy by inducing stretching motions with the FlexCell machine before vector treatment. The FlexCell produces cellular stretching through regulated fluctuations in air pressure. Skeletal myocytes were cultured on a clathrin coated bioflex plate and underwent cycles of equibiaxial cyclical stretch for a set time period. Cell cultures were then treated with the proposed myotubular myopathy AAV vector therapy. Transgene expression was compared using immunofluorescence between cultures that underwent mechanical stretch and cultures that did not to determine the effects of stretch in virus uptake and transgene expression. Increases in both will enable future attempts of gene therapy in animals or humans to use lower doses, which should be accompanied with lower toxicity and fewer and less severe adverse side effects.