Undergraduate Research Symposium May 19, 2017 Mary Gates Hall

Online Proceedings

POSTER SESSION 1

Balcony, Easel 100

11:00 AM to 1:00 PM

Paramater Estimation and Association between Different Growth Conditions for Cell Circuits

Keagan Moo, Senior, Bioengineering, Informatics
Mentor: Kyung Kim

The study of gene regulatory networks, both synthetic and natural, is at the center of modern medical and agricultural science. Gene regulatory networks – gene circuits – are the foundation of life and have been widely applied towards molding biology to better serve human interests. This potential, however, cannot be fully leveraged because of the complexity of genetic systems and the amount of time it often takes to get an accurate characterization of them. They are often complex, nonlinear, and responsive to a confounding number of environmental factors ranging from temperature, to light, to media composition. We aim to exploit data obtained from multiple kinds of experiments to enhance the accuracy of estimations of system parameter values of gene circuit mathematical models. For this aim, we first demonstrate a computational analysis method for automatic parameter estimation through use of Sequential Monte Carlo (SMC), a learning algorithm that uses randomness to estimate the parameters that govern a system. Using species input from real cell culture and a simulation model of the genetic circuit we are measuring, the algorithm can estimate parameter values over the course of many iterations. Though this computational method is not new, we will combine it with a transformation function that can compare estimated parameter distributions from different growth conditions in order to understand how the circuit in question has been affected by the change in environment. Often in research only one type of growth environment is used to demonstrate how cells or other biological functions work, but cells produce distinct results in different conditions. The transformation function will use the correlation and mean of predicted parameters for the same circuit in different growth conditions to understand how different factors influence the system and give researchers the ability to compare various kinds of experimental conditions.

POSTER SESSION 1

MGH 241, Easel 153

11:00 AM to 1:00 PM

Ndc80 Complex Length Affects Dam1 Complex Inter-Ring Distance and Cell Viability

Athena Gabrielle (Athena) Bollozos, Senior, Dance, Biochemistry
Mentor: Trisha Davis, Liberal Arts
Mentor: Jae ook Kim, Biochemistry

Living organisms are composed of cells; thus, cells are called the basic building blocks of life. In order for an organism to grow, cells undergo mitosis. During mitosis, chromosomal DNA is replicated and segregated into two daughter cells. Errors in segregation can lead to the development of some forms of cancer. Because of this, equal chromosome segregation is a critical mitotic process. Microtubules are fibril-like cytoskeletal structures that are responsible for chromosome separation. They originate from two centrosomes. The growth and extension of microtubules push the two centrosomes apart, creating two poles in a mitotic cell. Kinetochores bind to these microtubules and align at the center of the cell. Disassembly of these microtubules is coupled to the equal segregation of sister chromatids. In *Saccharomyces cerevisiae*, correct kinetochore-microtubule attachment is mediated by the Ndc80 and Dam1 complexes. The heterotetrameric and rod-like Ndc80 complex is the main microtubule-binding component of the kinetochore. The heterodecameric Dam1 complex forms oligomeric rings around microtubules and enhances the microtubule-binding capacity of the Ndc80 complex. In vitro, the Ndc80 complex bridges two Dam1 complex rings, yielding a specific 33 nm inter-ring distance. To investigate the importance of this distance, we constructed various mutant Ndc80 complexes to further increase the Dam1 complex inter-ring distance. Specifically, each Ndc80 complex mutant contained an 8, 10, or 12 heptad repeat insertion that elongated the length of the coiled-coil domain of the Ndc80 complex by 8, 10, and 12 nm, respectively. We tested if these mutant Ndc80 complexes support cell division by transforming a yeast strain that does not contain wild-type Ndc80 gene and selecting for growth on SD-ura low adenine plates. These produced non-sectoring red colonies; therefore, the constructs did not support cell division. This suggests the specific 33 nm Dam1 complex inter-ring distance is important for cell viability.

SESSION 1G

NEW CHEMISTRIES AND MATERIALS

MGH 248

12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Characterization of Poly(alkyl glycidyl ether) Homopolymers as a Platform for Use in Stimuli-Responsive Hydrogel Synthesis and 3D Bio-Printing

Cecilia G. (Cece) Martin, Senior, Biochemistry, Chemistry
Mary Gates Scholar
Mentor: Alshakim Nelson, Chemistry

Stimuli-responsive hydrogels represent a promising class of inks for use in 3D-bioprinting. These materials respond to external stimuli such as heat, light, pH, and pressure, and researchers have utilized these different stimuli responses to develop inks for direct-write 3D printing. The objective of our work is to understand how the composition of poly(alkyl glycidyl ethers)–more specifically, allyl-, ethyl-, isopropyl, n-propyl-, and methyl-glycidyl ether homopolymers–affects their thermal response in aqueous solutions. UV-Vis Spectroscopy was used to determine the lower critical solution temperature (LCST) of each homopolymer, varying the molecular weight, concentration and polymer composition to demonstrate poly(alkyl glycidyl ethers) versatility as a platform for synthesis of stimuli-responsive hydrogels. We determined that the identity of the alkyl group significantly affected the temperature-response of the respective hydrogel. These studies will guide our work toward developing temperature and shear-responsive inks for 3D-bioprinting. In particular, these materials can be used to develop printed hydrogel lattices for whole-cell catalysis to produce medicinal compounds.

POSTER SESSION 2

Balcony, Easel 120

1:00 PM to 2:30 PM

Effects of Drift Gas Selection on the Ion Mobility of Protonated Quinoline and Its Analogues

Anna Bakhtina, Senior, Biochemistry
Mentor: Matthew Bush, Department of Chemistry
Mentor: Kimberly Davidson

Ion Mobility Mass Spectrometry (IMMS) has become an important tool for separating small molecules such as peptides, glycans, and petroleum molecules. Ion Mobility is most often performed in He, N_2 or air. There is a great interest in using alternative drift gases to increase selectivity of ion mobility separations, specifically for complex mixtures like petroleum. In this study we measured arrival time distributions (ATD) of a set of double bond equivalents (DBE) in four different gases (He, N_2, CO_2, Ar) which span a range of masses and polarizabilities. Quinoline, isoquinoline, and their DBE were chosen because they are aromatic compounds that are easily protonated due to the presence of nitrogen and are similar to molecules found in petroleum. The measured ATDs were plotted as a function of reciprocal drift voltage; the resulting slope is inversely proportional to mobility. Using the mobility and the Mason-Schamp equation, we determined the collision cross section of each molecule with each drift gas. We also investigated the relationship between the dipole moment of an ion and its collision cross section. In general, collision cross sections increase with drift gas size and polarizability. Additionally some of the DBE were better separated with larger, more polarizable drift gases (N_2 and CO_2). We have shown that IMMS can be used with different gases to improve separation between ions of similar size and shape. This method can be used in future investigations of petroleum mixtures to potentially identify a wider range of molecules.

POSTER SESSION 2

MGH 241, Easel 123

1:00 PM to 2:30 PM

Fabrication of Tissue-Engineered Arterial Vessel

Shi Ying Calysta (Calysta) Yan, Senior, Bioen: Nanoscience & Molecular Engr
UW Honors Program
Mentor: Deok-Ho Kim, Bioengineering
Mentor: Alec Smith, Bioengineering

Preclinical screening and toxicity studies are crucial steps in the drug development processes to ensure safety of new compounds when exposed to patients during clinical tests. Cellular responses in blood vessels play an important role in drug screening in terms of permeability and control of blood flow. The structure of the smooth muscle cell layer is crucial to the function of vessels in terms of contractility, mechanical properties and extracellular matrix production. Through recapitulating native tissue properties, tissue-engineered vessel models provide insights to study cellular responses when exposed to different environments, such as drugs. Although much research has been done in the development of tissue-engineered blood vessels, limitations in current *in vitro* models are yet to be addressed. Existing models such as synthetic and autologous vessel grafts are limited by thrombosis, non-native cellular resonses and poor mechanical properties. Bioreactors or microfluidics device are limited in terms of the complexity of the model and the scale to mimic larger vessels. The project addresses the need for an *in vitro* tissue-engineered arterial vessel model that replicates *in vivo* functionalities of the vessel by developing a fabrication method to

form smooth muscle cell tubes that is reproducible and functional. The project is divided into three phases: (1) Formation of three-dimensional smooth muscle cell tubes using a cell sheet rolling method, (2) Optimization of the rolling method and (3) Validation of model by contractility analysis. Ultimately, the success of this project allows formation of vessel graft with functionalities in terms of mechanical properties, compliance and contractility. Vascular grafts can be created by human or patient derived sources and serve as drug screening or disease modeling purposes.

POSTER SESSION 2

MGH 241, Easel 124

1:00 PM to 2:30 PM

Bioprinting Viable Three Dimensional Aligned Tissue Constructs via Simple Biomaterial Post System

Joseph Long, Recent Graduate, Bioengineering, University of Washington

UW Post-Baccalaureate Research Education Program

Mentor: Deok-Ho Kim, Bioengineering

Mentor: Jonathan Tsui, Bioengineering

Heart disease and other cardiac degenerative diseases account for 1 in 4 deaths in the United States. Current *in vitro* tissue models for cardiac disease modeling and potential drug testing lack dimensionality, scale, biomimetic behavior, and longevity. Manipulation of biophysical cues via biomaterial scaffolds in 3D bioprinting methods can alleviate these limitations as have been shown in 2D models. By mechanically inducing alignment in 3D tissue models, cells will respond to extracellular cues and form more organized structures similar to *in vivo*. This project focuses on implementing cardiac muscle cells into a mechanically aligned structure using a decellularized extracellular matrix (dECM) "bioink". A BioBots extrusion based bioprinting system is utilized to manufacture cardiac cells laden in porcine left ventricle cardiac muscle dECM. We hypothesize that a polycaprolactone (PCL) post system design will induce cell alignment in a band-like tissue structure suspended in a Pluronic F-127 polymer bed along the axis of tension during tissue manufacturing. Induced bioink tension will enhance cardiac structure and function compared to current bioprinted models. This bioink composition and biomaterial process will provide cardiac cells with a better niche for development into a mature state that accurately mimics in vivo conditions. Bioprinted tissues will exhibit increased mechanical properties and structural organization with expression of contractile markers via uniaxial cell alignment upon bioink gelation in culture. This process is high throughput and provides a streamlined standard method for drug screening that more accurately tests possible side effects inside native heart tissue.

POSTER SESSION 2

MGH 241, Easel 125

1:00 PM to 2:30 PM

Heterogeneous Coating of Reduced Graphene Oxide and Polydopamine on Nanostructured Substrates Using Dopamine Chemistry

Evan Jihong (Evan) Lam, Senior, Chemical Engineering

Mentor: Deok-Ho Kim, Bioengineering

Mentor: Kevin Gray, Bioengineering

The use of graphene in nanoscale systems serves many applications not only in solar cells but also in biomaterials because of the combined optical, electrical, and structural properties. Conventional methods including chemical vapor deposition and electrospraying to produce a layer of graphene on nanostructures is costly. Reduction of graphene oxide using chemical reagents presents an easier and cheaper alternative while maintaining the unique properties of graphene. But these methods are typically solution-based and further steps must be taken to coat reduced graphene oxide onto nanostructured surfaces. Dopamine chemistry was applied in a simple simultaneous approach of self-polymerization to reduce graphene oxide while creating an adhesive to incorporate a heterogeneous coating of polydopamine and reduced graphene oxide on a nanostructure. Use of various concentrations of graphene oxide demonstrated control over the sample's surface conductivity profile. Surface analysis characterizations confirmed the presence of the coating through X-ray photoelectron spectroscopy (XPS) while the electrical properties were determined using conductive atomic force microscopy (cAFM). The overall topographical morphology will be confirmed with atomic force microscopy (AFM). A potential application of the proposed device serves to provide functionally and structurally mature human pluripotent stem-cell derived cardiomyocytes (hPSC-CMs) for use in research and clinical drug trials.

SESSION 2B

CHEMISTRY, BIOCHEMISTRY, AND MATERIALS SCIENCE

Session Moderator: Sharona Gordon, Physiology and Biophysics

MGH 228

3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Immune Modulation of Electrospun Nanofibers on Dendritic Cell Activation

Namratha Potharaj, Senior, Bioengineering
Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program
Mentor: Kim A. Woodrow, Bioengineering
Mentor: Jaehyung Park, Bioengineering

Vaccines save approximately 2.5 million lives every year, and vaccine development is an ongoing area of research in immunology. A vaccine is formulated with antigen and adjuvant, the latter of which enhances the host immune response to an antigen via stimulation of antigen presenting cells (APCs) like dendritic cells (DCs). Only a few adjuvants are currently approved for clinical use, posing a significant obstacle for new vaccine development. Multiple studies have investigated a diverse array of biomaterials and highlighted chemical analogues of pathogen associated molecular patterns recognized by immune cells. However, the immunogenic effects of physical properties like stiffness and porosity, which play an important role in cell function, are not fully understood. The goal of this project is to investigate how the stiffness and porosity of electrospun nanofibers modulate DC activation states. Recent studies showed that macrophages exhibit higher activation on stiffer substrates. Both macrophages and DCs are APCs derived from myeloid progenitors, thus motivating the hypothesis that stiffer nanofiber meshes could also induce higher DC activation. We investigated poly (vinyl alcohol) (PVA) and chitosan (CS) nanofibers that were cross-linked to modulate stiffness and porosity while also improving stability in water for cell studies. PVA and CS nanofibers were both thermally cross-linked at 150C. PVA nanofibers were additionally treated with methanol for 8 hours. Thermal treatment did not cause a significant change ($p>0.05$) in the stiffness of the PVA nanofibers whereas combined methanol and thermal treatment for 20 minutes produced PVA fibers that were twice as stiff. Cross-linked nanofibers will be incubated with murine bone marrow-derived DCs for cell viability and DC activation studies. DC activation state will be measured by cytokine secretion along with CD86 and CD80 surface marker expression. The results from this study have the potential to guide engineering of immune-modulating biomaterials for novel adjuvant development.

SESSION 2B

CHEMISTRY, BIOCHEMISTRY, AND MATERIALS SCIENCE

Session Moderator: Sharona Gordon, Physiology and Biophysics
MGH 228
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Development of a Nanofiber Micronization Process for Water-Soluble Drug Delivery

Christina Nhan, Senior, Bioengineering
Mary Gates Scholar, NASA Space Grant Scholar
Mentor: Kim A. Woodrow, Bioengineering
Mentor: Rick Edmark, Bioengineering

Current approaches to deliver prophylactic drugs against HIV are ineffective for many reasons, including drug leakage, low drug loading, and lack of drug release tunability. To protect against HIV infection, a versatile prophylactic drug delivery platform that offers flexible dosing schedules would be an attractive and empowering option for many women. In the Woodrow Research Group, we have developed a fiber-in-fiber (FIF) drug delivery platform that addresses these requirements. The primary benefit of FIF is that it provides both immediate and prolonged drug release to protect against HIV acquisition. To fabricate the FIF platform, two nanofibers are used: a rapidly dissolving burst release fiber with encapsulated, slowly-degrading sustained release nanofibers. To preserve the release properties of the sustained release nanofibers and to ensure that they are of an imperceptible size to the user, we micronize the sustained release nanofiber in a blender. While this process is effective for water-insoluble drugs, water-soluble drugs like tenofovir leak out during micronization. Thus, improving the encapsulation of water-soluble drugs in the sustained release nanofiber component is needed to expand the functionality of FIF. I have developed a new strategy using dry micronization to improve the encapsulation of water-soluble drugs, allowing the FIF system to deliver diverse classes of drugs to protect against HIV acquisition. Retention of water-soluble drug drastically increased from 0% using previous strategies to 90% with dry micronization. Current experiments are assessing the drug release profiles of the dry micronized fibers compared to those intact nanofibers. SEM images of dry micronized fibers show that nanofiber architecture still exists, meaning that the benefits of nanofiber technology apply to dry micronized fibers. Therefore, dry micronized fibers have the potential to be their own drug delivery platform that can be delivered orally, intravenously, as well as intravaginally as a part of the FIF system.

SESSION 2O

USING MODERN GENETIC APPROACHES TO INVESTIGATE DEVELOPMENT AND DISEASE

Session Moderator: Celeste Berg, Genome Sciences
MGH 389
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

From Genotype to Phenotype: Unraveling the Developmental Effects of *vent* and *eve1* Mutations Using CRISPR-Cas9

Alice Dong, Senior, Biochemistry
Mary Gates Scholar, UW Honors Program
Mentor: David Kimelman, Liberal Arts

During development, the repression of transcription factor *tbx16* allows embryos to dynamically allocate neural and muscle tissue in the correction proportion as the body axis is formed. Understanding how *tbx16* is regulated is crucial for identifying the underlying mechanisms of neuromuscular developmental defects. Currently, we are investigating how two genes of interest termed *eve1* and *vent* interact to regulate the expression of *tbx16* in zebrafish embryogenesis. Previous studies have relied on Morpholino anti-sense oligonucleotides (MOs), but MOs often create off-target effects and misleading phenotypes that don't reflect genetic mutants. Using the CRISPR-Cas9 system in zebrafish, we have created true genetic knockouts for *eve1* and *vent*. By analyzing the difference in phenotypes between *eve1* mutants, *vent* mutants, and *eve 1/vent* double mutants, we can determine if *eve1* and *vent* act redundantly to repress *tbx16*. Our goal is to uncover the mechanisms behind these mutant phenotypes in order to identify the role that *eve1* and *vent* play during embryogenesis. Our research aims to further our understanding about how serious birth deficits arise from gene deficiencies and complications in development.

POSTER SESSION 4

MGH 241, Easel 143

4:00 PM to 6:00 PM

Regulation of the Morphogenesis of the Early Vertebrate Embryo

Penghan (Peng) Yang, Senior, Biochemistry
UW Honors Program
Mentor: David Kimelman, Liberal Arts

The embryonic development of vertebrates involves the anterior movement of progenitor cells, which form and proliferate at the posterior end of the embryonic body and then move anteriorly to differentiate into neural and muscle cells. Our interest is in understanding how these progenitor cells migrate, and how their movement is regulated, using the zebrafish as a model system because of its outstanding optical qualities. In my project, I am examining the role of specific proteins that are hypothesized to be important in this migration. To alter the expression of these proteins, I injected messenger RNAs encoding these proteins and also some newly developed transgenic lines where the expression of these proteins is regulated by a short period heat shock. For the mRNA injections I examined the phenotype of the whole embryo, whereas with the transgenic lines I microdissected the most posterior end of the growing embryo (called the "tailbud") and imaged cell movement using a confocal microscope, with image analysis using software such as Slidebook and ImageJ. My results show that a change in the expression of several interesting proteins produced abnormal embryos and yielded abnormal movement of progenitor cells and impaired formation of the tail. These results provide useful information that helps us understand vertebrate development, including human embryogenesis.