CEMB

Center for Engineering MechanoBiology

Summer Research Exerience for Undergraduates

Research Symposium

AUGUST 1, 2019

9:00 AM - 1:30 PM

UNIVERSITY OF PENNSYLVANIA

SINGH CENTER FOR NANOTECHNOLOGY, GLANDT FORUM

SCHEDULE

9:00 AM	*Continental Breakfast*
9:20 AM	*Welcome Address* Rebecca Wells, PhD, Director of Education, CEMB
9:30 AM	*Studying Primary Sclerosing Cholangitis with a Vascular Biliary Model* **Dax Craig, Alabama State University** Wells Lab
9:45 AM	*Nuclear and cytoskeletal mechanical stress generated by short chain fatty acids in HuH7s cells* **Alejandra Jiménez Escobar, University of Mexico** Wells Lab
10:00 AM	*Hypotonic Versus Hypertonic Microenvironments Respectively Suppress or Enhance Nuclear Rupture During Cell Migration Through Micropores* **Emma Ricci-De Lucca, Swarthmore College** Discher Lab
10:15 AM	*In vitro Investigation of Eukaryotic Translation using Single-Molecule FRET* **Aaron Sykes. Villanova University** Goldman Lab
10:30 AM	*Integrated Sensing for Lung Fibrosis-on-a-Chip* **Milagros Fernandez Oromendia, University of Minnesota Twin Cities** Huh Lab
10:45 AM	*Recruitment of the Arp2/3 Complex Reverses Cadherin-driven Contractile Cytoskeletal Reorganization and Contributes to the Closing of Endothelial Gaps* **Tal Sneh, Arizona State University** Shenoy Lab

Alabama State University
Boston University
Bryn Mawr College
New Jersey Institute of Technology
University of Pennsylvania
University of Texas at Austin
Washington University in St. Louis

11:00 AM	*Osteoprogenitor Lineage Progression is Spatiotemporally Determined in Embryonic Bone Morphogenesis* **Caleb Jones, Kansas State University** Boerkel Lab
11:15 AM	**Break**
11:30 AM	*Substrate stiffness and contractility regulate Nesprin expression in 3T3 cells* **Gabriela Villalpando Torres, University of California Merced** Mauck Lab
11:45 AM	*Microstructured Hydrogels for Controlled Formation of Bronchial Organoids* **Christina Hummel, Clemson University** Burdick Lab
12:00 PM	*3D Printing Micron-Scale Molds* **Gustavo Soto, University of Puerto Rico Mayagüez** Stebe Lab
12:15 PM	*Molecular mechanisms regulating arterial stiffness in Hutchinson-Gilford Progeria Syndrome* **Naira Abou-ghali, New Jersey Institute of Technology** Assoian Lab
12:30 PM	*Graphene-Based Microdevices to Probe Effects of Electrical Stimulation on Stem Cell Behavior* **Sebastian Naranjo, Rowan University** Jariwala Lab
12:45 PM	*Determining Nanoparticle Biodistribution Using a Time Dependent Physiologically Based Pharmacokinetic Multi-Scale Model* **Emma Glass, The College of William and Mary** Radhakrishnan Lab
1:00 PM	*Closing Remarks* Annie Jeong, PhD, Assistant Director, Education and Diversity

ABSTRACTS

DAX CRAIG

Studying Primary Sclerosing Cholangitis with a Vascular Biliary Model

Primary sclerosing cholangitis (PSC) is a chronic liver disease in which inflammation and fibrosis lead to multi-focal biliary structures. PSC is commonly associated with damage to the barrier function of the epithelium. This project seeks to create an in vitro model of the vascular biliary system capable of replicating full barrier function of cellular monolayers. Cholangiocyte mice cells and human umbilical vein endothelial cells (HUVECs) were isolated, injected, and cultured within a three-channel microfluidic device. The cells formed confluent monolayers over 5-6 d. After the formation of confluent monolayers, the cells were fixed, permeabilized, and blocked before the addition of antibodies and fluorescent stains. Immunofluorescence microscopy was performed on the monolayers to observe the expression of antibodies--higher levels of expression being attributed to proficient barrier function. We found that cholangiocytes and endothelial cells could be cultured within the same in vitro environment in the form of a microfluidic device, along with the cells forming confluent monolayers that displayed full barrier function. These findings allow for further research in the pathogenesis of PSC, where injection of immune cells and a toxin can replicate transmigration within this vascular system.

ALEJANDRA JIMÉNEZ ESCOBAR

Nuclear and cytoskeletal mechanical stress generated by short chain fatty acids in HuH7 cells

Hepatocellular carcinoma (HCC) predominantly occurs in patients with cirrhotic liver and is associated with increased matrix stiffness. Yet, in patients with non-alcoholic fatty liver disease (NAFLD), HCC can occur without increased liver stiffness. In NAFLD, hepatocytes are characterized by lipid droplets that occupy most of their cytoplasmic space. Our group has hypothesized that accumulation of intracellular lipid may generate nuclear and cytoskeletal mechanical stress, either through the physical presence of lipid droplets or the disruption of cellular mechanosensing. As lipid accumulation is impacted by fatty acid composition, here we investigated the effect of three different short chain fatty acids (SCFAs) on mechanosensing in a HCC cell line. HuH7 cells were cultured in PAA hydrogels of 500 Pa (normal liver stiffness), 10 kPA (cirrhotic liver stiffness) and glass and they were treated with different SCFAs (acetate, propanoate, and butyrate). IImmunofluorescence staining was completed to mark actin fibers (rhodamine-phalloidin), lipid droplets (Bodipy) and the nucleus (DAPI). Somewhat surprisingly, we found that treatment with SCFAs does not induce lipid droplet formation in HuH7 cells; however, normal mechanosensing appears disrupted. Specifically, acetate treatment increases cell spread area on all stiffness substrates over BSA control. Cell area then linearly decreases as carbons are added to the fatty acid chain . Additionally, SCFA treatment decreases actin intensity. YAP nuclear intensity and localization increases with stiffness for all treatment groups, yet we also find that SCFAs may impact YAP localization on stiff substrates. Hence, we propose that SCFAs alter the mechanosensitivity of HuH7 cells.

EMMA RICCI-DE LUCCA

Hypotonic Versus Hypertonic Microenvironments Respectively Suppress or Enhance Nuclear Rupture During Cell Migration Through Micropores

During tumor growth and metastasis, cancer cells squeeze through interstitial pores, across basement membrane barriers, and into micron-sized blood capillaries. Along with these and other solid stresses, cancer cells also endure fluid stresses due to tumor microenvironments that can be dysregulated in terms of pH, osmolarity, and more. How osmolality influences a cancer cell's migration through a constricting pore, or how the combination of constriction and osmotic stress impacts the integrity of the nucleus, are poorly understood issues. U2OS human osteosarcoma and A549 human lung cancer epithelial cells were seeded on a Transwell migration assay and the cells were incubated in hypoosmotic (~120 mOsm/kg), hyperosmotic (~650 mOsm/kg), or normal (~300 mOsm/kg) culture medium. After migration, the pore membranes were formaldehyde-fixed, stained for DNA, lamin-A/C, and lamin-B1, and imaged using a Leica TCS SP8 confocal microscope. For both cancer cell lines tested, hypoosmotic stress causes elevated cell death on a pore membrane—or possibly failure to adhere to the membrane—as well as reduced migration rate through both constricting 3 μm and larger 8 μm pores. Importantly, hypoosmotic stress also reduces the frequency of nuclear envelope rupture during constricted migration, as indicated by a ~25-30% decrease in nuclear bleb formation. Tumor growth and metastasis depend on cell migration, and cancer cells that squeeze through stiffer tissues—and therefore smaller interstitial pores—experience greater mechanical stress, which can also be induced by varying the osmolalities of the solutions in which cells migrate. Cytoskeletal organization might be altered and could provide insight into these differential effects.

AARON SYKES

In vitro Investigation of Eukaryotic Translation using Single-Molecule FRET

Nonsense mutations lead to approximately 7000 genetically transmitted disorders including Cystic Fibrosis and Duchenne Muscular Dystrophy. Nonsense mutations give rise to premature termination codons (PTC), which are replacements of an amino acid codon in mRNA by one of the three stop codons, and lead to inactive truncated protein products. Sometimes, translational readthrough occurs where selected near cognate tRNAs at the PTC position insert the corresponding amino acids into the new polypeptide, restoring the production of full length functional proteins. However, the specific molecular mechanisms by which this process occurs are still not well understood. Studies of readthrough using animals, intact cells, or cell extracts show a variety of mechanisms of readthrough, and so attempts to determine the precise mechanisms of action are complicated. To try to investigate the details of the mechanisms of readthrough that directly affect the ribosome pathway, single molecule fluorescence resonance energy transfer (smFRET) on a highly purified, eukaryotic cell-free protein synthesis system is being developed. A critical component of this assay are the Glutamine and Tryptophan tRNAs labeled with fluorescent cyanine dyes, Cy3 and Cy5, that are used for the smFRET. To obtain large enough quantities for the assay, the optimization of the charging of these tRNAs is necessary. Different charging conditions were tested to optimize the charging conditions for Cy5-labeled Tryptophan tRNA including the incubation time of the charging reaction, concentration of ATP, pH of the Tris-HCl buffer solution, and concentration of tryptophanyl-tRNA synthetase. The amount of charging is then quantified by measuring the amount of ionizing radiation from the incorporation of a mixture of amino acids that are radioactive and non-radioactive. Preliminary results show that an incubation time of 10 minutes, buffer pH of 7.8, 10 mM of ATP, and equal volume of synthetase to tRNA yield the most amount of charged tRNA. Further work will be done to optimize these conditions and test their activity with an octapeptide synthesis assay and with the smFRET assay. Optimization of the preparation of the tRNA and the other components of this assay will allow the direct investigation of translation and translational readthrough on the single molecule level and lead to the development of new therapeutic agents to treat nonsense mutation related disorders.

MILAGROS FERNANDEZ OROMENDIA

Integrated Sensing for Lung Fibrosis-on-a-Chip

Lung fibrosis is a deadly disease that currently affects over 130,000 people in the United States. This disease is characterized by the stiffening of the lung tissue caused by the excess fibroblast deposition of extracellular matrix. Although there are currently two pharmaceuticals on the market to help reduce symptoms and slow down the progression of the disease, there continues to be no cure. Furthermore, there are still many gaps in our understanding of lung fibrosis as well as a lack of good in vitro models of the disease. Therefore, we developed an integrated sensor organ-on-a-chip model that allows for real time monitoring of the functional properties [ex. Contract stiffness] of engineered cell hydrogel constructs. The device fabrication process includes the creation of a PDMS deformable membrane followed by screen printing a carbon black-PDMS mixture over the membrane. After fabrication, the devices were calibrated using in-house engineered circuity, an ammeter, a pressure sensor and pump, and microscopy. In order to optimize the sensitivity of the sensors, we tested multiple carbon black to PDMS concentrations to find a ratio that was conductive enough to transmit a signal even when stretched, but resistive enough that even slight changes in membrane deformation can be detected. The optimal concentration for sensor sensitivity as found to be a 12% Carbon black to PDMS by weight ratio. Future work involves further optimization of the sensor geometry as well as the addition of hydrogels to the device.

TAL SNEH

Recruitment of the Arp2/3 Complex Reverses Cadherin-driven Contractile Cytoskeletal Reorganization and Contributes to the Closing of Endothelial Gaps

Endothelial junction gaps are involved in a range of processes, from angiogenesis to cancer cell extravasation, and have garnered interest as a potential target for the prevention of tumor metastases. It has been experimentally realized that chemo-mechanical positive feedback signaling regulates cell contractility and junction behavior, with high contractility facilitating the opening of regular gaps bordered by regions of highly aligned actin stress fibers. However, it remains unclear what produces cytoskeletal re-organization into stress fibers or by what mechanism these fibers start to reduce in contractility, eventually disassemble, and close endothelial gaps. The Arp2/3 complex has been observed to act as a nucleation site for actin polymerization near the cell boundary, with such polymerization producing forces that may act counter to stress fiber contractility. In this study, we develop a continuum model of a symmetric two-cell junction to investigate Arp2/3-induced polymerization as a candidate mechanism for countering the positive cell contractility feedback loop, leading to the closing of endothelial gaps. The model is solved using COMSOL finite element analysis software. We demonstrate that increased polymerization at the junction can disassemble stress fibers and allow endothelial gaps to close. We further show that junction stability requires a specific balance of contractility and Arp2/3 recruitment. This work provides an important mechanistic understanding of mechanical signaling and cytoskeletal endothelial restructuring, demonstrating the key role of cadherin-complex strain-stiffening behavior in determining cell behavior and explaining that of Arp2/3 in recovering paracellular junction gaps to maintain endothelial integrity.

CALEB JONES

Osteoprogenitor Lineage Progression is Spatiotemporally Determined in Embryonic Bone Morphogenesis

Long bone morphogenesis requires the spatiotemporal coordination of osteoprogenitor invasion and lineage progression. During endochondral ossification, osteoprogenitors mobilize into the hypertrophic cartilage anlage and differentiate into osteoblasts that highly express collagen 1. Osteoblast lineage progression is coordinated by a series of morphogenic and mechanical cues in vitro, however, the spatial regulation of osteoblast maturity in utero is unclear. Here, we tested the hypothesis that immature osteoprogenitors present preferentially near the line of remodeling cartilage within the primary spongiosa, relative to other bone regions. We used a dual transgenic fluorescent reporter mouse model that co-expresses cyan fluorescent protein (CFP) and green fluorescent protein (GFP) under the control of the 3.6kb (Col3.6) and 2.3kb (Col2.3) fragments of the collagen 1 promoter, respectively. The Col3.6-CFP reporter marks immature osteoblasts/precursors, while the Col2.3-GFP reporter marks mature, further differentiated osteoblasts. Using cryohistology and fluorescent microscopy, E17.5 femurs were evaluated in ImageJ. Reporter-positive areas were evaluated by region of interest analysis in the primary ossification center (POC) and the bone collar. The bone collar exhibited a higher proportion of Col2.3(+) and Col3.6(+) cells per area than the POC, with peak expression occurring at the distal and proximal ends. Immature osteoblasts were found at a higher rate in the POC and are primarily present at the leading edge of the primary spongiosa. Comparatively, the immature osteoblasts in the bone collar were more uniformly distributed axially through the tissue. Together, these data reveal that lineage progression of osteoblasts is spatially regulated along the primary axis of these regions. This enhanced understanding of spatiotemporal lineage progression will aid in further characterization of microenvironmental factors that facilitate proper bone formation.

GABRIELA VILLALPANDO TORRES

Substrate stiffness and contractility regulate Nesprin expression in 3T3 cells

Mechanotransduction is the process by which cells convert mechanical stimuli to biochemical cues1. The cytoskeleton transfers forces from the ECM to the nucleus via the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex. One component of the LINC complex is nesprin, a family of large proteins that can bind cytoskeletal elements and are required for cytoskeletal-nuclear mechanotransduction. While much is known regarding the role of the LINC complex in mechanotransduction, less is known about how the LINC complex itself is regulated by mechanical forces. To address this, we investigated nesprin expression in response to changing microenvironmental stiffness and alterations in cytoskeletal architecture and tension. Nesprin expression was measured by quantitative polymerase chain reaction (qPCR) with primers designed for each isoform of nesprin 1 and 2. Immunofluorescence (IF) was used to evaluate nesprin localization, nesprin-cytoskeletal engagement, and qualitative expression based on intensity. 3T3 cells were seeded on polyarylamide gels and stained by IF for cytoskeletal components and Nesprin 1/2 to investigate the effects of substrate stiffness. To study the effects of contractility, the same experiment was repeated with exposure to a contractility antagonist (Y-27632) or agonist (CNO3). Decreasing substrate stiffness (from glass to 5kPa) on either fibronectin- or laminin-coated PA gels resulted in an increase in nuclear nesprin 1 and 2 staining intensity. Inhibition and promotion of contractility led to similar increases on tissue culture plastic. Substrate stiffness also appears to be a regulator of nesprin expression and localization, and contractility impacts which cytoskeletal element engages with nesprins. It could be that decreasing substrate stiffness increases nesprin expression to help anchor the cells to a softer environment and contractility regulates which cytoskeletal element participates in this cytoskeletal-nuclear mechanotransduction.

CHRISTINA HUMMEL

Microstructured Hydrogels for Controlled Formation of Bronchial Organoids

Epithelial organoids are emerging as cell culture platforms for epithelial tissue disease modeling and regenerative therapy such as in acute and chronic lung injuries. However, current organoid systems are traditionally based on mixed cultures with mesenchymal cells. These approaches limit understanding mesenchyme-epithelial cell paracrine signaling events during epithelial organoid formation as well as contributions of biophysical cues such as matrix mechanics and topography. Thus, we designed a synthetic hydrogel platform containing microwells to generate lung epithelial organoids physically separated from mesenchymal cells and within a microenvironment that mimics aspects of the native distal lung microenvironment. The hydrogels were fabricated from norbornene-modified hyaluronic acid with various microwell sizes and a range of different elastic moduli with high patterning fidelity. Human lung fibroblasts (hLFs, Lonza) were encapsulated in the hydrogels at 5 million cells/mL and human bronchial epithelial cells (hBECs, Lonza) were seeded at 66 cells per microwell. Hydrogels promoted the formation of hBECs spheroids when cultured in 500/200 μm microwells, which depended on hydrogel mechanics. When compared to larger microwells (800/300 μm) hBECs formed organoid-like structures with higher cell viability and increased diameter after 3 days, indicating that matrix topography guides hBEC self-assembly and organization. The fabrication of microstructured hydrogels facilitated the formation of epithelial cell organoids physically separated from the mesenchymal cell population. Both microwell size and hydrogel stiffness determined cell fate, viability, and overall epithelial cell organoid formation, which can be extended to the generation of other epithelial organoids. Future work will elucidate the contribution of matrix mechanics and degradability and extend to alveolar epithelial cells for therapeutic applications.

GUSTAVO SOTO

3D Printing Micron-Scale Molds

Soft matter has been found to respond to curvature cues. These effects were initially observed in such materials as liquid crystals and particles trapped at a liquid-liquid interface, and they have now been extended to include the more complex responses of mammalian cells. Variation in surface topography can be used to determine how cells adapt to and influence their mechanical environment. Unlike isotropic, spherical particles, anisotropic particles affect their planar surroundings due to their variations in curvature field. The shape of a particle defines how it will interact with a surface or another particle, and a curved microfluidic interface is vital for the assembly and guidance of the particle structural formation. A computer-aided design program called FreeCAD was used to design molds with various curvature fields, such as sine waves, spheres-with-skirts, and cylindrical posts. The sine wave surfaces had a cross-sectional area of 1mm x 1mm and the sphere-with-skirt surfaces had a cross-sectional area of 2mm x 1mm. The feature size of these molds were at the micrometer scale, which prompted the use of the Nanoscribe Photonic Professional GT to 3D-print them on indium tin oxide (ITO) slides. A profilometer was used to measure the roughness of the curved surfaces after they were printed, and the results proved that the Nanoscribe printed smooth surfaces with radii of curvature at the micron scale. The ability to 3D print these smooth-curved surfaces gives us the opportunity to seed cells and particles to study how they interact with each other and their environment.

NAIRA ABOU-GHALI

Molecular mechanisms regulating arterial stiffness in Hutchinson-Gilford Progeria Syndrome

Hutchinson- Gilford Progeria syndrome (HGPS) is a rare autosomal genetic disorder characterized by premature aging in children and death by accelerated cardiovascular disease (CVD). Blood cholesterol levels are normal in HGPS children, but their arteries are abnormally stiff. Arterial stiffness is a major cholesterol-independent risk factor for CVD. Arterial stiffening is characterized by increased ECM production by vascular smooth muscle cells (vSMCs) of the arterial medial layer. vSMCs can exist in a contractile (differentiated) state, characterized by expression of differentiation markers such as smooth muscle myosin heavy chain (Myh11), and a synthetic (dedifferentiated) state that produces ECM and ECM-modifying proteins such as lysyl oxidase (LOX). Using an HGPS- mouse model, we have previously shown that decreased mRNA expression of Myh11 in HGPS-SMCs correlates with increased LOX expression. Here, we examine the role of Myh11 in regulating LOX production and explore the role of endothelium-derived nitric oxide (NO) in arterial stiffness. Knockdown of Myh11 in WT-vSMCs increased LOX mRNA expression, suggesting that reduced Myh11, in the absence of genetic variables intrinsic to HGPS-vSMCs, is sufficient to increase LOX levels. As recent studies by others have shown that NO is a regulator of Myh11, we also explored the role of eNOS and NO in Myh11 and Lox regulation. RT-qPCR showed that Nos3 expression is significantly downregulated in HGPS-arteries. Moreover, addition of an exogenous NO donor to isolated HGPS-vSMCs increased Myh11 and decreased LOX gene expression. Our results indicate that nitric oxide may regulate SMC phenotype and identify defective NO signaling as a potential mediator of arterial stiffness in HGPS.

SEBASTIAN NARANJO

Graphene-Based Microdevices to Probe Effects of Electrical Stimulation on Stem Cell Behavior

Graphene monolayer has been shown to not only promote hMSC adhesion, but also accelerated and controlled osteogenesis. In the presence of an applied voltage, the graphene and the Si substrate behave as capacitor plates while the SiO2 behaves as a dielectric. The 2D material (graphene) behaves as a leaky capacitor plate. The electrical field that penetrates through the graphene monolayer is easily modulated to produce an electrical stimulus that spans approximately 20 nm, giving the induced electrical field the capability to specifically probe cell transmembrane receptor-ECM binding junctions. Epoxy resin was used to seal off all device circuitry from the cell culture, leaving only the graphene monolayer in contact with the cell culture. Microwells were then manufacture by placing pieces of PDMS on top of the epoxy resin. A 170 mV/mm electrical field was generated using a wave function generator and determined to be far too high and resultant in cell death. However, preliminary results indicate that graphene promotes cell spreading compared to the polystyrene petri dish after overnight incubation. Enhanced migration and proliferation were also observed with the on the chip that was incubated the entire duration of the experiment compared to the petri dish. However, cells that were seeded in the PDMS microwells died due to excess drying. Graphene has been shown to accelerate a spindle shaped cytoskeleton and aggregation was also qualitatively and statistically observed throughout preliminary, but more elaborate immunostaining is necessary to confirm this phenomenon.

EMMA GLASS

Determining Nanoparticle Biodistribution Using a Time Dependent Physiologically Based Pharmacokinetic Multi-Scale Model

In translational settings, nanoparticles (NPs) are increasingly being explored as vehicles for targeted drug delivery to healthy and cancerous tissues. Because there are nearly endless NP constructs (e.g., rigid, spherical, polymeric, etc.), sizes (nm to microns), and experimental models for translational studies, researchers are beginning to turn toward physiologically based pharmacokinetic (PBPK) models to guide in vivo experimentation and understand NP targeting behavior and performance in the human body. The purpose of this study is to create a novel multiscale model that describes NP dynamics at the subcellular, cellular, and vascular/organ levels to determine temporal biodistribution of NP in five target organs. We first developed a multicompartment organ-scale model to describe the flow of an intravenous concentration of NPs through the body and organ tissue using a combination of algebraic and ordinary differential equations (ODEs). We then created a cellular-scale model consisting of three ODEs to describe the movement of the NPs from the capillaries through the ECs and ultimately into the organ tissue. The ODEs of both scale models are solved in a coupled fashion (using a stiff ODE solver in MATLAB) to determine the temporal biodistribution of the NPs. We have successfully developed and validated a biophysically inspired multi-scale model that can describe the temporal biodistribution of NPs using experimental data, and achieving high correlation values (R). In the future, this model could be modified to include arterial branching, which will include NP uptake constants. Using PBPK models to predict NP biodistribution will ultimately result in more effective drug therapy development for humans.

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