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New Treatments for Old Diseases

Session Moderator: Benjamin Freedman, Medicine/Nephrology
JHN 111
3:30 PM to 5:15 PM

* Note: Titles in order of presentation.

Development of a High-Throughput Fabrication Method for Collagen Gel with Aligned Collagen Fibers

Alice Anna Burchett, Junior, Bioengineering
Mentor: Chia-Yi Su, Bioengineering
Mentor: Deok-Ho Kim, Bioengineering

Cancer metastasis, or spreading to another site, is the leading cause of cancer-related deaths. Invasive cancer cells migrate through the extracellular matrix (ECM), a meshwork of protein fibers that gives structure to tissue. These fibers normally have a random arrangement, but as a cancer cell migrates it rearranges them, leaving a trail of aligned fibers that can induce other tumor cells to follow. Tumor-specific aligned matrix topography is critically lacking in current cancer models and drug screening platforms. Here we explored the different mechanical factors that can be used to induce type I collagen, an important ECM protein, to form aligned fibers as they polymerize. Our preliminary experiments showed the effects of fluid flow induced by mechanical motion on gel formation. Collagen gels were incubated in polydimethylsiloxane molds of varying dimensions on a rocking platform to guide the flow direction and collagen fiber alignment. We then imaged the gels with a second harmonic generation microscope and analyzed the images using the MATLAB-based program CT-FIRE. The deviation between the angle of fibers and the alignment were used to determine the parameters for optimizing the method. The results suggested that the dimensions of the chamber and amount of time on a rocker affect the alignment of the collagen fibers. Further experiments will be conducted to quantify the effects of fluid flow, shear force, and surface tension on collagen fiber alignment. We plan to determine and combine two parameters which are most effective to align collagen fibers to establish an easy and reproducible method. We will further optimize the protocol to achieve uniformly aligned collagen fiber in a high-throughput manner. The tumor matrix topography specific 3D model we develop in our study will be valuable as a platform to study the behavior of migratory cancer cells and to identify novel anti-metastatic therapeutics.

Chitosan-Based Tissue Scaffolds for High-Throughput Screening of Human Glioblastoma Therapeutics

Colin Alexander Lester, Senior, Mat Sci & Engr: Nanosci & Moleculr Engr
Mentor: Miqin Zhang, Materials Science & Engineering
Mentor: Olivia FC Chang, Materials Science and engineering

Glioblastoma multiforme (GBM) is a highly aggressive variant of brain cancer that has been a focal point of chemotherapeutic development for years. However, initial drug screening using traditional *in vitro* culture of GBM cells frequently produces encouraging results that do not translate well to animal models and clinical application. To address this disparity, implementation of three-dimensional tumor modeling can better emulate the microenvironment that tumor cells experience *in situ*, improving accuracy of early *in vitro* screening. We developed two chitosan-based polymer blends to produce biocompatible, porous scaffolds that mimic the extracellular matrix and promote cell adhesion. Scaffold production was done in 96-well cell culture plates for high-throughput drug screening with a large sample size. These scaffolds were used to grow human GBM cell lines U-118 MG, U-87 MG and GBM6 for 14 days, confirming cell compatibility with the materials and promoting formation of tumor spheroids. The cultures were treated with the established chemotherapeutic agent temozolomide (TMZ) for 72 hours, and cells were then tested for metabolic activity using the Alamar Blue resazurin assay. We demonstrated increased resistance to chemotherapeutics in cells with this induced morphology relative to cells grown in two-dimensions for all cell lines and both scaffold compositions. Additionally, based on gene and protein expression analysis, GBM cell spheroids more strongly expressed cancer stem cell characteristics and greater malignancy. The presence of GBM resistance to chemotherapy and enhanced characteristics associated with *in situ* tumors indicates the potential of using chitosan-based tissue scaffolds for more accurate high-throughput screening of novel GBM treatments.

Inducing Collecting Duct Precursors to Study Mitochondrial Metabolism in Polycystic Kidney Disease
Laura Victoria Islas, Senior, Biochemistry
Mary Gates Scholar
Mentor: Benjamin Freedman, Medicine/Nephrology

Polycystic kidney disease (PKD) affects nearly 600,000 people in the United States and it is caused by mutations in the genes that encode polycystin-1 (PC1) and polycystin-2 (PC2). These modifications are responsible for the growth of fluid-filled renal cysts that can impair kidney function over time, and lead to end stage renal disease. PC1 and PC2 have also been localized to the mitochondria, suggesting that they are responsible for regulating cellular metabolism and energy production, which can lead to mitochondrial dysfunction and increased PKD progression. Our lab uses human pluripotent stem cells (hPSCs) to generate kidney organoids that can be used to study the advancement of PKD, which contain the major proximal structures of the nephron including podocytes, proximal tubules, distal tubules, and endothelial cells. Orgnaoids that lack both PC1 and PC2 will develop cysts that can grow large enough to be seen by eye. Unfortunately, these organoids lack a collecting duct (CD) system, a crucial component of the kidney that expands greatly in PKD. To address this need, we developed a protocol to differentiate hPSCs into ureteric bud (UB) cells, the precursors of CD cells. First, we identified *Dolichos biflorus agglutinin* (DBA) as a key CD lineage marker in developing kidneys through immunofluorescence analysis on human kidney tissue. Then, we took undifferentiated hPSCs and investigated their ability to express DBA by treating them with a concentration gradient of small molecules known to induce kidney lineage cells. The resulting cells were stained with DBA and GATA 3, an additional, early CD marker, and we found that both markers were expressed *in vitro*. We will continue modifying this protocol to better study the increased metabolic rates seen in PKD, its effects in CD cells, and the mechanism behind the PC1 and PC2 pathway that leads to cyst formation.

Non-Muscle Myosin Activators as a Novel Form of Therapy for Polycystic Kidney Disease
Grace Jun, Senior, Bioengineering
Mentor: Benjamin Freedman, Medicine/Nephrology
Mentor: Nelly Cruz, Medicine

Polycystic kidney disease (PKD), commonly caused by defects in polycystin-1 or polycystin-2, results in the formation of fluid-filled cysts and progressive loss of kidney function. Our laboratory has established a cellular model for ADPKD using kidney organoids, multicellular tissue that functionally and structurally resembles the organ of interest, derived from gene-edited human pluripotent stem cells (hPSC). To address the need surrounding a cure for PKD, we have also discovered a basis for a form of treatment involving myosin. Non-muscle myosin is a protein that controls cell protrusion and adhesion; we have established that lack of adherence increases cystogenesis dramatically. We discovered blebbistatin, a non-muscle myosin (NMMII) inhibitor, is a robust inducer of cystogenesis. Our previous finding begged the question if activators of NMMII have the opposite effect and reduce cystogenesis in PKD organoids. To better understand the role of myosin, we differentiated kidney organoids from human PKD hPSCs in a 24-well plate coated with Matrigel. We proceeded with microdissecting the organoids at 3 weeks before they formed any cysts and transferred the organoids to suspension culture where they were treated with either DMSO as a control or a pharmacological myosin activator for one week. The organoids were then imaged and number of cystic organoids were quantified. The treatment using the myosin activator compound resulted in decreased cystogenesis, in terms of the size and number of cysts. Although we have yet to clarify myosin's effect in the PKD pathway, we have identified a myosin activator as a potential inhibitor of cystogenesis. Further experimentation is to be done with other compounds such as actin activators, and proceeding with experimentation on *ex vivo* kidneys of mice. Our current findings suggest that the polycystin proteins positively regulate actomyosin's contractility, therefore myosin may be an important factor for keeping kidney tubule integrity and preventing cystogenesis.

Optogenetic Maturation of Neural Stem Cells for the Study of Alzheimer's Disease
Kira M Evitts, Senior, Bioengineering
Mary Gates Scholar
Mentor: Jessica Young, Laboratory Medicine and Pathology
Mentor: Bonnie Berry, Pathology

Alzheimer's disease (AD) is a severe neurodegenerative disease and the most common cause of senile dementia in the United States. AD affects approximately 5.7 million Americans. Presently, there is no treatment for the disease and the mechanisms underlying the development and progression of the disease are not well understood. Human induced pluripotent stem cells (hiPSCs) are a promising technology for the study of Alzheimer's disease pathogenesis, but current stem cell maturation methods, used to produce functionally mature neurons for accurate disease models, are too time-consuming to allow for the efficient study of disease mechanisms. Therefore, there is a need for a more rapid maturation method to produce cultures of functional neurons without the extended time commitment that is standard for common differentiation protocols. To achieve accelerated neuron maturation, optogenetic stimulation will be used to periodically stimulate neural stem cells according to an optimized stimulation protocol. To accomplish this, we have created a lentivirus using a construct that expresses the channelrhodopsin, Chronos, under an EF1α promoter, designed RT PCR primers to measure Chronos expression, and collected RT PCR data on transduced cells. This virus will then be used to transiently trans-

duce hiPSC derived neurons that have previously been gene edited to express a NeuN (marker of maturity) RFP (Red Fluorescent Protein) tagged fluorescent reporter that will function as a cell maturity readout. This stem cell line will be used to develop an optimized protocol for the light driven maturation of stem cells *in vitro*. The maturity of the neurons will be measured using electrophysiology to evaluate cell function and RT PCR to monitor gene expression. The success of this project will allow for more efficient study of Alzheimer's disease in stem cell based disease models, which could lead to the discovery of disease mechanisms, revolutionizing the understanding of this burdensome disease.

Using *C. Elegans* to Study Human Brain Tissue in Alzheimer's Disease

Haoyi Lei, Senior, Neurobiology
UW Honors Program
Mentor: Matt Kaeberlein, Pathology
Mentor: Josh Russell, Pathology
Mentor: Su-In Lee, Computer Science & Engineering

Alzheimer's disease (AD) is the most common cause of dementia, a general term for memory loss and other cognitive abilities. Although this disease has been a major research focus since the 1980s the pathologic mechanisms are still not understood, and therapeutic interventions have been ineffective. The most definitive method for classifying AD is through identifying accumulations of toxic proteins amyloid-beta and tau proteins in post-mortem brain tissue. Dr. Su-in Lee's lab has developed a machine learning method that integrates the pathological tau phenotypes with gene expression levels in the same brain tissue. This analysis highlights the genes with expression level changes that correlate with the pathological protein aggregation phenotypes. For this proposal I will directly test the impact of these candidate genes on cellular pathologies resulting from aggregating human tau protein with a new *C. elegans* AD model in which human tau is expressed in the worm's muscle. This tau expression will likely result in premature paralysis because previous nematode AD models with human amyloid-beta have shown this phenotype. The results of my genetic screening will lead to a better understanding of the human genes that are dysregulated in human AD brains and provide a basis for genetically-dissecting the pathways that influence the mechanisms of tau toxicity.

Islet Amyloid Deposition in a Novel Mouse Model of Cystic Fibrosis

Brendy Sue Fountaine, Senior, Biomedical Sciences
Mentor: Rebecca Hull, Medicine

Treatments for cystic fibrosis (CF) have extended patients' lifespan, resulting in CF-related diabetes (CFRD) as a major CF complication affecting 30-50% of adults. A key pathological feature of CFRD is the deposition of islet amyloid polypeptide (IAPP) as amyloid in pancreatic islets. In type 2 diabetes, Islet amyloid is associated with decreased beta-cell mass and function. Current mouse models of CFRD do not develop all the pathological features observed in human CFRD, including islet amyloid. To generate a mouse model of CFRD which exhibits islet amyloid deposition, we crossbred a CF mouse (CftrF508del) and a human IAPP (hIAPP) transgenic mouse and compared amyloid deposition in cultured islets from the resulting offspring. Islets from each of the mice genotypes (NT.Cftrdel/del (n=2), NT.Cftr+/del (n=4), NT.Cftr+/+ (n=5), hIAPP.Cftrdel/del (n=2), hIAPP.Cftr+/del (n=5), hIAPP.Cftr+/+ (n=3)) were isolated and cultured for 7 days in high (16.7 mM) glucose to induce amyloid formation. Islets were fixed in neutral-buffered formalin, paraffin-embedded and sectioned. Sections were stained with thioflavin S (for amyloid) after which amyloid prevalence (% islets with amyloid), amyloid severity (% islet area occupied by amyloid) were quantified. Islets from hIAPP transgenic mice developed islet amyloid when cultured in vitro, while islets from non-transgenic mice did not. Among hIAPP transgenic mice with different CF genotypes (hIAPP.Cftrdel/del, hIAPP.Cftr+/del, hIAPP.Cftr+/+, respectively), islet amyloid prevalence ($74\pm13\%$, $73\pm11\%$, 81 ± 5 %; p=0.89 by ANOVA)[NE2] or severity ($3.4 \pm 1.7\%$, $2.0 \pm 1.1\%$, $3.57 \pm 0.4\%$; p=0.64 by ANOVA) did not differ. The presence of amyloid in islets of mice expressing hIAPP genotypes supports the utility of this new mouse model to study this aspect of islet pathology as seen in human CFRD.