

Stanford



**Cardiovascular
Institute**

CVI Summer Research Program Symposium

August 8th, 2022

**Please join us to celebrate the scientific accomplishments of our
2022 cohort of CVI Summer Research Program students.**

In-Person in LKSC LK101/LK102

or via Zoom

Monday August 8th, 2022

9am-12pm PT - Morning Session

1-4pm PT - Afternoon Session



Morning Session

9:00am PT	Opening Remarks – Dr. Joseph Wu
9:10am PT	Emily Hunt - Lab of Nazish Sayed Victoria Lam - Lab of Sean Wu Kasey Lewis - Lab of Joseph Wu Philip Dierks - Lab of Joseph Wu Sophia Parmisano - Lab of Detlef Obal Leahlyn Mamuyac - Lab of Helen Blau
10:00am PT	Joiliana Lecointe - Lab of Sushma Reddy Roshini Asirvatham - Lab of Fatima Rodriguez Paul Gonzalez - Lab of Shipra Arya Damini Patel - Lab of Abha Khandelwal Sahana Prasanna - Lab of Alison Marsden Krystal Rivera - Lab of Christopher Cheng Patrick Acholou - Lab of Brian Kim
11:00am PT	Agustin Rodriguez Lopez - Lab of Vinicio de Jesus Perez Amanda Pacheco - Lab of Vinicio de Jesus Perez Alkapriya Chaudhary - Lab of Kevin Wang Danielle Young - Lab of Katrin Svensson John Isaiah Jimenez - Lab of Katrin Svensson Giuliano Lobos - Lab of Joshua Knowles Keyana Zahiri - Lab of Craig Levin
12:00pm PT	Lunch in LKSC Herb Garden

Afternoon Session

1:00pm PT	Theodora Abah - Lab of Claire Watkins Alex Ladd - Lab of Joseph Woo Amanda S Padilla Lopez - Lab of Joseph Woo Catherine Beaudin - Lab of Jack Boyd Esteban Garcilazo - Lab of Michael Fischbein Madeleine Chai - Lab of Yasuhiro Shudo Simisola Ogundare - Lab of Doug Liou
2:00pm PT	Esteban Rivera - Lab of Mark Berry Alex Stephane Ewane - Lab of Dominik Fleischmann Nahom Zewde - Lab of Michael Salerno Emmanuel Fale - Lab of Kevin Alexander Bernardo Moreno - Lab of Karim Sallam Bongiwe Ncube - Lab of Kristy Red-Horse Suprina Neupane - Lab of Daniel Bernstein
3:00pm PT	Miriam Trigo - Lab of Sarah Heilshorn Arely Campos-Melendez - Lab of Christin Kuo Katie Gu - Lab of Marlene Rabinovitch Hao-Yu Huang - Lab of Marlene Rabinovitch Gabrielle Montenegro - Lab of Wah Chiu Gerbenn Seraphin - Lab of James Spudich Alyssa Cassandra Sales - Lab of Casey Gifford
4:00pm PT	Celebratory Refreshments in LKSC Herb Garden

Morning Session

Morning Session 1 - 9:10 am



hiPSC-derived organoid models allow for elucidating the role of CXCL9 chemokines in cardio-oncology and cardiovascular aging

Emily Hunt - Lab of Nazish Sayed

In 2017, an estimated 1.6 million new cases of cancer were diagnosed in the United States and 0.5 million people died from the disease. Human induced pluripotent stem cells

(hiPSCs) are often derived from skin or peripheral blood mononuclear cells in cardio-oncology and cardiovascular research. These hiPSCs have the ability to undergo in vitro reprogramming back into an embryonic-like pluripotent state, thus enabling the cells to undergo controlled differentiation into specific cell types via the Wnt signaling pathway. In this study, we induce the differentiation of hiPSCs into the mesoderm lineage to form cardiomyocytes (CMs) to construct cardioid models for elucidating the role of CXCL9 chemokines in vascular inflammation. By leveraging induced pluripotent stem cells (iPSCs)-derived cells, we can understand the effect of chemotherapy drugs such as Doxorubicin on CMs and other cells, including their effect on CXCL9 chemokines.



Characterizing gene expression of MYH6 and MYH7 throughout cardiac differentiation in MYH7-mutant iPSC lines

Victoria Lam - Lab of Sean Wu

Hypertrophic cardiomyopathy (HCM) is the most common inherited cardiac disease, affecting 1 in 500 individuals. Various mutations in the MYH7 gene, which encodes a sarcomeric protein called myosin heavy chain beta (β -MHC), have been described to cause HCM. In this work, we focus on how the R369Q mutation in the MYH7 locus affects expression of MYH7 and its isoform MYH6 in developing cardiomyocytes. Therefore, the mutation was introduced into a human induced pluripotent stem cell (hiPSC) line hetero- and homozygously using CRISPR/Cas9. Cells were subsequently differentiated into cardiomyocytes and expression of MYH6 and MYH7 was measured at different timepoints (days 0-30) using qPCR. Throughout differentiation we observed a continuous upregulation of MYH6 in the homozygous clone compared to the wildtype clone. MYH7 was less expressed in the homozygous clone compared to the wild type clone.



Utilizing iPSCs to investigate chemotherapy induced cardiotoxicity in African American patients

Kasey Lewis - Lab of Joseph Wu

Prostate cancer is the second most commonly diagnosed cancer in the US, but thanks to the development of more effective drug therapies there is a high survival rate. However, many life saving cancer drugs induce cardiotoxicity, or direct damage

to heart muscle or function, that can be irreversible or life threatening. The widely administered prostate cancer treatment Bicalutamide is an example of such a drug, causing a high risk of developing heart failure. Additionally, African Americans have been found to be at increased risk for developing chemotherapy induced cardiotoxicity. As a result, a cardioprotective drug, BiDil, was developed to treat heart failure in African Americans and shown to be effective in that population, but ineffective in other racial/ethnic groups. This project exhibits the cardiotoxic effect of the widely administered prostate cancer treatment drug Bicalutamide and the cardioprotective effect of BiDil through performing qualitative functional assays on iPSC derived endothelial cells.



Modeling radiation-induced cardiovascular disease using iPSC-ECs

Philip Dierks - Lab of Joseph Wu

Radiation therapy is a pillar of cancer therapy, yet its use has been hampered due to a strong association with cardiovascular disease. To study the molecular mechanisms of radiation-induced cardiovascular disease, we utilized induced pluripotent stem cell-derived endothelial cells (iPSC-ECs) which control vascular homeostasis including blood clotting, platelet adhesion, and vessel contractility. Using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), we have screened markers for senescence (CDKN2A, PTCHD4) and endothelial to mesenchymal transition (CDH5, FN1) in iPSC-ECs exposed to X-ray radiation delivered either single or fractionated at a total dose of 2 Gy. At 14 days post-irradiation, the expression of CDKN2A increased in response to both radiation types, but PTCHD4 only increased in iPSC-ECs after fractionated radiation. On the other hand, the expression levels of FN1 and CDH5 are comparable between control and irradiated samples. These results suggest that vascular senescence is a potential druggable target to treat radiation-induced cardiovascular toxicity.



Effect of opioids on cardiovascular development

Sophia Parmisano - Lab of Detlef Oba

The use of medically and non-medically prescribed opioids has increased significantly over the last two decades. The addictive properties of opioids often lead to the continuance of usage, even if individuals become pregnant. Consequently, an increasing number of embryos are exposed to opioids. Little is known about the potential impacts of fetal opioid exposure on cardiovascular development. In this project, I differentiated human embryonic stem cells into vascularized cardiac organoids to study the impact of DAMGO, formylfentanyl, and cocaine on cardiomyocyte and endothelial cells during early cardiovascular development. While cardiomyocyte and endothelial cell differentiation were opioid concentration and substance dependent, the recreational drug cocaine did not affect early cardiomyocyte and endothelial cell differentiation. Our data provide a first insight into how opioids impact early cardiovascular development during pregnancy. Further studies with this new model will evaluate the molecular mechanism of opioid induced cardiovascular malformation.



Microdystrophin gene therapy for Duchenne Muscular Dystrophy cardiomyocytes

Leahlyn Mamuyac - Lab of Helen Blau

Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by a lack of dystrophin, a protein that provides structural support between the contractile apparatus and the extracellular matrix. DMD results in severe muscle weakness, and heart failure is the major cause of death. While gene therapy approaches testing delivery of smaller versions of dystrophin have been extensively studied in the skeletal muscle, little is known about efficacy in the heart. To address this knowledge gap, we are comparing three dystrophin variants that are currently in clinical trials in cardiomyocytes differentiated from human induced pluripotent stem cells (iPSCs). We tested these variants in three DMD lines and three healthy controls in corresponding isogenic backgrounds. To confirm expression and localization of the dystrophin variants, we performed immunostaining of dystrophin and analyzed their expression levels. Further studies on the function of the variants on calcium handling and mechanical contraction will elucidate efficacy of these therapies in DMD cardiomyocytes.

Morning Session 2 - 10:00am



The Fontan circulation shows decreased cell survival and oxidative damage

Joiliana Lecointe - Lab of Sushma Reddy

Single ventricle congenital heart disease such as hypoplastic left heart syndrome (HLHS) is usually fatal without surgical correction. The Fontan surgery corrects the circulation of HLHS patients, but despite these advances, >50% of long-term survivors develop circulation failure. We sought to evaluate the mechanisms underlying Fontan-related circulation failure. Blood was collected from patients with HLHS s/p Fontan, and controls with normal cardiac anatomy and function. Plasma microvesicles (MV) were isolated and proteomics assessed using data dependent acquisition mass spectroscopy. Proteins were evaluated using DAVID and Ingenuity Pathway programs. We found that circulating MVs from patients with HLHS s/p Fontan were released from the heart, blood vessels, and liver, providing a noninvasive signature of organ remodeling. The MV protein cargo implicates heightened cell death, oxidative damage, and impaired cell survival, providing insight into the mechanisms of Fontan-associated circulation failure.



Readability and reliability of online patient educational materials on statins

Roshini Asirvatham - Lab of Fatima Rodriguez

Statins are the cornerstone for the prevention and treatment of cardiovascular disease, and are among the most commonly prescribed medications. Yet, patient adherence to statins remains low. Patients are likely to seek health information online. The readability and reliability of online patient educational materials (OPEM) related to statins is not yet understood. 17 search terms ("statins", generic

names of all 7 statins, and 9 brand names) were entered into Google. The top 20 results for each term were collected. OPEM were identified, categorized and run through a readability software. JAMA benchmark criteria and Health on the Net (HONCode) certification were used to determine reliability. Mean grade level across all sites (n=169) was 10.81 (95% CI 10.50-11.12). Results from industry were the most readable (10.13, 95% CI 9.61-10.65) but low in reliability (2.7% HONCode certified). Government sources were most reliable (71% HONCode certified). Dictionary/encyclopedia results were the least readable (13.02, 95% CI 11.12-14.92). Readability of all OPEM assessed exceeded the sixth grade reading level recommended by the American Medical Association. This has potential to disproportionately impact patients with low health literacy.



Development of automated peripheral calcium score in peripheral artery disease

Paul Gonzalez - Lab of Shipra Arya

Peripheral artery disease (PAD) affects the blood flow from the heart to the legs. Claudication can be felt within these patients causing leg pain. There are diagnostic tests for PAD such as ABI, TBI, a walking test but their results can be skewed. The main objective of this research project was to develop an automated peripheral calcium score (PCS), used to determine calcium deposits in peripheral arteries in PAD. PCS has been used for coronary artery disease but only rarely used PCS to diagnose PAD. However, PCS has been shown to be a useful technique for accuracy of stenosis prediction in peripheral arteries. We developed a new protocol for this project for the institutional review board (IRB) that captured how we would handle human-related data. Future work will incorporate lower extremity CT scans, and comparisons with ABI and TBI, which would reduce risk of radiation exposure.



Cardio-obstetrics case series: High-risk pregnancies

Damini Patel - Lab of Abha Khandelwal

A 25-year-old gravida-1 female with no past medical history presented with radiating chest pain and STEMI around 5-6 weeks gestation. Testing showed elevated troponin, reduced LVEF of 30-35%, and aneurysmal LAD. Unfortunately, she suffered a miscarriage at 21 weeks gestation. Her symptoms were attributed as sequelae of Kawasaki disease. A 33-year-old female presented with a stroke during pregnancy and later diagnosed with PFO. She has prior history of two miscarriages at 12 weeks. Ultimately, the decision for anticoagulation therapy, although not recommended during pregnancy, was made over surgical closure of PFO. Both patients were managed closely in their following pregnancies and delivered without complications. Careful preconception planning and multidisciplinary cardio-obstetrics are paramount to successful outcomes. More high-risk OB centers are required, especially in the current US climate. We presented two of many cases to raise awareness and are reviewing multiple high-risk pregnancy cases given limited literature and treatment guidelines.



Electrical dyssynchrony in HLHS patients

Sahana Prasanna - Lab of Alison Marsden

Hypoplastic left heart syndrome (HLHS) is a congenital heart defect where the left ventricle is underdeveloped and unable to pump blood to the rest of the body. In order to help heart function, multiple surgeries are conducted to bypass the left ventricle and allow the right ventricle to become the main pumping chamber. However, these surgeries do not result in a fully healthy heart, and over time, patients may develop hypertension in the right ventricle and various electrical and mechanical problems with the heart. This project aims to study the potential ventricular electrical dyssynchrony in HLHS patients after the Fontan surgery, the last of the HLHS surgeries. Using SimVascular modeling software and scans of HLHS hearts over several years, we created patient specific models of the right and left ventricles. We will implement electrophysiology modeling and a Purkinje network plugin to run the propagation of electrical signals in the hearts. Given the data, we will be able to gain more insight into whether there exists electrical dyssynchrony of HLHS hearts over time, and to what extent.



Computational Modeling of Diseased Thoracic Aorta and its Motion During a Cardiac Cycle

Krystal Rivera - Lab of Christopher Cheng

Thoracic aorta is a biggest artery in human cardiovascular system, transporting blood from the left ventricle of the heart to head, neck, chest, abdomen, and pelvis. Thoracic aortic dissection is a disease with aortic wall torn and expanded creating the false lumen. Its geometry is complicated, and expected to deform during a heartbeat. Three dimensional modeling of the dissected aorta helps visualize geometry and deformation and provide essential data for surgical planning. Previous research has evidenced that computational modeling of the patient-specific thoracic aorta enables geometry characterization of arclength, curvature, lumen volume, and branch angles. Yet, data variance is high owing to highly patient-specific aorta shape. Here, we show the new case of thoracic aorta using computational modeling and compare its geometry at systole and diastole.



Effects of E-cigarettes aqueous extracts on Aryl hydrocarbon receptor Pathway in vascular smooth muscle cells

Patrick Acholonu - Lab of Brian Kim

Approximately 17.9 million people worldwide die from cardiovascular diseases (CVDs) each year. Cardiovascular diseases affect the heart and blood vessels, which could cause coronary heart disease. The main underlying cause of CVDs is atherosclerosis, a chronic inflammatory disease marked by the formation of atheromatous plaques within the arterial wall. Cigarette smoke is one of the most important modifiable risk factors for cardiovascular diseases (CVDs) and it is responsible for 20% of deaths from coronary heart disease. The AhR is an environment-sensing transcription

factor that is activated upon binding of polycyclic aromatic hydrocarbons (PAHs) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) mediate their biological and toxic effects. In particular, the AhR pathway is believed to play a significant role in cardiovascular diseases. The AhR Pathway is currently being investigated to determine if there is an involvement with atherosclerosis.

Morning Session 3 - 11:00 am



Role of pericyte exosomal IncAPI5-6 in pulmonary endothelial cell function and vascular remodeling

Agustin Rodriguez Lopez - Lab of Vinicio de Jesus Perez

Pulmonary arterial hypertension (PAH) is a disease involving small vessel loss in the lungs. Pericytes (PCs) support to pulmonary endothelial cells (ECs) is critical to vascular integrity and becomes dysregulated in PAH. Exosomes are extra-cellular vesicles important in intercellular communication. Exosomal cargo includes long non-coding RNA (lncRNA), that can alter gene expression and cell function. This project aims to validate previous RNA-seq data that revealed low lncAPI5-6 expression in PAH-PC derived exosomes (PPDE). Exosomes were characterized by Western Blot. Exosomal and cellular lncAPI5-6 levels were quantified using qPCR. ECs function upon exposure with PPDEs was assessed using Caspase assay. Preliminary qPCR data demonstrated elevated levels of lncAPI5-6 in both PAH-PCs and PPDEs compared to healthy donors. Exposure to PPDEs caused increased apoptosis in ECs. These findings suggest that PC-derived exosomal lncAPI5-6 may play a role in modulating EC behavior. Future studies are warranted to clarify its role in PAH pathogenesis.



Understanding the link between ROR2 and endothelial cell dysfunction

Amanda Pacheco - Lab of Vinicio de Jesus Perez

Pulmonary arterial hypertension (PAH) is a rare disease which involves micro-vessel loss and endothelial cell dysfunction. Vascular lesions in patients' lungs show downregulation of a gene called ROR2 and upregulation of focal adhesions (FAs).

These structures, involved in cell adhesion, link the cell to the extracellular matrix. Previous RNA-sequencing data collected by our lab showed that the following genes: BICD2, IGFBP3, and FERMT2, all involved in trafficking and stabilization of FAs, are downregulated in ROR2-deficient cells. My project, therefore, aims to validate this previous data with proteomics. After using cultured pulmonary artery endothelial cells, western blot, and immunocytochemistry, our results show that protein expression of these three genes, just like gene expression, is also decreased in PAH endothelial cells. This finding will allow us to better understand the role of ROR2 in the dysregulation of focal adhesions which ultimately contributes to the endothelial cell dysfunction seen in PAH.



Using real-time PCR to observe lncRNA and cytokine expression in human PBMCs

Alkapriya Chaudhary - Lab of Kevin Wang

Heart disease remains the leading cause of death in the USA. Therefore, understanding the relationship between cytokine expression and the physiological risk factors that lead to cardiovascular diseases is crucial for prevention. The Wang Lab focuses on the expression of T helper 2 locus control region (TH2-LCR) long noncoding RNA (lncRNA). Previous research demonstrated interleukin-4 (IL4), IL5, and IL13 cytokines are regulated by TH2-LCR lncRNA, but insufficient evidence supports whether degradation of TH2-LCR lncRNA would also decrease cytokine expression. Here, we tested various GapmeRs designed to target and degrade TH2-LCR lncRNA in peripheral blood mononuclear cells (PBMCs). The levels of lncRNA and IL4, IL5, and IL13 were measured through quantitative polymerase chain reaction (qPCR), and we observed a decrease in TH2-LCR lncRNA and IL4 with one set of GapmeRs. This initial research could potentially lead to new treatments for cardiovascular diseases that are present with Th2 cytokine expression.



Sex-specific differences in mammalian energy metabolism

Danielle Young - Lab of Katrin Svensson

Regulation of energy metabolism is essential for survival. Dysregulated energy metabolism can lead to aberrant fat accumulation, insulin resistance, and hyperglycemia, which all increase the risk for developing metabolic diseases. Among other factors, one major regulator is biological sex. There is a higher incidence of metabolic diseases within males compared to females. Previous work in the lab has demonstrated that males develop glucose and insulin intolerance after just one week on a high-fat diet whereas females do not. Thus, my project aims to determine whether a high-sugar diet will also lead to a male-specific development of metabolic dysfunction. Our findings demonstrate that the rapid development of metabolic dysfunction in males but not females is nutrient specific. This provides insight into how males and females differently respond to excess nutrients in their diet and will ultimately help us understand the mechanisms underlying the sex differences in developing metabolic dysfunction and disease.



Determining hepatic GLUT8 as a fructose transporter *in vivo*

John Isaiah Jimenez - Lab of Katrin Svensson

Excessive and prolonged fructose consumption may stimulate the progression of hepatic steatosis leading an individual to be diagnosed with non-alcoholic fatty liver disease (NAFLD). The relevancy of fructose in NAFLD is due to the lipogenic precursors formed by fructose catabolism thereby stimulating downstream de novo lipogenesis and the ChREBP pathway. Though several Slc2a family members such

as GLUT2 & GLUT5 are well understood as fructose transporters in the intestine, it is still unknown which transporters are responsible for hepatic fructose uptake. Preliminary studies in the lab show that GLUT8 serves as an avenue of fructose entry into hepatic cells in vitro. However, the hepatic GLUT8 remains elusive regarding its function in vivo. Here, we generate liver-specific GLUT8-KO mice using the Cre-loxP system and trace fructose fate upon intraperitoneal delivery of 3H-fructose. Hepatic 3H-fructose uptake is slightly decreased in GLUT8-KO alluding to the transporter's function, though further investigation is advised.



Validation of the role of novel receptors in adipogenesis using CRISPRi

Giuliano Lobos - Lab of Joshua Knowles

The differentiation of preadipocytes into adipocytes plays a vital role in the regulation of metabolism. However, not much is known about the genes which code for receptors vital to adipogenesis. In previous studies 353 genes of interest encoding receptors were selected based on single-cell RNA-Sequencing and genome-wide association studies on body mass index-adjusted waist-hip ratio. Next, utilizing CRISPR interference (CRISPRi), a gene knockdown technique, 8 novel genes involved in adipogenesis were discovered. In this project, the goal was to utilize quantitative real-time PCR to validate knock-down of cell surface receptor genes involved in the regulation of pro and anti-adipogenic signaling in 3T3-L1 cell line at selected time periods of adipogenesis. In addition to validating our system, this analysis showed that our knock-down of the genes was efficient throughout adipogenesis. Further understanding of the molecular function of these genes, along with viewing the effects on mouse models could not only help us further understand the process of adipogenesis, but also has the potential to become therapeutic targets to combat adipose-related metabolic diseases.



Investigating deep generative models for domain adaptation in mitral valve surgery

Keyana Zahiri - Lab of Craig Levin

Minimally invasive mitral valve surgery has become the leading method for mitral valve repair and replacement (MVR) worldwide due to its numerous clinical benefits. Usage of 3D, as opposed to 2D, endoscopes in video-assisted MVRs is a recent development aiming to improve depth perception in the surgical field and visualization of anatomy for intraoperative decision-making. To mitigate depth perception challenges when only 2D endoscopic visualization is available, this project aimed to develop a program for domain translation of MVR endoscopic images using artificial intelligence methods. A conditional generative adversarial network (cGAN) was developed and trained in image-to-image translation of left and right endoscopic stereo images from simulation and real intraoperative data from various phases of MVR surgeries. Ultimately, the model predicts corresponding images for left or right endoscopic angles to facilitate 3D visualization of the surgical field. Future work will focus on further improving image-to-image translation for higher quality visualization.

Afternoon Session

Afternoon Session 1 - 1:00 pm



Diastolic heart failure predicts poor outcome following thoracic endovascular aortic repair for type B dissection

Theodora Abah - Lab of Claire Watkins

Background: Common among aortic disease patients, hypertension can lead to increased afterload on the heart, resulting in diastolic heart failure (dHF). Standard perioperative care involves permission hypertension for spinal cord protection which may exacerbate dHF. Additionally, thoracic endovascular aortic repair (TEVAR) changes the compliance of the aorta and systemic vascular resistance. The effect of diastolic heart failure on outcomes following aortic surgery is not well known. **Hypothesis:** Our hypothesis is that dHF, from both concomitant hypertension and TEVAR, correlates with poorer clinical outcomes and increased heart failure readmissions. **Methods:** Clinical data of patients undergoing TEVAR for type B dissection between 2000 and 2022 was coupled with institutional echocardiogram and heart failure databases. Pre- and postoperative clinical, echocardiogram and CT data was collected. Patients were compared according to NYHA classification, and grade 2 or greater diastolic dysfunction. **Results/Conclusion:** Diastolic heart failure may be an overlooked risk factor for complications following TEVAR.



3D bioprinting vasculature for coronary artery disease

Alex Ladd - Lab of Joseph Woo

Coronary artery disease is a common heart problem, in which blood vessels have difficulty delivering blood to the heart due to cholesterol buildup. A coronary artery bypass graft (CABG) surgery restores blood flow to an obstructed area and can help improve survival inpatients with advanced coronary artery disease. Complications such as infections at the incision site can cause health risks that can endanger the patient. In the last few years, 3D bioprinting vasculature and organ tissues have been investigated because it offers the potential to create patient-specific tissue and personalized treatment plans. 3D bioprinting of vasculature could offer the chance to produce functional tissue to be used in a CABG surgery. This would eliminate the need for a second incision site. The purpose of this experiment is to produce a bioink blend that will be biocompatible and biodegradable so that the printed scaffold can degrade and the perfused extracellular matrix (ECM) and smooth muscle cells (SMC) of the patient will remain as an intact vessel. The results should yield a 1cm vessel of the patient's cells that were grown from the printed scaffold.



Dietary and socialization effects in novel murine model of coronary atherosclerosis

Amanda S Padilla Lopez - Lab of Joseph Woo

As the COVID-19 pandemic emerged, human lifestyle changed in order to follow the prevention guidelines. While the pandemic itself is considered a stress factor, studies suggest that SARS-CoV-2 may have underlying cardiovascular

implications related to the effects of social isolation and diet alterations. This study investigates the independent roles of diet and socialization on the development of cardiovascular disease in the novel diet-inducible fatal atherosclerotic mouse strain, SR-B1ΔCT/LDLR KO. To understand socialization effects, the models were isolated or grouped into smaller (2-3) or larger (4-5) groups, where survival and cardiac function increased with socialization. Meanwhile, to research dietary significance, mice were switched to a normal diet after being fed a high fat diet to analyze the heart damage and further characterize potential cardiac remodeling. Overall, the findings suggest that lifestyle factors affect the progression of heart disease, highlighting the importance of analyzing human habits and behavior to understand heart health.



Biodegradable external sheath to improve saphenous vein graft patency following coronary artery bypass surgery

Catherine Beaudin - Lab of Jack Boyd

Coronary artery bypass grafting (CABG) remains the treatment of choice for patients suffering from severe coronary artery disease (CAD), the leading cause of death worldwide.

However, long-term outcomes are limited by the poor longevity of saphenous vein grafts (SVGs), the most commonly used conduits in CABG, with over 50% of SVGs becoming occluded within 10 years. This has been attributed to early structural remodeling of SVGs upon exposure to arterial circulation and the development of intimal hyperplasia, subsequently leading to accelerated atherosclerosis. This research aims to develop a biodegradable external sheath to support SVGs for six to twelve weeks after CABG. Different sheath prototypes have been tested during CABGs conducted in 50 rabbit models. Results suggest that, relative to controls where no sheath was used, including an external sheath around SVGs may help mitigate factors leading to vein graft failure, thereby potentially improving long-term SVG patency and CABG outcomes.



Induced pluripotent stem cell differentiation into vascular smooth muscle cells

Esteban Garcilazo - Lab of Michael Fischbein

We demonstrate that human induced pluripotent stem cells (hiPSC) appropriately differentiated into vascular smooth muscle cells (VSMC). Immunofluorescence staining (IF) for pluripotent markers such as OCT4, SOX2, and TRA-A-60

was positive in hiPSCs, confirming that these cells retain their ability to differentiate into any cell type. hiPSC-derived VSMC positively stained for VSMC markers such as ACTA2 and TAGLN. qPCR for pluripotent cell markers was greatly elevated in hiPSCs but VSMC markers were not expressed. On the contrary, hiPSC-derived VSMCs expressed ACTA2, TAGLN, and CNN, but not pluripotent stem cell markers, implying that the VSMCs are terminally differentiated. Using a combination of IF and qPCR, we confirmed that hiPSC-derived VSMC are differentiated into the correct cell type. Harnessing the power of hiPSCs, we can now derive aortic smooth muscle cells to study disease severity and treatment response before the patient undergoes an operation, displacing the requirement for surgical samples.



Three-dimensional culture of human mesenchymal stem cells (hMSCs) on microcarriers

Madeleine Chai - Lab of Yasuhiro Shudo

Myocardial Infarction (MI), the death of the heart muscle due to decreased blood flow, is a major cause of death worldwide. Previous studies in rats have shown that transplantation of bi-layer cell sheets of Endothelial Progenitor Cells (EPCs) along with Smooth Muscle Cells (SMCs) differentiated in-vitro from Mesenchymal Stem Cells (MSCs) leads to improved outcomes following MI. However, translating these studies into large animal models has been challenging due to the much larger quantity of cells required. Three-dimensional (3D) cell culture using microcarriers provides a greater surface area for cell growth compared to traditional monolayer culture. Because of this, it may be a more efficient alternative in the largescale expansion of cells. In this study, bone marrow-derived Human Mesenchymal Stem Cells (hMSCs) were incubated on plastic microcarriers within spinner flasks, which provided continuous agitation to maintain the microcarriers in suspension. After multiple trials with varying conditions and protocols, a detectable cell count could not be obtained, perhaps due to failure of the cells to adhere to the microcarriers. Further optimization is necessary to achieve successful culture through this method.



Impact of age on long-term survival following endoscopic resection versus esophagectomy in patients with high-risk cT1bN0 esophageal cancer

Simisola Ogundare - Lab of Doug Liou

Although esophagectomy is preferred over endoscopic resection (ER) for cT1bN0 esophageal cancers that have high risk features for occult nodal metastasis, long-term outcomes following treatment may be impacted by patient factors such as age or co-morbidities. This study tested the hypothesis that esophagectomy for high-risk cT1bN0 esophageal cancer is associated with better survival compared to ER in the younger patient population. 797 patients with high-risk cT1bN0 esophageal cancer from the National Cancer Database who underwent ER or esophagectomy between 2010-2017 were included in this study. Survival was compared between esophagectomy versus ER in two patient cohorts stratified by age: <70 years and 70 years or older. Patients <70 had significantly improved 5-year survival with esophagectomy compared to ER. In contrast, patients 70 years or older had comparable 5-year survival with either treatment modality. Esophagectomy is the preferred treatment modality for younger patients with high-risk cT1bN0 esophageal cancer, however ER is a reasonable option for patients 70 years and older.

Afternoon Session 2 - 2:00 pm



The utility of PET scan in predicting pathologic complete response after chemoradiation for esophageal squamous cell carcinoma

Esteban Rivera - Lab of Mark Berry

Locally advanced Esophageal Squamous Cell Carcinoma (ESCC) generally requires multimodality therapy, but surgery may not be necessary for cure in all patients. Testing for residual cancer after chemoradiation and thus a need for surgery is currently limited. This study aimed to examine if changes in PET activity of the primary tumor after chemoradiation correlated with pathologic complete response (pCR). We retrospectively reviewed all patients (n=30) who underwent chemoradiation followed by esophagectomy for ESCC between 2009-2022 and had pre and post treatment PET scans. Overall pCR occurred in 53% (n=16). The pCR rate in patients who had complete PET activity resolution was 50% (2/4), which was not statistically significantly different from patients who had >50% reduction (58%, 11/19) ($p=0.77$) or patients who had <50% reduction in PET activity (43%, 3/7) ($p=0.82$). Changes in PET activity is limited in predicting pCR, and should not be used alone to determine which patients need esophagectomy.



Manual labeling of CTA scans of patients with type b aortic dissection compared to Deep Reinforcement Learning

Alex Stephane Ewane - Lab of Dominik Fleischmann

Aortic branch localization in patients with type B aortic dissection (TBAD) is crucial to evaluate false lumen outflow. Deep reinforcement learning (DRL) could provide a more time-efficient method than manual labeling. We aim to provide training data for DRL by manually labeling aortic branches on CTAs. From a multicenter database, we retrospectively included 164 patients who underwent a CTA scan for acute TBAD between 2006 and 2018. Two readers were trained by an expert in cardiovascular imaging to identify and label the aortic center line, annulus, and major branches from left subclavian to the bifurcation. We tracked completion time and compared it to DRL. Of the 164 cases, 12 were excluded and 66 were tracked for completion time with a mean of $846+/-357$ sec. Preliminary results for DRL on the IMA show a 0.9 sec to detect and seems to offer a faster method to label major aortic branches in TBAD patients.



Evaluation of CMR perfusion image quality using ari DL for image denoising

Nahom Zewde - Lab of Michael Salerno

Coronary artery disease (CAD) is the leading cause of death and is responsible for 1 in 7 deaths in the US. Vasodilator stress CMR perfusion imaging has been shown to be highly accurate for assessing CAD and can permit quantification of

myocardial blood flow (MBF). CMR perfusion imaging is a challenging application due to the high demands on temporal and spatial resolution which can lower the signal-to-noise ratio (SNR). New deep learning (DL) techniques can enhance image quality by denoising the images permitting higher temporal and spatial resolution. The goal of this research was to evaluate a DL algorithm, AIR™ Recon DL for perfusion imaging in patients undergoing CMR. Images were reconstructed with different denoising strengths, and image quality was graded on a 5-point scale by two cardiologists. Future directions will use the results of this preliminary study to apply AIR™ Recon DL for quantitative adenosine stress CMR.



Deciphering the role of cigarette smoking in transthyretin cardiac amyloidosis

Emmanuel Fale - Lab of Kevin Alexander

Transthyretin cardiac amyloidosis (ATTR) is a particularly lethal amyloid disease that primarily affects middle-aged and older adults, causing cardiomyopathy (ATTR-CM). V122I is the most common variant in the United States, affecting 3-4% of African Americans. There is currently no effective treatment for ATTR-CM due to a lack of understanding surrounding pathogenesis. Past studies have linked cigarette smoking to increases in the prevalence of amyloid diseases. More specifically, cigarette smoking promotes the formation of Transthyretin amyloid resulting in cardiomyopathy. We propose to investigate the effects of cigarette smoke on Transthyretin cardiac amyloidosis using our established ATTR-CM mouse model and in vitro assays. Furthermore, we will look at how tobacco smoke affects cardiac morphology and function, as well as cardiac gene and protein expression. The results of this study will reveal important mechanisms by which cigarette smoking contributes to cardiac amyloidosis and potential therapeutic targets.



Visually directed protocol for hiPSC-CMs differentiation

Bernardo Moreno - Lab of Karim Sallam

Induced pluripotent stem cells (iPSC) have the ability to differentiate into any cell type and have proven an indispensable tool to study human physiology and disease. iPSC-derived cardiomyocytes (iPSC-CMs) have revealed novel potential in studying human cardiac disease. Monolayer cardiac differentiation protocols have evolved to produce a high yield of cardiomyocytes, but published protocols have a variable level of efficiency and frequently requires protocol optimization for individual iPSC lines. This results in loss of significant time and resources to produce a large number of high quality, pure cardiomyocyte populations. Most protocols for efficient differentiation towards cardiomyocytes have been developed using a time-based approach. We propose an adapted differentiation protocol for iPSC that relies primarily on the visualization of mesoderm induction rather than a time-dependent approach. In this study will compare the relative efficiency in a visualization based approach to a time-based approach in iPSC cardiac differentiation.



Comparative analysis of CXCR4 pathway activation between postnatal day 3 (P3) murine cardiac tissue and adult murine cardiac tissue

Bongiwe Ncube - Lab of Kristy Red-Horse

Coronary artery disease (CAD) ranks among the leading causes of death in adults globally. However, CAD patients with collateral arteries are observed to have decreased long-term mortality rates. Understanding the pathways implicated in collateral artery formation at a single-cell scale using the CXCR4/TANGO mouse line may offer an approach to treating CAD. The aim is to know which cardiac cell types utilize the CXCL12/CXCR4 signaling pathway with respect to developmental stage. The rationale is that coronary arteries express CXCR4 which is likely activated by CXCL12 in the formation of collaterals. However, their formation after a myocardial infarction occurs in neonatal mice only. We discovered that pericytes, smooth muscle cells, non-erythrocyte hematopoietic cells and arterial endothelial cells are positive for this signaling. It appears that there are no differences in the cell types that are CXCR4-activated between healthy neonatal and adult mice, implying that the formation of collaterals is disease-related



Potential drug for heart disease: Studying the effect of the drug mavacamten on hypertrophic cardiomyopathy in stem cell derived cardiomyocytes

Suprina Neupane - Lab of Daniel Bernstein

Hypertrophic cardiomyopathy (HCM) is a leading cause of sudden cardiac death and is characterized by hypercontractility and hypertrophy. We studied how mavacamten, an FDA approved myosin inhibitor, reduces the hypercontractility and hypertrophy in human-induced pluripotent stem cell (hiPSC) derived cardiomyocytes with the P710R mutation in β -cardiac myosin by quantifying its acute and longitudinal effects 30 minutes, 24 hours, and 21 days post-treatment by video analysis and immunostaining. We used micropatterned hydrogels to measure single cell traction forces after acute treatment and confirmed the hypertrophic signaling activation of ERK and AKT of the P710R cells via western blot. The results showed that P710R cells respond to mavacamten but are less sensitive than the control cells. We are repeating these experiments in additional biological replicates and other HCM mutations and measuring metabolism and structure to understand mechanisms of mavacamten short and long term to help patients with HCM.

Afternoon Session 3 - 3:00 pm



Catheter-Injectable engineered gels for the delivery of gene-based therapy after a myocardial infarction

Miriam Trigo - Lab of Sarah Heilshorn

For myocardial infarction (MI) survivors, treatment for heart tissue damage after MI is limited due to 1) low retention of therapeutics in the myocardium and 2) degradation of therapies after MI. Research shows that delivery of stromal cell-derived factor 1 (SDF-1 α) after MI improves cardiac function in preclinical models, but this strategy requires prolonged activity to be therapeutic. In response, we have developed a catheter-injectable gel from recombinant Hyaluronan and Elastin-Like Protein (HELP) that has outstanding cargo retention in mechanically active myocardium. This hydrogel can be optimized to carry a mini circle of DNA coding for SDF-1 α (MC) and provide sustained delivery through programmed enzymatic release in the myocardium. To this end, MCs were expressed from E. Coli and used to transfect human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes. GFP fluorescence after transfection was assessed through confocal microscopy. Preliminary results indicate MC uptake, but transfection protocol requires optimization. Next, cardiomyocytes will be encapsulated in HELP, and transfection and survival in gel will be assessed.



Serotonin expression in mice pulmonary neuroendocrine cells

Arely Campos-Melendez - Lab of Christin Kuo

Pulmonary neuroendocrine cells (PNECs) are located in the airway epithelium, often clustered together as neuroepithelial bodies (NEBs). PNECs play a homeostatic role as sensors of environmental stimuli and have been previously associated with the release of the hormone serotonin. However, little is known about the level of expression, or the presence of serotonin in rodents. We conducted a temporal and spatial analysis of serotonin expression in mouse PNECs to determine if and when serotonin is expressed in mice. By immunohistochemistry and confocal imaging, we identified serotonin expression across three developmental stages, and found the highest level of serotonin expression in embryonic tissue. We also investigated the role of tryptophan hydroxylase (TPH1), a gene responsible for the rate-limiting step in the biosynthesis of serotonin, to determine if the hormone is synthesized by the PNECs themselves. Together, these results describe the presence and activity of serotonin in mice PNECs.



Human Endogenous Retrovirus-K mediates inflammation in PAH through TLR activation

Katie Gu - Lab of Marlene Rabinovitch

Despite inflammation playing a role in pulmonary arterial hypertension (PAH) pathophysiology, precise inflammatory mechanisms remain unclear. Our lab previously reported increased human endogenous retrovirus K (HERV-K) in PAH monocytes. We also

found that HERV-K dUTPase (deoxyuridine triphosphate nucleotidohydrolase) secreted by PAH monocytes engaged toll-like receptor 4 (TLR4), which induced cytokine release including interleukin-6 (IL-6) and inflammation in pulmonary arterial endothelial cells. We hypothesize that released HERV-K from monocytes can activate monocyte-specific TLRs and downstream inflammatory pathways. Our GEO analysis of a previously published database revealed elevated TLR7-9 levels in PAH peripheral blood mononuclear cells. Experimentally, we found that HERV-K-dUTPase-transfected monocytes exhibited higher levels of TLR9 expression among the TLRs of interest. Addition of HERV-K-antibody prevented TLR9 activation, indicating that the effects are not intracellular but autocrine. HERV-K dUTPase may induce inflammation through TLR9 activation. Further studies are warranted to determine whether targeting downstream signals of TLR9 will attenuate PAH inflammation.

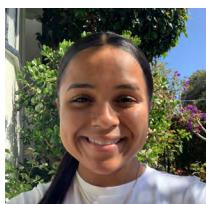


Characterizing T-box transcription factor (TBX4) in the pathogenesis of pulmonary arterial hypertension (PAH)

Hao-Yu Huang - Lab of Marlene Rabinovitch

Mutations in the T-box transcription factor (TBX4) are associated with pediatric pulmonary arterial hypertension (PAH). TBX4 gain-of-function (GOF) or loss-of-function (LOF) mutations can lead to PAH that may cause alterations in protein-DNA and protein-protein interactions. Despite these observations, it remains unclear how the transcriptional activity of TBX4 regulates the dynamic chromatin environment and, ultimately, the pathogenesis of PAH. We aim to characterize TBX4 activity in a GOF or LOF TBX4 mutation background. Since prior work has implicated the role of TBX4 in development, we will differentiate TBX4 KO/WT iPSCs into smooth muscle cells (SMCs) to determine if TBX4 is necessary for this transition.

Leveraging immunofluorescence imaging, we can stain for contractility markers to assess the quality of SMC development. With validated cell lines, we can introduce novel TBX4 variants using overexpression plasmids of BioID tags, assaying with ChIP-Seq and mass spectrometry to reveal the functional significance of TBX4 in the onset of PAH.



A machine learning approach to annotating macromolecules in cryo-electron tomography datasets

Gabrielle Montenegro - Lab of Wah Chiu

The mammalian heart depends on tightly controlled contraction and relaxation cycles, which are coordinated from the molecular to organ level. Sarcomeres within cardiomyocytes produce the contracting force of the heart. In the Chiu Lab at Stanford University, I worked under the mentorship of Rahel Woldeyes, PhD to investigate cardiomyocyte sarcomere using cryo-electron tomography and machine learning. Using rat and human iPSC cardiomyocytes, we used a supervised deep learning algorithm to train a neural network to recognize and accurately label sarcomeric proteins within

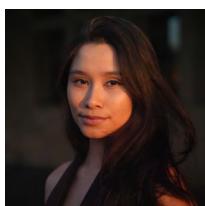
tomograms. We found that optimization of parameters and an increased number of manual annotations resulted in better labeling performance by the neural network. Using these approaches, we expect to discover structural differences between healthy and diseased cardiomyocytes, which can ultimately improve our understanding of the heart and improve patient care.



Properties of myosin that contribute to hypertrophic cardiomyopathy

Gerbenn Seraphin - Lab of James Spudich

Hypertrophic cardiomyopathy (HCM) is a form of heart disease that affects 1 in 500 individuals. More than 90% of HCM is inherited as an autosomal dominant disease, and it is the most common cause of sudden cardiac death in young athletes. To understand HCM our research in the lab focuses on studying the human beta cardiac myosin, which is the mechano-enzyme that converts the energy from ATP hydrolysis into the mechanical force that drives the contractility in the cardiac muscle. β -cardiac myosin mutations are believed to make up 40% of genetic mutations associated with HCM. We demonstrate using in-vitro motility and ATPase assays and comparing HCM mutations to WT myosin the properties of human β -cardiac myosin that contribute to HCM. The current work in the lab confirms the importance of analyzing myosin at the sarcomeric level to further develop effective cardiac myosin inhibitors in the future to reduce contractility and improve the relaxation of the heart as a whole.



Using cardiac organoids as a model to study congenital heart disease

Alyssa Cassandra Sales - Lab of Casey Gifford

Congenital heart disease (CHD) is one of the most common birth defects, affecting 1% of the population. The molecular mechanisms underlying CHD are poorly understood. However, there is strong evidence that dysregulation of the endocardium can contribute to CHD. To further investigate the role of the endocardium on cardiovascular development, we utilized hiPSC derived cardiac organoids (cardioids) that contain endocardial-like cells to model heart development in vitro. Specifically, we will evaluate how perturbation of endocardial genes, such as NOTCH1, using CRISPR interference machinery affects cardioid development compared to wildtype controls. Preliminary studies showed that NOTCH1 knockdown led to delayed beating compared to wild type cardioids. To identify the cause of these patterns, we will perform FACS and immunohistochemistry to investigate differences in the structural and cellular makeup of the cardioid following NOTCH1 perturbation. Defining the cellular interactions necessary for cardiac development is critical for developing precision medicine approaches to treat CHD.

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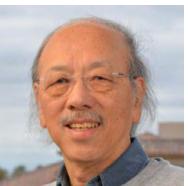
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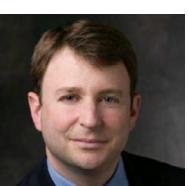
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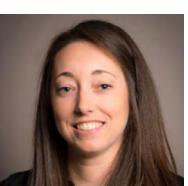
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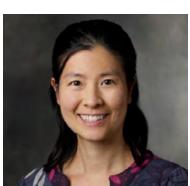
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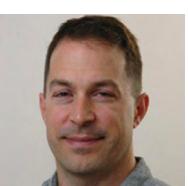
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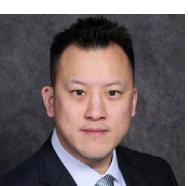
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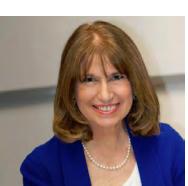
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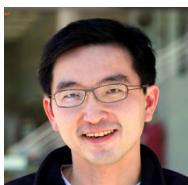
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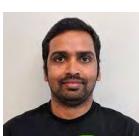
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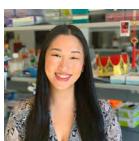
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