FIU | Engineering & Computing

Department of Biomedical Engineering

Friday, February 16, 2024

Florida International University

PVH 100, Parkview Hall - Modesto A. Maidique Campus

MIAMI
HEART DAY SYMPOSIUM

DISCUSSIONS OF CURRENT RESEARCH AND CHALLENGES IN CARDIOVASCULAR MEDICINE

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BIOMEDICAL ENGINEERING PRESENTS

Miami Heart Day Symposium

Friday, February 16, 2024

The Department of Biomedical Engineering at FIU has established the Miami Heart Month to coincide with the American Heart Month recognized nationally by the Florida Heart Research Foundation, AHA, CDC, and NHLBI. For three consecutive Fridays in February, we invite renowned investigators to Miami for a series of seminars focused on cardiovascular research. The goal of the Heart Day Symposium is to gather cardiovascular researchers from across South Florida to learn from each other and discuss current research and lingering challenges in cardiovascular medicine.

WELCOME

Joshua Hutcheson, Ph.D.
Associate Professor and
Graduate Program Director

Welcome to FIU's seventh annual Miami Heart Day! When we hosted our inaugural Heart Day event, the capstone event of Miami Heart Month, the goal of the Heart Day Symposium was to gather cardiovascular researchers from across South Florida to learn from each other and discuss current research and lingering challenges in cardiovascular medicine. In recent years, we have welcomed researchers from several departments within FIU, as well as researchers from the University of Miami, University of Florida, Nova Southeastern University, Florida Atlantic University, Joe DiMaggio Children's Hospital, and Baptist Health South Florida. We are excited to continue this tradition, bringing together many individuals from several different institutions and a broad range of disciplinary backgrounds. We are coming together to connect as cardiovascular researchers, medical practitioners, educators and members of our vibrant South Florida community. Through these connections and conversations, we hope that this venue helps us all find new ways to use our research and engagement efforts to impact cardiovascular medicine and promote healthier communities.

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We have developed an outstanding program to meet those objectives. This year, we are delighted to welcome Dr. Kartik Balachandran from the University of Arkansas to deliver the morning's keynote lecture. Dr. Balachandran has pioneered research efforts in how altered mechanics and structure contribute to the biological progression of cardiovascular, neurovascular and epithelial diseases, and how this knowledge can be utilized to develop therapeutic strategies and early disease detection strategies. His work has led to new insights into mechanobiology, mechanics, structure-function relationships, and organ-chip engineering. Following Dr. Balachandran's lecture, we will gather for lunch and a poster symposium that will highlight cutting edge cardiovascular research being performed in South Florida. Our afternoon session will provide an opportunity for members of our broader community to learn more about efforts to improve cardiovascular health in South Florida and beyond. The day will be capped off with flash talks and a discussion on current challenges and the future of cardiovascular research and medicine. We would like to thank our sponsors, notably the Florida Heart Research Foundation, for their support of this event. We are happy that you have decided to join us, and we hope that you will join us again in the years to come!

Joshua D Hutcheson

Joshua Hutcheson, Ph.D.

AGENDA

Florida International University | MMC (Main) Campus

PVH 100, Parkview Hall 1599 SW 113th Ave, Miami, FL 33199

8:00 am – 8:45 am	**Breakfast and Poster Setup**
9:00 am – 10:00 am	**Keynote lecture by Dr. Kartik Balachandran, Professor of Biomedical Engineering at the University of Arkansas**
10:15 am – 12:00 pm	**Networking and poster symposium featuring trainees and cardiovascular research and medicine**
12:00 pm – 1:00 pm	**Lunch**
1:30 pm – 2:30 pm	**Community Engagement Session with exhibits by FIU researchers, clinicians and symposium sponsors**
2:30 pm – 3:30 pm	**Flash Talks and Discussion on Cardiovascular Research and Health**

FIU | Engineering & Computing

Department of Biomedical Engineering

ABOUT OUR

Biomedical Engineering Program

The Department of Biomedical Engineering at Florida International University (FIU), located in Miami, is committed to preparing ambitious students who want to combine their love of problem-solving with their desire to help others through a fascinating and growing field that applies cutting-edge technologies and modern engineering techniques to improve healthcare.

Our College of Engineering and Computing is ranked #1 for bachelor's degrees awarded to Hispanics, #1 for Bachelor's degrees awarded to Underrepresented minorities by total, #2 for master's degrees awarded to underrepresented minorities by total, and our department is ranked #56 among graduate programs in the country*. Nationally, we are among the Top 30 to award undergraduate degrees and Top 80 for research expenditures*. Florida International University is designated a Carnegie Highest Research (R1) and Carnegie Community Engaged Institution.

*US News 2023 *ASEE 2022 and NSF HERD 2019-2021

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To Our Sponsors and Participants

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Florida International University

To The Founders and Organizers

Joshua Hutcheson, Ph.D.
Associate Professor and
Graduate Program Director

Healthy Hearts – Florida Int'l University

Jorge Riera, Ph.D.
Interim Chair of
Biomedical Engineering

FLASH TALKS

EGFR INHIBITION PREVENTS CAV1-DEPENDENT CALCIFYING EXTRACELLULAR VESICLE BIOGENESIS

Authors: Sophie Ashbrook, Jazlyn Hernandez, Alexandra Rodriguez, Joshua Hutcheson

Abstract: Vascular calcification represents the most significant predictor of cardiovascular events with no current therapeutic options for prevention or treatment. Osteogenically-differentiated vascular smooth muscle cells (VSMCs) release calcifying extracellular vesicles (EVs), which nucleate nascent mineral. Caveolin-1 (CAV1), a plasma membrane scaffolding protein residing in caveolar domains, plays a critical role in the formation of calcifying EVs. Previous studies have reported interactions between CAV1 and epidermal growth factor receptor (EGFR) in cancer pathology. Given the nature of these reported interactions, we hypothesized that EGFR inhibition may prevent the biogenesis of calcifying EVs by altering CAV1 trafficking. We assessed the potential of EGFR tyrosine kinase inhibition (AG1478 and PD153035, 2.5 uM, N = 3) to prevent calcification in vitro using VSMCs cultured in osteogenic media (OS) for 28 days and in vivo (AG1478 and PD153035, 10 mg/kg, N = 40) using a chronic kidney disease (CKD) diet model to induce medial calcification. In vitro, EGFR inhibition significantly prevented the release of calcifying EVs in OS cultures ($p < 0.05$ and $p < 0.001$). In vivo, calcification was significantly decreased by EGFR inhibition ($p < 0.05$). The decrease in vascular calcification by EGFR appeared independent from effects on kidney function. EGFR inhibition also decreased the release of calcifying EVs when given after 14 days of OS treatment in vitro, a time point at which VSMCs have adopted a pro-calcific phenotype and continued for an additional 14 days ($p < 0.01$). Our results suggest that EGFR interferes with trafficking mechanisms that are required for calcifying EV biogenesis. EGFR inhibition effective at preventing calcification prior to pro-calcific stimuli in vitro and in vivo and following initiation of calcification in vitro. Future studies will test the efficiency of different EGFR inhibitors, both tyrosine kinase and ligand binding, at different concentrations, to determine the most effective therapeutic options. Given that EGFR inhibitors exhibit clinical safety, the current data show that EGFR may be a propitious target in preventing vascular calcification.

THE EFFECT OF RIGHT CORONARY OSTIUM HEIGHT ON CHRONIC KIDNEY DISEASE INDUCED MOUSE AORTIC VALVE CALCIFICATION

Authors: Daniel Chaparro, Asad Mirza, Valentina Dargam, Lucas Menendez, Ian Chen, Sharan Ramaswamy, Joshua Hutcheson

Abstract: Aortic valve (AoV) calcification is a condition where bone-like mineral deposits form on the AoV. These deposits render the valve dysfunctional and if left untreated can lead to heart failure and death. Variations in the hemodynamic environment of valvular tissues can increase the calcification potential of the tissue. Areas of high shear tend to have lower amounts of calcification while areas of low or disturbed shear tend to have more calcification. In one of our previous studies, we show that mice on a high adenine high phosphate diet develop AoV calcification in an asymmetric manner. In these mice, the right coronary cusp (RCC) is more calcified than the left. These mice also have high take off right coronary ostia (RCO) which would have an impact on the hemodynamic shear experienced by the RCC. In this study we aim to determine the effect of RCO height on RCC calcification. We hypothesize that a higher RCO origination will induce lower shear stresses and higher calcification burden on the RCC. Computational fluid dynamics of shear stresses on the AoV reveal that there is a significant drop off of wall shear stress during diastolic loading on the RCC when the RCO originates above the sinotubular junction. From our mouse studies, we show that there is a positive correlation between RCO origination height and RCC calcification but a negative correlation between RCO origination height and KLF2 expression. This suggests that hemodynamic shear differences between the aortic valve cusps due to coronary orifice origination heights may play a significant role in the asymmetry in calcification burden seen in calcific aortic valve disease (CAVD).

DEVELOPMENT AND CHARACTERIZATION OF DESIGNED ELECTROSPUN NANOFIBERS FOR CARDIOVASCULAR APPLICATION

Authors: Alexi Switz, Anamika Prasad, Darryl Dickerson

Abstract: Structure and fiber orientation dictate the hierarchical structure of biological materials and impact their mechanical and physiological outcome. Structures with coiled fibers, for example, promote elasticity and flexibility, whereas those with aligned structures promote higher strength. Helically coiled nanofibers are suitable for cardiac applications due to their inherent elasticity in a dynamic environment, along with other functionality such as hydrophobicity and large surface area for biomolecular interactions. While directed fibers are the hallmark of biological systems, replicating and controlling fibrous structures, specifically helically coiled fibrous structure, in engineered systems is challenging. We focused on using electrospinning as a manufacturing platform to produce and characterize directed fiber mats. Through this work, our key contribution is towards the development of electrospinning as a reliable, reproducible manufacturing method as well as establishing its significance and relevance in designing electrospun fibers for tissue engineering applications. We developed a manufacturing method using an affordable in-house electrospinning platform to create aligned and helically coiled fibrous mats for cardiovascular tissue engineering applications. We used a bioink comprised of polycaprolactone and dichloromethane as our polymer solution. Polycaprolactone is biocompatible and biodegradable, making it a suitable polymer for tissue engineering applications. Testing was conducted on the fibers to evaluate their mechanical properties and physiological outcome. Fiber structure was characterized using scanning electron microscopy and post-processed using ImageJ software. The composition of the fibers was assessed using Raman spectroscopy. The mechanical response was characterized via tensile testing. Limited tests were performed to evaluate the fiber's biocompatibility and capability to support cardiomyocyte activity. Imaging showed the in-house electrospinning platform and associated parameters resulted in reliable, replicable and accurate production of aligned and helically coiled fibers. Testing data showed promising results for fibrous mats applications in the development of a cardiac patch to treat deceased tissue resulting from a myocardial infarction.

INNOVATIVE MARKERS AND TECHNIQUES FOR HEART FAILURE DETECTION

Authors: Valentina Dargam, Lin Tong, Aashiya Kolengaden, Yency Perez, Rebekah Arias, Christopher Tarafa, Ana Valentín Cabrera, Alexandra Coba, Nathan Yapaolo, Daniel Chaparro, and Joshua Hutcheson

Abstract: Despite recent advancements in surgical interventions and pharmacotherapies used to treat heart failure (HF), HF outcomes remain poor and hospitalizations continues to rise. Early diagnosis and management of the conditions that cause HF could prevent adverse cardiac remodeling and irreversible functional impairment that lead to poor patient outcomes. The goal of this study is to identify new markers of cardiac remodeling and develop advanced techniques to analyze cardiac signals to detect early HF.

We use two different mouse models to study the progression of heart failure and associated biomarkers. Mice were fed special dietary regimens to induce either chronic kidney disease (CKD) with cardiac dysfunction or HF with preserved ejection fraction (HFpEF). We assessed changes in cardiac structure and function via echocardiography, electrocardiography, phonocardiography, cardiac catheterization, plasma biomarkers, and histology.

Preliminary results show that early markers of HF can be identified prior to functional changes. Mice in the CKD regimen had significantly higher RV pressure at week 6 when compared to control mice. However, echocardiogram-based cardiac functional changes were only observed at week 12 weeks with an increase in stroke volume and ejection fraction. Our electrocardiogram data showed that the duration of Speak-p markers was also significantly higher than controls at week 6 of the CKD diet. In the HFpEF model, higher RV pressure was observed at week 3, prior to echocardiogram-based functional changes.

So far, our results show the potential of using new, noninvasive biomarkers to detect early signs of HF.

DYNAMIC SHIFTS IN NEUROLOGICAL DYSREGULATION AND COUPLING LINKING CEREBROVASCULAR MODULATION AND ONSET OF POST-TRAUMATIC EPILEPTOGENESIS

Authors: Md Adil Arman, Pritom Kumar Saha, Biswajit Maharathi, Oleksii Shandra

Introduction: Traumatic subarachnoid hemorrhage (SAH), often accompanying TBI, leads to complications like delayed cerebral ischemia (DCI) and seizures due to brain structure and function changes. Continuous EEG monitoring in TBI patients reveals neurovascular changes, although the relationship to post-traumatic epileptogenesis in a prospective study is not clear. Studying these biomarkers in an animal model can improve understanding and treatment of post-traumatic epilepsy (PTE).

Methodology: We induced repetitive diffuse TBI (rdTBI) in 12–16-week-old male mice using a non-invasive impact acceleration model, mimicking key features of human non-lesional TBI like diffuse axonal injury. Continuous video-EEG monitoring over four months post-TBI was used to assess seizure onset and power spectrum changes, comparing TBI mice with sham controls to understand neurological dysregulation and coupling.

Results: After rdTBI, 25% of mice developed late-onset (>1-week post-TBI) PTE. These mice, demonstrated significant decrease in the power of alpha-to-delta ratio during the first two weeks post-TBI, indicating similarities with SAH in which 20–30% of patients develop DCI within the first 3–14 days. The power spectrum changes shifted to increased high frequency oscillations power from 14 days post-TBI and onwards, indicating hyperexcitable state and likely onset of post-traumatic epileptogenesis. Sham mice did not show such changes.

Discussion: Our study reveals a crucial link between rdTBI-induced neurological dysregulation and PTE, with EEG changes in TBI mice reflecting those in SAH patients, highlighting a shared pathway. These findings will pave the way for testing new treatments and prevention strategies in a clinically relevant model, advancing TBI management.

EFFECTS OF EARLY LIFE LEAD EXPOSURE ON THE CARDIOVASCULAR SYSTEM IN A TRANSGENIC MOUSE MODEL OF ALZHEIMER'S DISEASE.

Authors: Sarah Hardin, Valentina Dargam, Joshua Hutcheson, Tomas Guilarte

Abstract: Lead (Pb2+) is a well-documented neurotoxicant. Children in critical developmental periods are particularly susceptible to Pb2+ neurotoxicity with lasting effects on cognition. Evidence indicates significant detrimental effects of Pb2+ exposure on the cardiovascular system. Impaired cardiovascular health is also an associated risk factor for Alzheimer's Disease (AD), a deadly neurodegenerative disorder affecting cognitive, social, and behavioral function. The negative health consequences induced by chronic early-life Pb2+ exposure (CELLE) are also observed in AD subjects. We addressed a critical knowledge gap by investigating the effects on neurological/cardiovascular health influenced by CELLE in a familial AD animal model.

These studies utilized the 5XFAD transgenic mouse model and wildtype controls. 5XFAD mice exhibit an aggressive AD phenotype. We employed ultra-high frequency ultrasound techniques to study the effect of CELLE on the cardiovascular system as a risk factor for late-life AD.

Our preliminary findings in female mice indicate that exposure from birth to 3 months of age to a Pb2+-containing diet results in environmentally relevant levels of blood Pb2+ at 3 months (14.45 ± 1.107 µg/dL) relative to non-exposed controls (<1.9 µg/dL). In 3 month old female mice, we observed that CELLE results in significant increases in body weight, skull thickness, and posterior cerebral artery peak velocity, hypertrophy of left ventricular mass, and a decrease in mitral valve A-wave velocity.

Our research on CELLE seeks to characterize the relationship between Pb2+-induced cardiovascular deficits and its ramifications on the central nervous system, shedding light on the potential risk of AD onset later in life.

THE MYOGENIC REGULATION OF CEREBRAL BLOOD FLOW: A MATHEMATICAL MODEL FOR ELECTROMECHANICAL COUPLING IN ARTERIOLAR SMOOTH MUSCLE AND CAPILLARY PERICYTES

Authors: Niloufar Khakpour and Nikolaos Tsoukias

Abstract: Capillary-mediated signaling has emerged as a significant component of neurovascular coupling (NVC), allowing blood perfusion to match local demands in the brain. Capillary Pericytes (PCs) can actively regulate capillary tone and diameter and respond to electrochemical signals or changes in pressure. In this study, we present an integrated modeling approach that can link macroscale flow responses to cell-level dynamics, and we explore the role of capillary PCs in coordinating local blood flow distribution and mediating NVC. The cell level models describe membrane potential (Vm) and Ca2+ dynamics in capillary endothelial cells (cECs) and pericytes (PCs), as well as in endothelial (ECs) and smooth muscle cells (SMCs) of parenchymal arterioles (PAs) and pial arteries. Biomechanical models of arterioles and capillaries are employed to translate Ca2+ signals into changes in vessel diameter. The model is compared against experimental data capturing arteriolar and capillary responses to pressure or extracellular K+ challenges. Network level simulations show how myogenic autoregulation maintains a relatively constant brain perfusion as blood pressure increases. Interestingly, the model suggests significant contribution by contractile capillaries in addition to arterioles in this phenomenon. Simulations further explore the physiological relevance of PCs regulating capillary diameters, identifying two potentially critical regulatory roles. First, PC-mediated, capillary adaptation can promote more uniform blood flow distribution when arterioles constrict. This can preserve blood supply to the deeper and more vulnerable regions of the brain. Second, capillary-level myogenic autoregulation can promote ‘‘blood stealing’’ by redistributing perfusion from unstimulated brain regions towards regions of neuronal activity, maximizing resource utilization.

POSTER PRESENTATIONS

OPTIMIZATION OF A MOUSE AORTIC VALVE LEAFLET TENSILE TESTING METHOD

Authors: Andrea Rivera, Daniel Chaparro, Joshua Hutcheson

Abstract: Calcific aortic valve disease (CAVD) is the most prevalent valvular disease in the United States. It is distinguished by the deposition of calcium in the leaflets of the aortic valve (AoV) that render it stiffer, preventing it from opening and closing properly. The role of biomechanics in CAVD initiation and progression remains unclear. Mouse models present the unique opportunity to measure temporal changes as the disease progresses. However, due to their microscopic size, standard uniaxial and biaxial tensile testing modalities are not suitable to assess mouse aortic valve leaflet (MAVL) tissue mechanics. Our lab came up with a novel method of quantifying the tensile properties of MAVLs by resecting the tissues, placing them on a silicone rubber membrane, stretching the composite and tracking its deformation. However, the stiff elastomer used previously can mask subtle differences in tissue properties. Therefore, an elastomer that is more compatible with the elastic modulus of mouse aortic valve leaflets is necessary to improve the sensitivity of the analysis. We have manufactured polydimethylsiloxane (PDMS) membranes using commercially available elastomers that have a lower apparent stiffness ($P < 0.0001$) than the standard membranes we have used so far for this method. Current studies aim to assess the sensitivity of these membranes by conducting tensile testing of MAVL on the PDMS membranes and the standard membranes before and after fixing the tissues with paraformaldehyde. Completion of this project will improve the sensitivity of our tensile testing method, allowing us to identify subtle changes during disease progression.

INVESTIGATING THE POTENTIAL OF EGFR INHIBITION AS TO REVERSE VASCULAR CALCIFICATION IN VITRO

Authors: Jazlyn Hernandez, Sophie Ashbrook, Joshua Hutcheson

Abstract: Cardiovascular disease is the leading cause of death worldwide, and vascular calcification is the most significant predictor of cardiovascular events. Currently, there are no therapeutic treatment options for vascular calcification. In diseased arterial tissues, vascular smooth muscle cells (VSMCs) develop an osteoblast-like phenotype and release calcifying extracellular vesicles (EVs), mimicking bone mineralization. Caveolin-1 (CAV-1), a membrane scaffolding protein linked to the biogenesis of calcifying EVs, plays a critical role in this process. The epidermal growth factor receptor (EGFR) modulates CAV-1 trafficking, making EGFR a promising target for preventing vascular calcification. This study investigated the potential of EGFR inhibition to reverse calcification in vitro using VSMCs cultured in an osteogenic media, which mimics osteogenic conditions for 28 days. EGFR tyrosine kinase inhibitors (AG1478, 2.5 uM, N=3 and PD153035, 2.5 uM, N=3) were added to the media after 7 and 14 days of osteogenic culture and continued for the duration of the 28 days. Alizarin red staining was used at the endpoint to observe the degree of calcification. Although calcification did not decrease compared to the osteogenic group, we observed that the addition of EGFR inhibitors prevented further development of calcification. Future studies will determine the effects of the inhibitors in reversing phenotypic changes in VSMCs and in arresting and reversing calcification in vivo.

DOSE DEPENDENCY OF PD153035 TO TREAT VASCULAR CALCIFICATION

Authors: Alexandra Rodriguez, Sophie K. Ashbrook, Joshua Hutcheson

Abstract: Cardiovascular disease is the primary contributor to global mortality. Vascular calcification (VC) increases cardiovascular risks by altering the mechanics and function of the arterial wall. Inflammation and altered calcium and phosphate levels in the blood cause vascular smooth muscle cells (VSMCs) to differentiate into osteoblast-like cells inducing VC. Our lab established that the scaffolding protein, caveolin-1 (CAV1), and the epidermal growth factor receptor (EGFR) regulate VC mechanisms. EGFR tyrosine kinase inhibition reduced VSMC calcification in vitro by altering CAV1 trafficking through the cell. These previous studies were performed with single dosage of the EGFR inhibitor AG1478. In the present study, we examined the effects of an additional EGFR inhibitor, PD153035, at 5 different doses to assess the specificity of our observations and to find the most effective dose at decreasing calcification identified via alizarin red staining. We found that PD153035 ($p < 0.001$) and AG1478 ($p < 0.01$) both reduce calcification compared to positive controls cultured in osteogenic media for 28 days. However, PD153035 exhibited a slightly higher efficiency compared to AG1478. The PD153035 treatment was most effective at a concentration between 2.5 uM and 4 uM. Our study established that the tyrosine kinase inhibitors effectively mitigate VSMC calcification, with an optimal PD153035 dosage of 2.5 uM. Future studies will explore the in vivo efficacy of these treatment strategies.

FREE FLOATING AMULET DEVICE IN PATIENT WITH COEXISTING ATRIAL FIBRILLATION AND THROMBOCYTOPENIA

Authors: Amina Namrouti and Nish Patel

Introduction: Atrial Fibrillation affects as many as 33 million worldwide and is the leading cause of cerebral vascular accidents. The most common location for thrombus formation and source of embolism is the left atrial appendage. Left atrial appendage closure devices, notably the Amulet device offers a noninferior alternative for those intolerants to anticoagulation therapy. Complications including dislodgement or migration of device may arise requiring prompt intervention. This paper aims to describe a case of free-floating Amulet devices following implantation in a patient with coexisting thrombocytopenia and atrial fibrillation.

Case Presentation: An 81-year-old woman with history of persistent atrial fibrillation status post cardioversion and chronic immune thrombocytopenic purpura presented for evaluation of left atrial appendage closure. Patient with CHA2DS2 VASc Score of 4 and a HASBLED of 3 indicating necessity for lifelong anticoagulants. With platelet levels of 47,000; decision was made for amulet device placement. One day following successful placement of amulet device patient was afebrile, hemodynamically stable. TEE revealed a free-floating amulet device in left atrial appendage and Echo with grade II diastolic dysfunction. Percutaneous retrieval of the free-floating device employing the Amplatz Gooseneck Snare and MitraClip transseptal sheath was successfuly completed.

Discussion: To our knowledge, there are limited cases describing the complication of free-floating amulet device. This case displays the complexities that may arise. Further studies and ongoing research about managing device-related complications should be explored to continue to refine strategies and protocols for patients who require alternative stroke prevention strategies due to contraindications to anticoagulation therapy.

QUANTIFICATION OF LEFT VENTRICULAR REMODELING IN A MOUSE MODEL OF HEART FAILURE

Authors: Alexandra Coba, Nathan Yapaolo, Valentina Dargam, Joshua Hutcheson

Abstract: In this study, we assess the extent of left ventricular (LV) remodeling due to heart failure. The risk of developing heart failure increases due to changes in the myocardial interstitium that cause increases in cell size and reduced contractility. Our lab previously showed that mice with chronic kidney disease (CKD) develop LV dysfunction, as measured by echocardiography. However, it is unknown whether LV hypertrophy develops. In this study, we quantify LV remodeling via histology to determine whether the LV hypertrophies and characterize cardiac remodeling in the CKD mouse model. Adult HFpEF mice were fed a standard chow diet or high adenine diet to induce CKD and cardiac dysfunction. Following 12 weeks of the regimen, the heart was dissected and the LV was prepared for hematoxylin and eosin (H&E) staining. To evaluate differences in LV remodeling, H&E images were analyzed to determine myocyte cell count and cross-sectional area (CSA). Our quantitative analyses are ongoing. We expect to see a decrease in myocyte quantity and an increase in CSA following the adenine regiment compared to the control group. An increase in myocyte CSA at 12 weeks of the high adenine diet would indicate cardiac thickening due to CKD, leading to a hypertrophic cardiomyopathy. Our results could help characterize cardiac dysfunction in this mouse model and determine whether it can be used to study heart failure.

THE PREVALENCE OF CARDIOVASCULAR DISEASE IN NON-HISPANIC BLACKS LIVING IN THE U.S. TERRITORIES COMPARED TO THOSE IN STROKE BELT AND NON-STROKE BELT STATES

Authors: Hana Shah, Namra Khan, Carolina Fernandez, Georgeta Vaidean

Introduction: Limited research exists surrounding cardiovascular disease (CVD) prevalence in the US territories. This study compared the prevalence of CVD in the Non-Hispanic, Black population residing in the U.S. territories, to those living within and outside the U.S. Stroke belt.

Methods: Non-Hispanic, Black Adults (18+) with complete information from 2021 cross-sectional data from the Behavioral Risk Factor Surveillance System (BRFSS) were included. The prevalence of CVD was defined as self-reported coronary artery disease, stroke, or myocardial infarction. Multivariable logistic regression was used.

Results: Of the 31,246 individuals included in our study, 87 (8.7%) residing in the US territories reported experiencing a cardiovascular event, as compared to 1487 (10.1%) in the Stroke belt and 1872 (8.2%) in non-stroke belt regions. Compared to Blacks residing in the US non-stroke belt, those living in the Stroke belt had 23% higher odds of reporting CVD (OR 1.23, 95% CI 1.10-1.44), while the odds were similar for the Blacks living in the US territories (OR 1.07, 95% CI 0.66-1.73), after adjusting for age and gender. Independent of residence, individuals with high blood pressure (OR: 2.85, 95% CI:2.05-3.96), diabetes (OR: 1.45, 95% CI:1.20-1.75), and those with high cholesterol (OR: 1.55, 95% CI:1.27-1.89) had significantly higher odds of CVD.

Conclusion: Non-Hispanic Black adults residing in the US territories had similar self-reported CVD with those living in the U.S. non-stroke belt regions. Further research is needed to investigate the socio- behavioral factors influencing cultural and historical disparities among Non-Hispanic Black individuals in the US and its territories.

BIOMARKERS TO PREDICT RISK OF CARDIAC DYSFUNCTION IN A PRE-CLINICAL MODEL

Authors: Lin Tong, Valentina Dargam, Joshua Hutcheson

Abstract: It is difficult to follow the progression of cardiovascular disease and biomarkers in humans from early to late stages. Tracking a healthy person from the onset of plaque formation to heart failure can take decades. Most clinical trials of cardiac biomarker risk stratification have only one baseline measurement in frozen serum samples. However, baseline levels of biomarkers may vary from person to person, depending on age, body mass index, gender, race, and some chronic conditions such as diabetes. Additionally, many measures of disease stage depend on patient self-reporting.

Our laboratory has extensive expertise in using mouse models of vascular remodeling and heart failure. Mice with persistent heart failure induced by an adenine diet helped us establish correlations of cardiac biomarkers with disease status with less variation. We collected blood samples and measured cardiac function such as right ventricular pressure (RVP) at different time points to correlate disease progression/stages with biomarker concentrations. We found that circulating baseline levels of NT-proBNP increase with age and with changes in cardiac function. Likewise, we observed a strong correlation between RVP and circulating levels of cTnI, NT-proBNP, sortilin.

ASSOCIATION OF PREOPERATIVE FUNCTIONAL STATUS WITH SHORT-TERM MAJOR ADVERSE OUTCOMES AFTER CARDIAC SURGERY

Authors: Julio Sanchez Gonzalez, Isabel Diaz, Barbara Chiu, Pura Rodriguez de la Vega, Rupa Seetharamaiah, Georgeta Vaidean

Introduction: The prevalence of cardiovascular disease results in an increasing number of cardiac surgeries each year. Implementing effective screening techniques can identify high-risk patients, allowing for better pre/postoperative management. Our study investigates the association between preoperative functional status and adverse outcomes after cardiac surgery.

Methods: We conducted a retrospective cohort study of 42,917 adult cardiac surgery patients from 2011 to 2022 utilizing the American College of Surgeons National Surgical Quality Improvement Program Database. Patients with missing data in the primary composite outcome were excluded. Totally and partially dependent groups were combined to increase statistical power. Adverse outcomes including stroke, sepsis, and pneumonia within 30 days of surgery were assessed while controlling for confounders. Unadjusted and adjusted odds ratios and 95% confidence intervals were obtained by multiple logistic regression.

Results: Compared to those with independent functional status, dependent participants experienced the primary outcome at greater frequencies (35.68% vs 20.93%), and were associated with a crude OR of 2.09 (95% CI 1.85-2.37) and an adjusted OR of 1.21 (95% CI 1.04-1.41). Additional factors associated with greater odds of the primary outcome included age >80 years, female sex, BMI <18, black race, emergency case, pre-operative blood transfusion, and sepsis.

Discussion: Our findings revealed higher odds of postoperative complications in dependent patients. Assessing preoperative functional status could help deliver more personalized care and decrease financial burden associated with surgical complications.

THERAPEUTIC EFFECTS OF RELAXIN RECEPTOR AGONISTS ON IN VIVO AND IN VITRO MODELS OF VASCULAR CALCIFICATION

Authors: Ana Valentín Cabrera, Courtney Myhr, Roxana Melo, Katelan Sugrim, Alexander Gonzalez, Kenneth Wilson, Juan Marugan, Joshua Hutcheson, Alexander Agoulnik

Abstract: Vascular calcification contributes to the rupture of atherosclerotic plaques—the leading cause of heart attacks. No therapeutics exist to treat vascular calcification. We suggested that relaxin, a vasoprotective and anti-fibrotic small peptide hormone of the insulin/relaxin family, may affect this condition. However, recombinant relaxin has short stability in vivo, poor bioavailability, and is expensive to synthesize, limiting its clinical utility for chronic conditions such as vascular calcification. As an alternative, ML290 is a biased allosteric agonist of the human relaxin receptor (hRXFP1) previously shown to attenuate vascular calcification in Apoe-/- mice. This study aimed to determine if ML290 may reverse arterial remodeling with lifestyle interventions and develop an in vitro assay to compare multiple relaxin agonists on alkaline phosphatase activity, a key enzyme in the development of vascular calcification. A high-fat diet-induced atherosclerosis and vascular calcification in humanized (hRXFP1/hRXFP1) Apoe-/- mice for 15 weeks. To simulate lifestyle changes, mice were returned to the chow diet for the last 10 weeks of the experiment. Mice were divided into four groups: vehicle with atherogenic diet, ML290 with atherogenic diet, vehicle with chow diet, and ML290 with chow diet. In addition, we developed an in vitro assay to compare multiple relaxin agonists on alkaline phosphatase activity in human aortic vascular smooth muscle cells. Preliminary data suggest that relaxin agonism reduces alkaline phosphatase activity dose-dependently in haVSMCs. This study demonstrates the potential to determine the most suitable small-molecule relaxin agonists for vascular calcification treatment.

DETERMINING THE ROLE OF MELANOCYTES IN ELASTINOGENESIS OF ALBINO AND K-5 ENDOTHELIN3 MICE

Authors: Rosali Nodarse, Daniel Chaparro, Joshua Hutcheson

Abstract: The aortic valve (AoV) promotes unidirectional blood flow from the left ventricle to the rest of the body, however this can be affected by aortic valve disease (AVD). AVD is most notably caused by pathological remodeling of the extra cellular matrix (ECM), a trilaminar microarchitecture of elastin, collagen and glycosaminoglycans. A diverse population of valvular interstitial cells (VICs), including melanocytes, maintain the ECM. There is a striking correlation between melanocytic pigment and elastin fibers abundance in AoV leaflets. Mice with Keratin-5 mediated endothelin 3 overexpression are hyperpigmented, both on their skin and heart valve, and show vast amounts of elastin in a disorganized network. While albino mice who do not have any pigment production, have very minimal elastin in the leaflets. Despite the observed correlation between pigmentation and elastin fiber abundance and organization the direct role of melanocytes on elastinogenesis remains unknown. Melanocytes do not seem to be producing the main component of elastin (tropoelastin) but could be involved in other mechanisms of elastin fiber maturation. We hypothesize that melanocytes produce other proteins necessary for elastinogenesis such as EPB, LOX, or FBLN5. We will test this hypothesis through immunofluorescent assays of whole mount mouse AoV leaflets. These will be co-stained with a melanocyte specific marker such as DCT and we will quantify the amount of co-expression. If completed as proposed, these findings of this study could provide insight into the role of a largely overlooked and understudied subpopulation of VICs within the AoV leaflets.

DECELLULARIZED ELASTIN-RICH VALVE FOR THE TREATMENT OF CRITICAL CONGENITAL VALVE DISEASE (CCVD) IN CHILDREN

Authors: Daniela Alvarado, Claudia Ponce, Ariadna Herrera, Sharan Ramaswamy

Abstract: Critical Congenital Valve Disease (CCVD), when one or more of the heart's four valves don't develop properly before birth, affects around 44,000 children annually in the United States. CCVD leads to inadequate valve closure, resulting in life-threatening complications. CCVD lacks effective treatment options other than heart transplants or compassionate care due to the shortage of suitable prosthetic valves that can support somatic growth. The current compassionate care solution being used is the use of raw porcine small intestinal submucosa (PSIS) valves; however, these valves have limitations. Our study explored if using a bio-scaffold valve that was deposited with tissues from human bone marrow-derived mesenchymal stem cells (hBMMSCs), cultured statically and dynamically to enhance extracellular matrix (ECM) constituents, and subsequently decellularized, could be an effective treatment for CCVD. hBMMSCs were cultured until confluent, harvested, passaged continuously, and then seeded onto PSIS bio-scaffold valves. The valves were cultured statically, then subjected to dynamic culture via physiologically relevant oscillatory flow with shear stresses and were later safely decellularized to remove the hBMMSCs while keeping their secreted ECM. We were able to regenerate a thin layer of engineered human ECM on the PSIS bio-scaffold valve that was rich in elastin content, which promotes cellular chemotaxis. Preliminary hydrodynamic testing and immunofluorescence found that our elastin-rich valves had ~8% elastin compared to ~10% elastin found in native heart valves. Our results reveal that decellularized elastin-rich valves could be a potential treatment for CCVD in children, offering them a normal, healthy life following heart valve replacement surgery.

AN ADAPTIVE ALGORITHM FOR DETECTION OF AORTIC FLOW ONSETS

Authors: Mohammadreza Kazemi, Taylor Elise Baum, Emery Neal Brown

Abstract: Information about the cardiovascular system (e.g., duration of the cardiac cycle, heart rate, cardiac output, etc.) can be obtained from aortic blood flow (ABF) waveforms. Accurate characterization of such information requires extraction of each individual pulse from the ABF waveform. This task is difficult due to variability in ABF pulse morphology over time because of the changing dynamics of the cardiovascular system. We propose an adaptive pulse detection algorithm capable of detecting the onset of ABF pulses. By introducing an adaptive refractory period and thresholds, the proposed framework is robust to changes in ABF pulse shape and heart rate. For an ABF pulse to be detected, the amplitude of the pulse should cross a certain threshold which is determined by the algorithm based on the amplitude of the previous ABF pulses. Additionally, after each pulse is detected, there is a refractory period reflective of the average heart rate to prevent detection of false positive pulses during the same cardiac cycle. Preliminary validation of our algorithm on ABF recordings yielded high sensitivity and positive predictability. Overall, our method provides a highly reliable and computationally efficient framework for detecting the onset of ABF pulses.

TRITIUM BETAVOLTAIC-POWERED LEADLESS PACEMAKER BATTERY

Authors: Johann Hernandez and Peter Cabauy

Abstract: Leadless cardiac pacemakers (LCP) represent a significant leap in cardiac pacing devices. These circumvent lead-associated complications (e.g., infection, migration, dislodgement), the greatest contributor to transvenous pacemaker (TVP) complications. As such, LCPs are greatly preferred to TVPs. However, their use is bottlenecked by lithium-based batteries' technological limits of ~0.6 cc for use in LCPs and a useful life of only 7-10 years. Leadless pacemakers are extremely difficult to retrieve, so they are primarily used in 70+ year-old patients who are not projected to outlive the device. City Labs is developing a small betavoltaic power source with sufficient current density to power an LCP. The battery's volume can be as small as 0.1 cc, while providing a consistent ≥3.8 microwatts for a fixed 20 years, as informed by tritium's decay curve. A decrease in size grants both the manufacturer of delivery catheters and the clinicians implanting the LCP them a higher degree of flexibility, both in developing the device design process and implantation protocol. From a clinical standpoint, a longer-lived pacemaker expands the use of leadless pacemakers to younger demographics and reduces the frequency of LCP replacement surgery. City Labs plans to generate a revenue stream from the commercialization of the LCP betavoltaic power source initially through direct sales of prototype devices to a partner LCP OEM. The medical tritium battery will be brought to market as a component of an LCP system.

CHANGES IN HEMODYNAMIC CORRELATION MAPS IN MICE WITH VASCULAR CALCIFICATION

Authors: Aasma Dahal, Daniela Leizaola, Faiza Nazir, Valentina Dargam, Joshua Hutcheson, Anuradha Godavarty

Abstract: Chronic kidney disease (CKD) increases the risk of vascular calcification (VC), a leading predictor of cardiovascular morbidity and mortality. Our previous study suggested hemodynamic changes in the peripheries were different with and without calcification during peripheral imaging of murine tails. However, these results were based on hemodynamic changes at point locations, and the overall changes in the entire peripheral tail were not determined. Our current work focuses on understanding the overall changes in hemodynamic correlation throughout the murine tail in the presence and absence of VC.

Here, ten-week-old adult mice were placed on a special diet for 12 weeks to induce CKD and VC (CKD+ VC group) and CKD with no VC (CKD group). Murine tails were imaged using an in-house near-infrared optical scanner (NIROS) and the spatiotemporal diffuse reflected NIR signals were obtained in response to occlusion. Occlusion-induced changes (during the first occlusion cycle) in hemoglobin-based parameters were obtained and analyzed to determine the hemodynamic correlation maps.

The hemodynamic correlation maps based on effective oxy- (ΔHbO) and total-hemoglobin (ΔHbT) changes exhibited a predominantly negative correlation in the CKD+VC group compared to the CKD group at the end of week 12. This suggests a disruption of flow patterns due to the presence of VC in the CKD+VC group compared to the CKD group. These observed changes may be due to calcification in altering vascular compliance and blood flow. Ongoing work will focus on a more thorough comparison across weeks.

THE ROLE OF ADENOSINE KINASE IN NEUROVASCULAR FUNCTION AFTER TRAUMATIC BRAIN INJURY

Authors: Pritom Kumar Saha, Md Adil Arman, Nija White, Heidy Umana, Sofia Torrech, Ivanna Zambrano, Oleksii Shandra

Introduction: Traumatic brain injury (TBI) is a major cause of cerebrovascular dysfunction in the neurovascular unit. Acutely after TBI, a surge of an endogenous vasodilator is associated with neuroprotection and maintenance of the blood brain barrier. Yet, this transient effect is later replaced by chronic overexpression of adenosine kinase (ADK), the key adenosine-metabolizing enzyme. The resulting reduced levels of brain adenosine levels may lead to impaired neurovascular coupling, compromised blood-brain barrier and increased susceptibility to seizures. Understanding the critical timeline of the shift from an early adenosine surge to sustained ADK overexpression in a clinically relevant animal model first, would be vital for optimizing the intervention strategies that may prevent long-term neurovascular complications.

Method: We used Western blotting, immunohistochemistry and confocal brain imaging in male and female mice after repetitive diffuse TBI (rdTBI). Data between TBI and control mice were performed with one-way ANOVA analysis.

Results: We determined progressive increase in cortical expression of ADK and a two-fold increase in the reactive astrogliosis and ADK overexpression in the hilus region of the mouse dentate gyrus in the hippocampus within 2 weeks post-rdTBI compared to Sham mice. A subset of mice with TBI and ADK overexpression also demonstrated spontaneous, electroclinical seizures, supporting a complex interplay between neurovascular health and seizure susceptibility following rdTBI.

Discussion: Our results underscore a critical shift in adenosine homeostasis post-rdTBI, potentially leading to impaired neurovascular function. The 2-week post-TBI phase appears to be pivotal point in ADK overexpression, suggesting a window for therapeutic intervention.

FSI MODELS OF EARLY-STAGE CALCIFIED AORTIC VALVES SHOW HEMODYNAMIC BIOMARKERS

Authors: Asad Mirza, Chia-Pei Hsu, Andres Rodriguez, Paulina Alvarez, Lihua Lou, Matty Sey, Arvind Agarwal, Joshua Hutcheson, Sharan Ramaswamy

Abstract: Diagnosis of calcific aortic valve disease (CAVD), the most prevalent human adult heart valve disorder, is generally done after symptoms present themselves. Indeed, before diagnosis, hydrodynamic quantities, such as pressure gradient (ΔP), mean flow rate (Q_RMS), effective orifice area (E.O.A), and regurgitant fraction (RF), are often indistinguishable from a healthy valve. Our goal was to identify if there existed hemodynamic biomarkers, such as time-averaged wall shear stress (TAWSS), attained through fluid-structure (FSI) simulations of blood and tissue of a bioengineered valve, that present at early-stage CAVD even if there were no changes in hydrodynamics.

Porcine small intestinal submucosa (PSIS) bio-scaffold valves (N=9) were seeded with valvular interstitial/endothelial cells (VICs/VECs). They were then equally cultivated under a calcifying media with either static or high oscillatory flow, compared to a raw PSIS control group. Hydrodynamic and nanoindentation assessment was done to record pressure, flow, and tissue stiffness per valve. Valve and surrounding geometry were modeled in SolidWorks 2022 and meshed using ANSYS LS-PREPOST. FSI simulations were then conducted using a strong coupling algorithm in ANSYS LS-DYNA.

Hydrodynamic assessments showed parameters were below thresholds associated for even mild calcification. Young's modulus, however, was 73.25 kPa in cultured valves versus 55.10 kPa control. FSI simulations of these valves agreed within 15% of hydrodynamic assessment and showed an increase in TAWSS between calcified, 18.96 dynes/cm2, and raw valves, 12.41 dynes/cm2.

This study demonstrates that following changes in hemodynamics, such as TAWSS, might be a numerical metric for diagnosing early-stage valve calcification, facilitating patient monitoring through clinical imaging and computational modeling.

THE DEVELOPMENT OF A DYNAMIC PHANTOM FOR PPG SIGNAL VALIDATION WITH SKIN TONE AND OBESITY VARIATIONS

Authors: Tananant Boonya-ananta, Andres Rodriguez, Ajmal, Marianne Porras Bouzas, Amanda Sanchez, Jessica Ramella-Roman

Abstract: Cardiovascular disease is one of the leading causes of death in the United States. Currently in the U.S. over 40% of the population suffers from some form of cardiovascular condition including hypertension. These conditions make it very desirable to provide a non-invasive point of care health technology system to help monitor cardiovascular health conditions. Photoplethysmography (PPG) responds to volumetric changes in the blood volume and generates a waveform representing the cardiovascular performance. When probing deeper arteries, the PPG signals are affected by factors such as skin tone and obesity levels. We have developed a dynamic phantom with various form factors which generates a pulsatile fluid flow representing blood flow in the radial artery at the wrist. The phantom is developed using rapid prototyping and mold casting methods to be able to control the mechanical and geometric properties, alongside design elements which allow the manipulation of optical properties of the medium. The pressurized flow system allows for the generation of a controlled pressurized continuous flow at various pressures ranging from 30mmHg to 200mmHg. PPG measurements were measured using the NellCor Pulse Oximeter device at 633nm and 890nm wavelengths. PPG signal at different pressure readings were gathered and evaluated for pulsatile signatures at the associated peristaltic pump cycle frequency while controlling optical properties, interfaces and geometrical form factors associated with obesity and skin tone at the radial artery.

MELANOCYTES PLAY A ROLE IN THE ELASTOGENESIS PROCESS WITHIN THE MURINE AORTIC VALVE LEAFLETS

Authors: Perony Nogueira, Daniel Chaparro, Sana Nasim, Joshua Hutcheson, Lidia Kos

Abstract: We have previously shown that elastic fiber synthesis in aortic valve leaflets is associated with the presence of pigment-producing melanocytes. However, the mechanisms through which melanocytes contribute to the formation of elastic fibers remain unknown. To better understand elastin production in the murine aortic valve (AoV) and establish the role that melanocytes and pigment play in this process, we employed lineage tracing methodologies with transgenic mice, including the Wnt1-Cre and Nkx2.5-Cre systems to trace the developmental origins of melanocytes from the neural crest and second heart field, respectively. Using in situ hybridization followed by immunofluorescence, we showed that fibroblasts and endothelial cells produce elastin. We also found that a small number of cells that express melanocytic markers also produce elastin. Markers associated with these populations of cells were also identified in spatial transcriptomic analyses. Cells that were identified as melanocytes, also expressed markers for smooth muscle cells, suggesting phenotypic plasticity not commonly observed in other melanocyte populations. The melanocytic cells co-expressed markers for all three precursors lineages: neural crest, second heart field, and endothelial, supporting the lineage tracing analysis results. These studies will provide critical insights into the complex processes underlying AoV elastin production and the development of potential clinical applications.

CAV1 (KO) MOUSE BONE ANALYSIS

Author: Anthony Camaraza Díaz, Katherine Kaiser, Joshua Hutcheson

Abstract: Vascular calcification, characterized by the deposition of mineral in arterial walls, poses a significant threat to cardiovascular health, potentially leading to cardiac complications and heart attacks. A comprehensive understanding of the underlying mechanisms of vascular calcification is crucial in addressing this health concern. Caveolin 1 (CAV1), a protein located in the lipid rafts of cell membranes, has been identified as essential for the formation of vascular calcification. Previous experiments showed that siRNA knockdown of CAV1 prevents vascular smooth muscle cells (VSMCs) from forming calcification in vitro. However, knockdown of CAV1 in osteoblast cultures resulted in no change to mineral formation. These results indicate that targeting mechanisms related to CAV1 may be a promising avenue for preventing vascular calcification without causing bone mineralization complications.

The primary objective of this study is to further explore the role of CAV1 in bone and vascular calcification in an in vivo model. CAV1 knockout (KO) mice and wild-type (WT) mice will be fed a high phosphate/high adenine diet to induce chronic kidney disease diet and vascular calcification. After 16 weeks, vascular calcification will be visualized using Osteosense to quantify mineral deposits. Additionally, bone composition and structure will be analyzed using Raman microscopy. This investigation aims to elucidate the specific role of CAV1 in both vascular and bone calcification processes, providing insights that may contribute to future pharmaceutical developments.

QUANTIFYING PROPERTIES OF CALCIFIC EXTRACELLULAR VESICLES FROM OSTEOBLASTS AND VASCULAR SMOOTH MUSCLE CELLS

Authors: Nicole Mendoza, Katherine Kaiser, Joshua Hutcheson

Abstract: Patients with osteoporosis frequently exhibit signs of vascular calcification, which is termed the "calcification paradox." These conditions traditionally have been considered independent processes related to aging, although recent studies revealed a close relationship between the loss of bone mass and vascular calcification. Despite widespread acceptance of the calcification paradox, the specific mechanisms underlying the divergence in vascular smooth muscle cell (VSMC) and osteoblast (HOB) calcification remain unknown. This project addresses this gap by analyzing calcifying extracellular vesicles (EVs) isolated from cultured HOBs and VSMCs using Tunable Resistive Pulse Sensing (TRPS) to accurately measure EV size, concentration, and charge. TRPS uses the Coulter Principle, where disruption of electric current due to EV movement across a nanopore provides physicochemical information. Osteoblast-derived EVs that mediate bone mineralization, have previously been reported to be within the ectosome size range (10-400 nm). We hypothesize that VSMCs produce smaller, exosome-like EVs (40-160 nm) as a result of their unique mechanism of formation. We expect to observe increased calcifying EVs in cultures exposed to osteogenic stimuli. Analyses of EV duration within the TRPS pore provides insight on EV charge, a critical property associated with mineral formation. Negatively charged phospholipids help immobilize calcium ions to begin the mineral nucleation process. This research aims to further clarify the mechanisms behind the calcification paradox and contribute to the broader understanding of the similarities and differences in the mineralization processes of bone and vascular cells. The findings could have significant implications for developing therapeutic strategies against vascular calcification and bone-related diseases.

KRUPPEL-LIKE FACTORS AND VALVULAR HOMEOSTASIS

Authors: Lucas Menendez, Daniel Chaparro, Joshua Hutcheson

Abstract: Vascular Endothelial Cells can respond to various physiological signals such as hemodynamic shear, or blood flow. Kruppel-like factors-2 (KLF2) is a hemodynamic shear-dependent transcription factor responsible for homeostatic maintenance. In cardiovascular disease, increased KLF2 expression is correlated with decreased inflammation, vascular tone, and thrombosis. Moreover, KLF2 overexpression may prevent mice from acquiring atherosclerosis (Fan, 2017). An increase in hemodynamic shear is associated with KLF2 overexpression (Huang, 2021). In vasculature tissues, it has been observed that a decrease in hemodynamic shear is correlated with KLF2 downregulation and is associated with higher calcification potential. Although KLF2 has been studied extensively in vasculature, the role of KLF2 in aortic valve (AoV) calcification remains unknown. KLF2 likely plays a similar role in both vascular and valvular tissues. Each AoV leaflet exhibits different levels of calcification which may be due to changes in hemodynamic shear profiles caused by the presence or absence of coronary ostia. We hypothesize that the absence of an ostium decreases laminar flow, which reduces the levels of flow dependent KLF2 and associate with increased levels of calcification. We analyzed the amount of KLF2 in calcified mouse AoV leaflets and our preliminary data suggests there is an inverse relation between calcification and KLF2 expression. We can then relate the differences in laminar shear stress between each and determine if there is a relationship between stress and calcification. Elucidating the role of KLF2 in valvular calcification could serve as a springboard for future therapeutic targets to combat early-onset pathological remodeling before the need for surgical intervention.

CAVEOLIN-1 TRAFFICKING IN CALCIFYING EXTRACELLULAR VESICLE FORMATION

Authors: Stephania Barroso, Katherine Kaiser, Joshua Hutcheson

Abstract: Calcification by vascular smooth muscle cells (VSMCs) is the process of deposition of mineral within the arterial walls, which can result in increased vascular stiffness. Calcification by osteoblasts (HOBs) is the deposition of calcium by bone-forming cells, essential for skeletal structure and function. Both processes rely on the formation and release of calcifying extracellular vesicles (EVs), however, their unique mechanisms of formation are not well understood. Caveolin-1 (CAV1) is a protein essential for VSMC calcification, found in low-density plasma membrane regions known as caveolar domains. Our previous data show that during VSMC calcification, CAV1 relocates from caveolar domains into higher density membrane regions. Inhibition of calpain, an intracellular protease, prevents the cleavage of Filamin A (FLNA), which links CAV1 to the actin cytoskeleton. VSMCs cultured in media supplemented with calpain inhibitor are unable to form mineralization, while the same treatment has no effect on HOB mineralization.

We hypothesize that by stabilizing the association between CAV1 and actin, calpain inhibition arrests CAV1 in caveolar domains and prevents the biogenesis of calcifying EVs within VSMCs, which occurs through intracellular trafficking. HOB EVs result from direct membrane budding, so the stabilization of CAV1 is hypothesized to have no effect on HOB EV formation. Density gradient ultracentrifugation will be employed to separate regions of VSMC and HOB plasma membranes. The CAV1 content of the resulting layers will then be measured using western blotting. We expect that CAV1 will remain in low-density fractions in calpain inhibitor treated VSMC groups. In HOBs, we expect no change in CAV1 location as their EV formation appears independent of CAV1 translocation.

LIPIDOMIC AND PROTEOMIC CHARACTERIZATION OF CALCIFYING EXTRACELLULAR VESICLES

Authors: Katherine Kaiser and Joshua Hutcheson

Abstract: Vascular calcification is the most significant predictor of an individual's risk of heart attack. In response to pathological conditions, such as hypertension and mechanical stress, vascular smooth muscle cells (VSMCs) that line the artery wall transition to an osteoblast (HOB) like phenotype. This allows VSMCs to produce similar hydroxyapatite mineral that HOBs produce naturally, for the purpose of potentially stabilizing atherosclerotic plaques and resisting vascular deformation. In both forms of mineralization, cells release small membrane bound compartments called extracellular vesicles (EVs) that carry pro-calcific cargo into the extracellular space to nucleate mineral formation. Despite the similarity of these processes, recent data suggest that the mechanism through which VSMCs create calcifying EVs is distinct from that of HOBs. To further investigate this, our group will isolate EVs from VSMCs and HOBs grown in osteogenic media and identify their lipidomic and proteomic composition. We aim to use this profile of lipids and proteins to identify unique characteristics between the two EV populations, thus providing more insight into their individual mechanistic origins.

MATHEMATICAL MODELING OF HEMODYNAMICS IN A DYNAMICALLY CHANGING CEREBRAL MICROVASCULAR NETWORK

Authors: Michelle Wiese and Nikolaos Tsoukias

Introduction: The brain is supplied with nutrients and oxygen by a complex microvascular network, mostly consisting of capillaries. Neurovascular coupling, the signaling of active neurons to the blood vessels, affects blood perfusion. Neurodegenerative diseases, including Alzheimer's and dementia, may compromise neurovascular coupling and blood flow control.

Oxygenation of cerebral tissues depends on red blood cell (RBC) perfusion throughout the tissue. RBC spread is dynamically changed by blood flow and hematocrit (HD), which vary based on stimuli. Capillary stalling, the absence of RBCs within a vessel, has been noted in capillaries and could impair cerebral tissue function.

Methods: Utilizing a microvascular network of mouse cerebral cortex, hemodynamic simulations of pressure, flow, and HD distributions are calculated. Flow is calculated in each vascular segment using the Hagen-Poiseuille equation. The non-continuum nature of blood is accounted for according to the Fahraeus-Lindqvist and phase separation effects. Conservation laws are observed at bifurcations. Transient capillary HD is accounted for through the tracing of individual RBCs throughout the network. The stochastic nature of RBC movement necessitates the use of Poisson entry times and path selection at bifurcations based on the phase separation effect.

Results and Discussion: Previous multiscale modeling of cerebral tissue perfusion and cell signaling does not capture local hematocrit changes due to dynamic vessel diameter change. Implementation of RBC tracking addresses this issue by transiently predicting HD and viscosity. Our introduction of stochastic RBC movement, based on the stated bifurcation laws, gives the HD distribution predicted by the Pries and Secomb empirical correlations.

PREDICTING NITRIC OXIDE BIOTRASPORT IN MICROVASCULAR NETWORKS USING THE GREEN'S FUNCTION METHOD

Authors: Mahsa Saadat and Nikolaos Tsoukias

Abstract: Nitric oxide (NO) plays a pivotal role in a variety of physiological processes, including in regulating blood flow and pressure. Understanding NO transport and signaling in the microcirculation can elucidate its biological functions and therapeutic possibilities. This study presents a computational approach to describe NO release and transport in three-dimensional microvascular networks using the Green's Function method. This method efficiently accounts for NO release by endothelial cells and consumption by Red Blood Cells flowing in complex vascular network geometries. Integration of the proposed approach with detailed models of blood flow in brain will provide a quantitative framework to elucidate mechanisms of blood flow control in health and their dysregulation in pathological conditions.

CHARACTERIZATION OF EXOSOMES INVOLVED IN VASCULAR CALCIFICATION USING MULTIFUNCTIONAL NANOPIPETTES

Authors: Trisha Chapagain, Santosh Khatri, Jin He

Abstract: Chronic kidney disease (CKD) can lead to early cardiovascular disease, which is the leading cause of death in the world. The high phosphate imbalance present in CKD accelerates calcification nucleation in exosomes. These exosomes, in turn, nucleate pathological calcification in the arterial wall. In CKD, the stretching of the vascular smooth muscle cells (VSMCs) due to hypertension may lead to a higher number of exosomes being secreted from the cells, accelerating cardiovascular calcification. The objective of this study is to use multifunctional nanopipettes, a novel biophysical technique, to compare and characterize exosomes secreted from mechanically stretched vs. unstretched VSMCs. The multifunctional nanopipettes, fabricated by pulling quartz theta capillary tubes with a laser pipette puller, consist of a nanopore and a carbon nanoelectrode containing a pyrolytic carbon deposit. The exosomes are loaded inside the nanopore barrel and driven out with an electric potential, allowing for the nanopore to detect the size and quantity of the exosomes while the carbon nanoelectrode is able to detect its charge. This procedure is conducted for both stretched and unstretched VSMC exosomes. Compared to the exosomes collected from the unstretched VSMCs, exosomes from the stretched VSMCs exhibit a higher frequency of signals collected by the nanopipette. They also exhibit a greater charge than their counterparts. This suggests that the stretching of vascular smooth muscle cells leads to a direct increase in secreted exosomes and plays a great role in the calcification of the arterial wall.

ISOLATION OF CEREBRAL MICROVASCULAR NETWORKS TO INVESTIGATE CAPILLARY-MEDIATED NEUROVASCULAR COUPLING

Authors: Katherine Lopez, Karen Colmenares, Nikolaos Tsoukias

Abstract: Blood flow regulation in the brain is essential for delivering oxygen and nutrients to active neurons. Neurovascular Coupling links neural activity to changes in cerebral blood flow, a process often impaired in neurodegenerative disorders like dementia and Alzheimer's. Recent research indicates that direct communication occurs between neurons and the smallest blood vessels, capillaries, in addition to established signaling with feeding arterioles. This study aims to investigate a novel mode of neurovascular communication by adapting a capillary-arteriolar ex-vivo preparation. The experiments involve using a C57BL6 mice and genetically modified Kir2.1 knockout mice. Brain isolation is performed following euthanization, and the middle cerebral artery is dissected to isolate pial arteries and parenchymal tissue containing capillary networks. The isolated vascular network is stretched and transferred for electrophysiology and fluorescence microscopy. The ex-vivo preparation offers a valuable tool for understanding capillary dynamics and signaling in blood flow regulation. The study focuses on the electrical properties of capillary networks, including signaling dynamics and communication with upstream arterioles, with a specific interest in the Kir 2.1 channel. Preliminary work indicates the feasibility of isolating and characterizing long capillary networks. The Kir-KO mice will help explore the role of the Kir 2.1 channel in neurovascular coupling. This ex-vivo model offers insights into the electrical properties of the capillary network and its significance in neurovascular regulation, with implications for both normal brain function and neurovascular disorders.

MULTISCALE MODELING OF ARTERIOLAR AND CAPILLARY FACILITATED SIGNALING INFLUENCE ON BLOOD FLOW REGULATION IN THE BRAIN

Authors: Dabasish Kumar Saha and Nikolaos Tsoukias

Abstract: Neurovascular communication (NVC) is essential for normal brain function but becomes compromised in brain disorders. Capillaries, located near neurons, play a crucial role in monitoring neuronal activity. Recent evidence suggests that capillaries can detect and initiate vasoactive signaling. However, the coordination of blood flow distribution in the microvascular network through arteriolar and capillary mediated NVC remains unclear. The structural complexity and challenges of experimental observation make modeling necessary to gain insights into these mechanisms. In this study, we incorporate detailed cell-level models into multicellular in-silico representation of capillaries and arterioles within a reconstructed large-scale vascular network. Biomechanical models translate electrical and calcium signals into changes in vessel diameter, predicting network hemodynamics in macroscale tissue volumes. We simulate the effects of local stimulation by applying a hyperpolarizing current to an arteriole or capillary segment, examining electrical conduction along the network, and resulting changes in blood flow distribution. At low levels of stimulation, hyperpolarizing signal is confined to the stimulated region, inducing a modest increase in local blood flow perfusion. Higher stimulation is required for the vasodilatory signal to travel over significant distances and reach upstream feeding vessels. Simulations also explore the role of K+ as an NVC mediator in arterioles and/or capillaries. The spread of the electrical signal depends on Kir channel density, which serves a dual role as a K+ sensor and an amplifier of propagating electrical signals. The model investigates the biophysical determinants of capillary and arteriole-initiated vasodilatory signaling and their contributions to blood flow control in the brain.

MEET OUR GUEST SPEAKER

Kartik Balachandran, Ph. D.

Presenting

LABEL-FREE ASSESSMENT OF EARLY CALCIFIC AORTIC VALVE DISEASE PROGRESSION – THE ROLE OF ALTERED METABOLISM

Kartik Balachandran, Ph.D., received his bachelor's degree in Mechanical Engineering from the National University of Singapore, and his master's degree in Mechanical Engineering and Ph.D. in Bioengineering from Georgia Institute of Technology. His primary expertise is in mechanobiology, mechanics, structure-function relationships, and organ-chip engineering. His research group is interested in how altered mechanics and structure contributes to the biological progression of cardiovascular, neurovascular and epithelial diseases, and how this knowledge can be utilized to develop therapeutic strategies and early disease detection strategies. His research is funded by NIH, NSF and the DoD. He joined the University of Arkansas in 2012, where he is currently Professor and Graduate Program Director in the Department of Biomedical Engineering.

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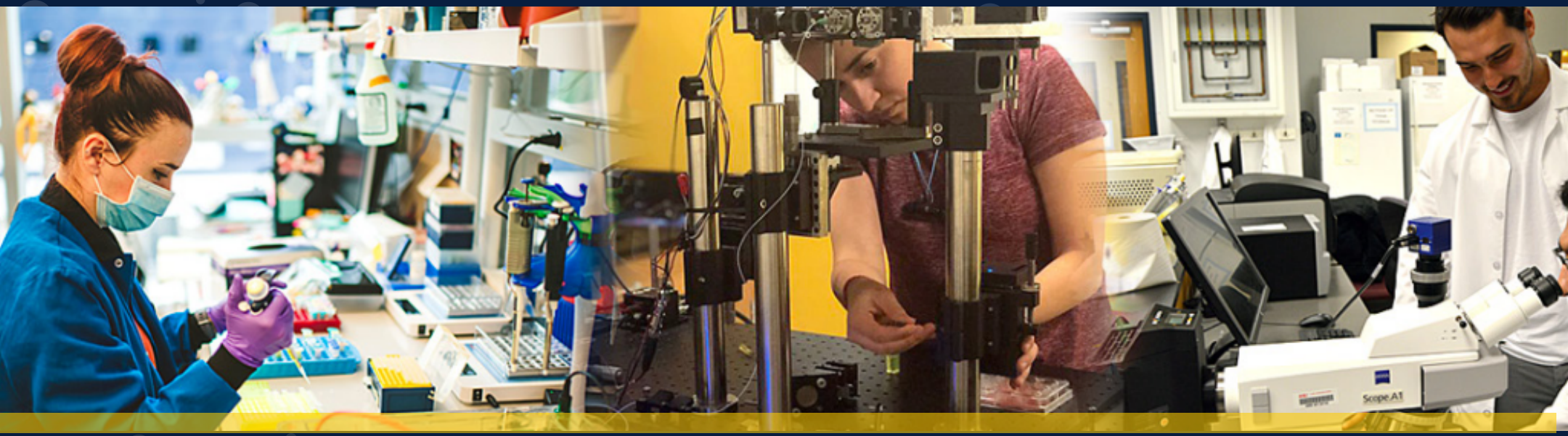

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