FLORIDA INTERNATIONAL UNIVERSITY

Miami, Florida

DEVELOPMENT OF VALVULAR CELL AND TISSUE CALCIFICATION FROM FLUID-INDUCED OSCILLATORY SHEAR STRESSES

A proposal submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

In

BIOMEDICAL ENGINEERING

By

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1. Introduction/Statement of Problem

Calcific aortic valve disease (**CAVD**) is one of the most prevalent chronic heart problems that involves hardening of aortic valve leaflets due to build-up of calcified deposits, eventually leading to valve failure. Statistical data show a global increase in CAVD by 124% between 1990 and 2017 [1]. Current treatment options for early and intermediate stages of CAVD are not available, and the main factors associated with early/intermediate CAVD remain unclear. Severe CAVD treatments include bioprosthetic or mechanical valve replacements, which are limited to a selective patient subset. A major obstacle in developing therapeutic targets for early/intermediate CAVD intervention is an absence of human tissue model systems that can assess the responses to the treatment, recognizing that animal models are unable to adequately mimic the human response [2]. Based on our preliminary data that show vulnerability to calcification at high levels of temporal flow oscillations, understanding the specific effects of flow oscillations on valve pathology development can help establish the foundation for developing a human engineered tissue model system for early and mid-stages of CAVD, thereby forming a testbed for effective drug discovery. The **overall goal** of this project seeks to correlate oscillatory shear index (**OSI**) with progression of CAVD by developing a bioreactor model system with controlled oscillatory flow. The platform developed in this study also enables longitudinal mechanistic studies with evolving calcification levels in engineered valve tissues. In addition, it can be used as an *in vitro* engineered tissue model system to facilitate expedited drug discovery that is needed for the long-term care of children and adults who are more susceptible to CAVD.

2. Background/Theory

2.1 Aortic Valve Function and Anatomy

The aortic valve facilitates unidirectional blood flow from the heart to the aorta for systemic blood distribution. Aortic valve biomechanical function relies on the action of thin, membranous leaflets that open and close the valve orifice over the course of the cardiac cycle. Each leaflet consists of three layers: ventricularis, spongiosa, and fibrosa (**Fig. 1**). The ventricularis faces the ventricular compartment of the heart and is mainly comprised of elastin aligned in the radial direction, while the fibrosa layer is located on the aortic side of the leaflet and consists of mainly collagen aligned in the circumferential direction [3]. The spongiosa layer is sandwiched between the ventricularis and fibrosa layers, and is mainly comprised of glycosaminoglycans (GAGs) [4]. A monolayer of valve endothelial cells (**VECs**) reside on the outer surfaces of the aortic valve leaflets. Underneath the VECs is a sublayer of valve interstitial cells (**VICs**). Flow environments on valve tissues can significantly affect cellular interactions, such as communication between VECs and VICs. VECs are known to respond to hemodynamic stimuli, and studies have shown that VECs exposed to disturbed flow can result in pro-inflammatory phenotypic changes and endothelial-mesenchymal-transition [5], which can initiate calcification [6] [7]. Valve endothelial dysfunction can also lead to phenotypic switching of quiescent VICs to osteogenic VICs, resulting in valve calcification [5] [8].

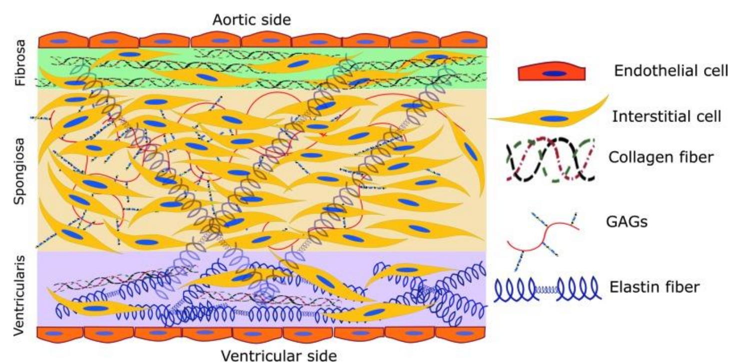

Figure 1: Structure of valve leaflets [4]

2.2 Aortic Valve Hemodynamics

Valves are subject to fluid shear stress, bending stress, and axial stretch. Shear stress is mainly experienced by leaflet surfaces, bending stress occurs as leaflet curvature changes during opening and closing, and axial stretch allows leaflet cusps to seal during coaptation. Due to valve structure and direction of blood flow, laminar flow accompanied by

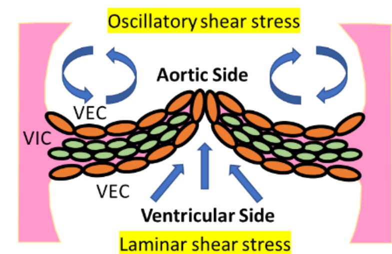

Figure 2: Valve hemodynamics

high shear stress is mainly observed on the ventricularis surface, while the fibrosa layer is dominated by low shear stress with various levels of oscillatory flow [9] (**Fig. 2**). The intensity of flow oscillation can be quantified using the oscillatory shear index (**OSI**). OSI (**Fig. 3**) is a parameter that measures flow disturbance by quantifying the ratio between forward shear (τ_w) and total shear [10]. Previous research show low shear stresses (<4 dynes/cm^2) [11] [12], specifically in vascular bifurcation sites, are commonly associated with lesions and calcification [13] [14]. Studies are needed to determine the effect of low shear stress and high flow oscillation in the development of CAVD. There is a lack of understanding between precise flow oscillations, specifically OSI regulation, and development of CAVD. A major obstacle in developing therapeutic targets for CAVD is an absence of human tissue model systems that can reliably assess the response to the treatment, recognizing as well that animal models are unable to adequately mimic the etiology of human CAVD.

$$OSI = \frac{1}{2}\left(1 - \frac{\left|\int_0^T \tau_w dt\right|}{\int_0^T |\tau_w| dt}\right)$$

Figure 3: OSI Equation [10]

3. Main Objective of Thesis Proposal

The research objective of this study seeks to correlate oscillatory shear stresses with progression of CAVD in a bioreactor environment with controlled oscillatory flow. Not only would this oscillatory bioreactor model system provide mechanistic and longitudinal studies of CAVD at the organ-level, but will also provide a platform to accelerate drug discovery for effective pharmaceutical management to prevent or slow the progression towards severe valve calcification.

4. Specific Aims and Hypothesis

To further determine the level of oscillatory flow patterns under a physiologically-relevant level of time-averaged valve leaflet shear stress (1 dyne/cm^2) and its association with CAVD, an OSI parameter is used to quantify changes in the direction of shear stress [10]. The effect of valve tissue calcification due to OSI as a key regulator of shear-dependent mechanotransduction has not been studied. **We hypothesize that a combination of high degrees of flow oscillations with pro-calcific (PC) biomolecular cues promotes aortic valve calcification, as oscillatory flow in blood with high calcium concentrations are known to trigger inflammation on the valve fibrosa layer** [5]. Our specific aims are as follows:

Aim 1: To evaluate the extent to which paracrine signaling-mediated events from VECs cultured under dynamic conditions in low (OSI=0), moderate (OSI=0.25), and high (OSI=0.50) OSI environments lead to VIC calcification.
Experimental Strategy: VECs will be conditioned under different OSI environments at a physiologically relevant magnitude of time-averaged shear stress (1 dyne/cm^2) for aortic valve leaflet tissues. To establish paracrine communication by exposing biochemical end-products from VECs to VICs, conditioned media of each OSI group from VECs will be collected and subsequently reused to culture VICs with equal volume of fresh PC media.
Expected Outcome: We expect that VICs exposed to high OSI VEC end-products in conjunction with PC environments will exhibit significantly higher calcification levels compared to other OSI groups. The goal of this aim is to identify OSI-mediated paracrine signaling between VECs and VICs that promotes calcification.

Aim 2: To assess calcification in tri-leaflet engineered valve tissue (3-dimensional) culture consisting of bio-scaffolds seeded with valvular cells conditioned *in vitro* under dynamic fluid induced mechanical environments with time-averaged shear stress and OSIs from Aim 1 in PC media in a bioreactor system.
Experimental Strategy: Using computational fluid dynamics (**CFD**), OSI environments in the bioreactor chamber will be quantified and validated using pressure and flow probes. Next, VECs and VICs will be seeded in cylindrical porcine small intestinal submucosa (**PSIS**) bio-scaffolds in tri-leaflet configurations to be conditioned in PC environments under a valve-relevant magnitude of time-averaged shear stress

(1 dyne/cm^2) in conjunction with low, moderate, and high OSIs in the bioreactor. Gene expression and histological assessments will be performed on conditioned tissues to determine level of calcification.
Expected Outcome: Under a physiologically relevant magnitude of time-averaged aortic leaflet shear stress, we expect that tissues exposed to high OSI in a PC environment will exhibit significantly higher calcification compared to low or moderate OSI conditions. The goal of this aim is to identify a specific OSI condition to pair with PC media, as an *in vitro* framework for our engineered tissue model system for re-creating the etiology of aortic valve calcification.

Aim 3: Identify spatial calcification and impact on valve function via hydrodynamic performance and strain mapping of bio-scaffold valves seeded with VECs and VICs. The valves will be sutured in a tri-leaflet configuration to mimic aortic valve function and conditioned in a high calcium phosphate environment in a bioreactor system under the most calcific OSI flow profile (as determined from Aims 1 and 2).
Experimental Strategy: VECs and VICs will be seeded in cylindrical PSIS bio-scaffolds in tri-leaflet configurations to be conditioned in PC environments under a valve-relevant magnitude of time-averaged shear stress (1 dyne/cm^2) in conjunction with low, moderate, and high OSIs in the bioreactor. Histological assessments on valve tissues will be performed to relate spatial calcification to OSI on the respective fibrosa and ventricularis surfaces. Valve hydrodynamic performance with strain mapping will be conducted to assess valve functionality with quantification of the transvalvular pressure gradient and the valve's regurgitation fraction.
Expected Outcome: For valve tissues exposed to high OSI (OSI=0.50) in PC media, we expect more calcification and a higher degree of non-uniform leaflet strains, resulting in poorer hydrodynamic performance. The goal of this study is to quantify early, intermediate, and severe stages of calcification via a mechanical metric (leaflet strains), while establishing a correlation with the valve's corresponding hydrodynamic parameters (effective orifice area, EOA, transvalvular pressure, and regurgitation fraction). Once identified, this platform can be used as an *in vitro* engineered human tissue model system to facilitate expedited drug discovery that is needed for earlier disease management in patients with CAVD to prevent or delay the need for a surgical valve replacement.

5. Research Strategy

Aortic valve remodeling often involves paracrine signaling between VECs and VICs, and diseases such as CAVD can result from improper communication between these cells. Studies have shown that VECs exposed to oscillatory flow can result in pro-inflammatory phenotypic changes and endothelial dysfunction [5]; endothelial transition is a significant modulator of progression of CAVD through VIC activation and calcification [8].

Preliminary Data

Two-dimensional cellular studies using rat VECs and VICs show that exposure of high OSI to VECs in combination with pro-calcific media (PC, control media supplemented with 1.8 mM $CaCl_2$, 3.8 mM NaH_2PO_4, 0.4 units of inorganic pyrophosphate 5% FBS, and 1% P/S [15] [16]) on VICs leads to significant calcification. Results from alizarin red staining of VICs (**Fig. 4**) show highest calcification under 0.50 OSI conditions. Statistical analyses of extracted alizarin red dye (**Fig. 5**) confirmed a significantly increased calcification ($p<0.001$) in the 0.50 OSI group compared to static, 0 OSI, and 0.25 OSI. On the other hand, comparisons of VIC calcification between 0.25 OSI vs. Static and 0.25 OSI vs. 0 OSI were not significant ($p>0.05$). Our preliminary gene expression data (**Fig. 6**) also show an upregulation of Bmp2 and Runx2 in VICs exposed to pro-calcific media in addition to conditioned media from VECs from the high OSI group. These genes are commonly associated with calcification responses.

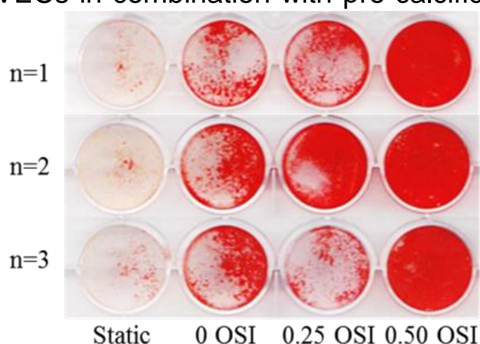

Figure 4: Alizarin red staining of VIC in oscillatory flow-conditioned VEC media with pro-calcifying components

Previous studies have shown an upregulated αSMA gene and protein expression in VICs at wound sites [17]. This correlates with our findings where groups that exhibited high calcification also experience upregulation of αSMA. The findings suggest that VECs exposed to low-to-moderate levels of flow oscillations permit VICs to maintain a quiescent phenotype via paracrine signaling. However, augmented extracellular matrix calcific conditions coupled with high oscillatory flow regions (OSI=0.50) on VECs lead to substantial risk of increasing VIC calcification. This corroborates current theories of increased VIC calcification under oscillatory flow and the fact that calcific aortic valve diseases are mainly associated with the fibrosa layer, where flow oscillation is commonly observed. ***A key point from our findings is that the quantification of this oscillatory flow environment is necessary, and this can be done via OSI. In addition, a maximum OSI value of 0.50 is specifically responsible for inducing VIC calcification via VEC-based paracrine signaling when the relative concentration of pre-calcifying agents is high within the circulation.*** The proposed project will build upon our preliminary data to elucidate the linkage between calcific valves and various oscillatory flow environments. The project will also provide a tissue model platform as a tool for longitudinal studies that will facilitate therapeutic discovery, which will thereby enable more effective management of patients with higher vulnerability to CAVD in adulthood.

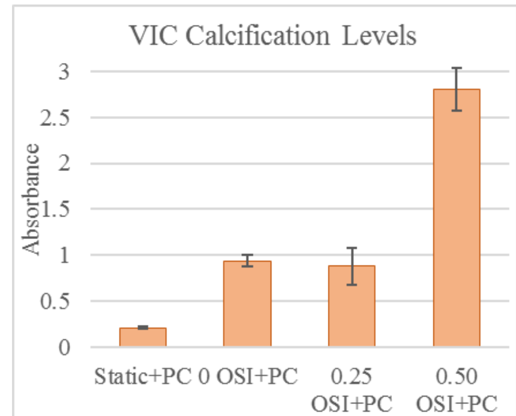

Figure 5: Absorbance at 405 nm after alizarin red dye extraction. Plot data are expressed as mean (n=3) with error bars representing standard error of the mean.

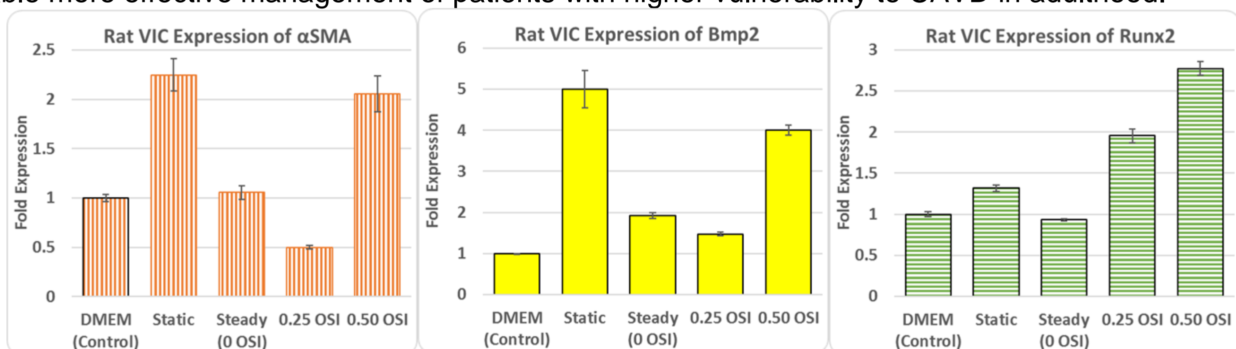

Figure 6: Rat VIC expression of αSMA, Bmp2, Runx2 after 7 days of culture in VEC conditioned media. Regular DMEM without VEC media was used as control. Fold change is expressed as $\Delta\Delta C_T$, or Livak method [18].

Research Plan

Aim 1: To evaluate the extent to which paracrine signaling-mediated events from VECs cultured under dynamic conditions in low (OSI=0), moderate (OSI=0.25), and high (OSI=0.50) OSI environments lead to VIC calcification.

Cell culture and expansion: Commercially available rat VECs will be purchased from Celprogen, Inc. (Torrance, CA) and expanded with Rat VEC Culture Complete Growth Media with Serum and Antibiotics in T75 flasks coated with extracellular matrix. Commercially available rat VICs will be purchased from Innoprot (Bizkaia, Spain) and expanded in T75 flasks using regular Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S).

Conditioning VECs in OSI environments: VECs will be seeded for 24 hours at 2.0×10^5 cells per channel in 24-well Bioflux plates consisting of 8 microfluidic channels per plate (Fluxion Biosciences, Inc., Alameda, CA). Using OSI as a parameter to quantify the change in direction of shear stresses, VECs will be conditioned for 48 hours in a shear stress cell assay system (Bioflux 200, Fluxion Biosciences, San Francisco, CA), at an average shear stress magnitude of 1 dyne/cm^2 under the following OSIs: static (no

flow, 0 OSI), steady flow (0 OSI), 0.25 OSI (moderate oscillation), and 0.50 OSI (full oscillation), where regular DMEM without VEC media will be used as control.

VEC-VIC paracrine regulation: Conditioned media from VEC groups will be collected and subsequently used to culture VICs in 12-well plates with equal volume of pro-calcific media (PC) consisting of control media supplemented with 1.8 mM $CaCl_2$, 3.8 mM NaH_2PO_4, 0.4 units of inorganic pyrophosphate, 5% FBS, and 1% P/S. VIC conditioning will last for 7 days with one media change on day 4.

VIC calcification assessments: Upon termination of VIC exposure to various VEC flow group media, VIC calcification will be measured by alizarin red staining (ARS). The alizarin red dye will then be extracted and quantified with a microplate reader. In addition to ARS, alkaline phosphatase (ALP) activity will be assessed in VIC conditioned media collected on day 7. Alkaline phosphatase is an enzyme involved in bone formation by increasing the availability of phosphates used for hydroxyapatite synthesis [19]. The increase in availability of phosphates also increases formation of calcium phosphates, or the mineral commonly found in valvular calcification [20]. Commercially available Alkaline Phosphatase Activity Colorimetric Assay Kit (BioVision, Inc., Milpitas, CA, USA) will be used to perform the assay, which will quantify level of calcium deposits in each sample using a microplate reader set to 405 nm wavelength. Values obtained from both ARS and ALP assays will be in triplicates per sample (n=3/group). ALP data will be normalized to VIC RNA concentration.

Analysis of cellular responses to environmental cues: Cellular responses to environmental cues involve conversion of DNA into working proteins, and this process of translation utilizes messenger RNA transcription as well as transfer RNA. To evaluate the gene expression step that precedes protein translation, RNA will be extracted from the cells followed by quantitative Polymerase Chain Reaction (qPCR). Upon termination of the 48-hour flow conditioning of VECs and 7-day conditioning of VICs, TRIzol Reagent (Invitrogen, Thermo Fisher Scientific) will be applied to collect VEC and VIC RNA. RNA will be extracted and quantified using NanoDrop™ One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific). Power SYBR Green RNA-to-CT 1-Step Kit (Applied Biosystems, Foster City, CA, USA) with forward and reverse primers sequenced in the NCBI GenBank (National Center for Biotechnology Information, Bethesda, MD, USA) and purchased from IDT PrimerQuest® Tool (Integrated DNA Technologies, Coralville, IA, USA) will be used to perform qPCR. Fold change will be computed using the ΔΔCT Livak method [18] with the static group as control. Genes of interest include and are not limited to Tnap, vWF, αSMA, Runx2, Bmp2, Col1a1, Mmp2.

Potential pitfalls and alternative approaches: Our preliminary data show that VECs exposed to a high OSI (at a maximum value of OSI=0.5) leads to significantly increased VIC calcification after paracrine regulation. However, it is possible that such VIC calcification may be due to exposure to low shear stress magnitude, since *in vivo* average physiological, aortic valve leaflet shear stresses can be higher (compared to our application of 1 dynes/cm^2) in magnitude [21]. In this case, we will conduct an additional test group on VECs using a higher shear stress in the Bioflux system.

Aim 2: To assess calcification in tri-leaflet engineered valve tissue (3-dimensional) culture consisting of bio-scaffolds seeded with valvular cells conditioned *in vitro* under dynamic fluid induced mechanical environments with time-averaged shear stress and OSIs from Aim 1 in PC media in a bioreactor system.

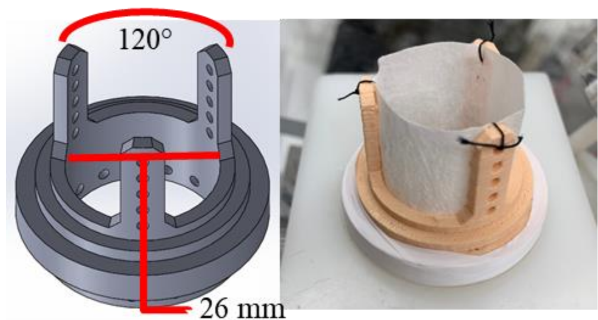

Figure 7: 3D printed tricuspid valve holder (left). Suturing of PSIS valves onto valve holder and mounting of the valve in the aortic position of the Vivitro pulse duplicator (right).

Culturing valve tissues using VECs and VICs: VECs and VICs will be seeded for 8 days in a 26-mm PSIS cylindrical bio-scaffold (CorMatrix Cardiovascular Inc., Roswell, GA). The cylindrical PSIS will be sutured to a custom, 3D printed valve holder along its ring and posts (**Fig. 7**), forming a tri-leaflet geometry under flow conditions. Seeded valve tissues will be subject to low

(OSI=0), moderate (OSI=0.25), and high OSI (OSI=0.50) with pro-calcific media in a novel bioreactor for 7 days, where static (no flow) will be used as control.

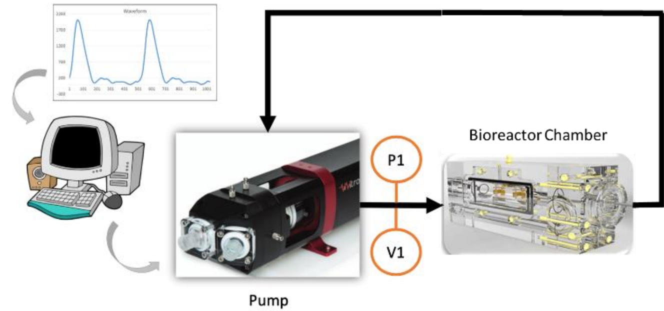

Figure 9: Bioreactor Schematic

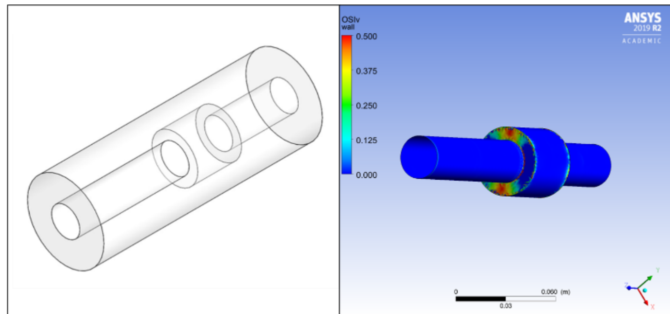

Figure 8: Bioreactor chamber and CFD

Bioreactor design using CFD and subsequent dynamic OSI-based conditioning: A novel pulsatile flow bioreactor system that facilitates oscillatory flow conditions will be designed and constructed using acrylic or similar transparent material (**Fig. 8**). CFD modeling (**Fig. 9**) will be carried out using commercially available software SolidWorks (Dassault Systemes, Waltham, MA, USA) to create the three-dimensional geometry and ANSYS (Ansys, Inc., Canonsburg, PA, USA) for fluid flow simulations within Windows 10, 64-bit operating system (Microsoft, Inc., Redmond, WA, USA). Flow waveforms delivering moderate and high OSIs will be generated from CFD and delivered by Vivitro Superpump (Vivitro Labs, Victoria, BC, Canada). Flow rate and pressure in bioreactor chambers will be verified using a magnetic volumetric flow probe and three pressure transducers from the Vivitro Pulse Duplicator System (Vivitro Labs). Pro-calcific media will be used to induce calcification under different OSI environments.

Valve tissue calcification assessments: Samples conditioned in the bioreactor will be processed for histology, ARS, qPCR, media ALP assay, and fluorescent detection of calcification with OsteoSense. To identify important features of cells and tissue samples, histological stains will be performed. Histological samples will be melted on glass slides for hematoxylin and eosin (H&E) staining upon cryostat sectioning at 10 microns per slice. To detect presence of calcification, tissue samples will be immersed in OsteoSense 680EX, which binds to calcium nodules. The samples will then be washed with PBS and placed in Li-Cor Odyssey System to be imaged at 680 nm.

Potential pitfalls and alternative approaches: Our preliminary data show that VECs exposed to high OSI (at a maximum value of OSI=0.5) lead to significantly increased VIC calcification after paracrine regulation. However, *in vitro* hemodynamic design may not fully recapitulate the complex flow environment of in vivo circulation. In the case that tri-leaflet PSIS scaffolds seeded with VECs and VICs exposed to different OSIs in the bioreactor system do not show difference in calcification, we may conduct a simpler bioreactor test using rectangular PSIS tissue strips as scaffolds for VEC and VIC seeding.

Aim 3: Identify spatial calcification and impact on valve function via hydrodynamic performance and strain mapping of bio-scaffold valves seeded with VECs and VICs. The valves will be sutured in a tri-leaflet configuration to mimic aortic valve function and conditioned in a high calcium phosphate environment in a bioreactor system under the most calcific OSI flow profile (as determined from Aims 1 and 2).

Spatial calcification of valves and impact on valve function: Using CFD, spatial OSI of cylindrical PSIS valve leaflets will be quantified on valve surfaces. VECs and VICs will be seeded for 8 days in a 26-mm cylindrical PSIS bio-scaffold. The cylindrical PSIS will be sutured to a custom, 3D printed valve holder along its ring with three posts prior to seeding (**Fig. 7**), forming a tri-leaflet geometry. Seeded valves will be conditioned in bioreactor system for 7 days under a pulsatile flow profile with the most calcific OSI (as determined from Aims 1 and 2). A co-relation between CFD spatial quantified leaflet OSI to experimentally measured calcified maps on the corresponding leaflet spatial locations will be investigated upon termination of the bioreactor experiments. Hydrodynamic testing will be performed using the Vivitro pulse

duplicator system containing 0.9% saline solution. Conditioned samples will be mounted in the aortic position with a flow probe affixed between the aortic and ventricular chambers. Three pressure transducers will be placed in the atrial, ventricular, and aortic locations. Valve performance tests will utilize a stroke volume of 71.4 mL, heart rate of 70 beats per minute, and input flow waveform comprising of a 35% systolic-65% diastolic configuration (S35 Waveform, Vivitest software, Vivitro Labs). A non-seeded PSIS valve and a bi-leaflet mechanical valve will be used as control.

Mechanical assessments of calcified valve tissue: Valve structures will be assessed by strain mapping (in Matlab, Mathworks, Natick, MA) derived from high speed camera images (**Fig. 10**) collected during valve hydrodynamic testing. Images will be used to measure valve leaflet deformation after different time points of accumulated tissue calcification. The strain maps will be used to identify structural reasons for compromised valve function and subsequently to relate tissue heterogeneity to the underlying valve microstructure. A non-seeded raw PSIS valve and a native uncalcified porcine valve will be used as controls.

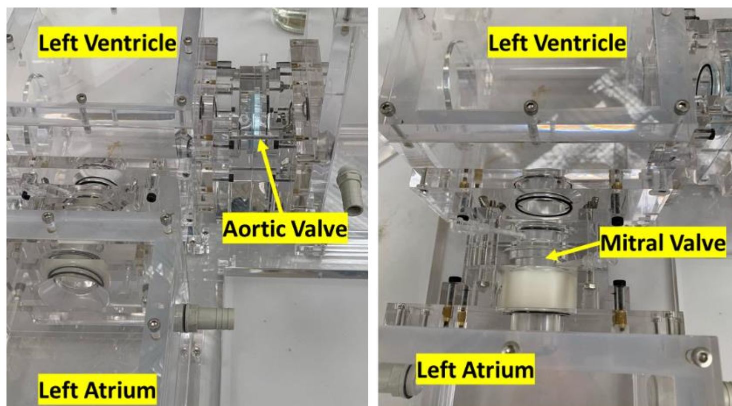

Figure 10: Apparatus of a left-side heart chamber with transparent material for strain map imaging. Test position of aortic valve is shown on the left, in addition to test position of the mitral valve to the right.

Potential pitfalls and alternative approaches: Our preliminary data show that VIC calcification is associated with exposure to high OSIs (at a maximum value of OSI = 0.50). The association of valve calcification to hydrodynamic performance and valve deformation has not been studied, and a relatively low level of calcification may not result in a significant difference in valve function. In such case, we may need to increase the severity of calcification by elevating concentration of pro-calcific ingredients in addition to an increased OSI-based bioreactor culture period.

Statistical Analysis: Each flow group experiment will be conducted at minimum of n=3. All graphs and numerical values will be presented as the mean ± standard error of the mean. Data will be evaluated using analysis of variance (ANOVA) in SPSS Software (IBM Corporation, Armonk, New York, USA) to analyze variations within the group and between the groups. Probability values less than 0.05 ($p<0.05$) will be considered significant and the null hypothesis will be rejected. To achieve a high confidence interval (≥95%) and low type II error (≤0.20), a reasonable sample size will be determined per conditioning group via power analysis.

Milestones and Timeline

TASK	*Prelim. Data*	*Spring 2021*	*Summer 2021*	*Fall 2021*	*Spring 2022*
Aim 1: 2D cell paracrine regulation assessments					
VEC-VIC calcification assessments	■				
VEC/VIC gene expression	■				
Aim 2: 3D valve tissue assessments after conditioning in bioreactor					
CFD modeling		■			
Bioreactor fabrication and verification		■	■		
Bioreactor conditioning and tissue assessments			■	■	
Aim 3: Spatial calcification in tri-leaflet valves and hydrodynamic function					
Conditioning of valves in bioreactor				■	
Strain mapping and hydrodynamics assessments				■	■

Graduate Student Diversity & Inclusion

Mentoring:
I have been a Teaching Assistant (TA) in Dr. Ramaswamy and Dr. Hutcheson's classes since I joined FIU as a PhD student, specifically for the Engineering Transport and Cell/Tissue Engineering classes. I have also been working closely with three undergraduate lab assistants. Many students are from traditionally underrepresented groups and I try to assure that their academic experience is a positive one. I would offer assistance and encouragement to others when needed, and my overall interactions with members of the BME department have been a wonderful experience.

Outreach:
As an E-board and active member for Alpha Eta Mu Beta (AEMB, BME honor society) and Tau Beta Pi (TBP, Engineering Honor Society), we have been organizing on and off campus outreach events every semester. I first joined the Florida Theta (TBP, FIU Chapter) E-board as the Professional Development Chair in early Fall 2018. I was elected the President in the middle of Fall 2018 and continued with the position until end of Fall 2020. I have now transitioned to a student advisor role for our chapter, and one of our missions is to host MindSET (https://www.tbp.org/memb/MindSETbrochure.cfm) events every semester to promote interest in STEM disciplines amongst K-12 students. As for AEMB, we've been conducting Engineers on Wheels (FIU's outreach program: https://succeed.fiu.edu/research/cd-ssec-projects/engineers-on-wheels-eow/index.html) a few times each semester, and we plan to continue participating in these events throughout the semesters.

Service:
To promote inclusion by making everyone feel welcome, the abovementioned honor societies also host social events throughout the semesters including movie nights, game nights, and sometimes off-campus activities. To dismantle barriers to people who are historically excluded from opportunities, the should-be plan is to overlook history and focus on what is happening *now*, and what can we do at the *present moment*. I do not always really know what I am doing, but somehow this leads to less biases and a higher level of serendipity, one of the greatest advantages of data-driven science, in which can also be applied to one's way of living. I am originally from Taiwan and my K-12 years were spent overseas across seven different schools in multiple countries and cities, from Taipei to California, to Singapore, and to Shanghai. As I switched from one school to the next, I learned about different cultures, languages, and the importance of making everyone feel welcome. I believe that maintaining good communication is one way to strengthen an inclusive learning community. Words cannot describe everything, but fortunately we live in a world where we can express things with art, music, and actions (even in academia and/or presenting scientific/research data!) I intend to contribute to a diverse and interactive learning environment, specifically in the STEM field, through art, such as drawings, figures, captions, and board games. I sincerely believe this will enhance our learning experiences from each other, in addition to minimizing exclusion.

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