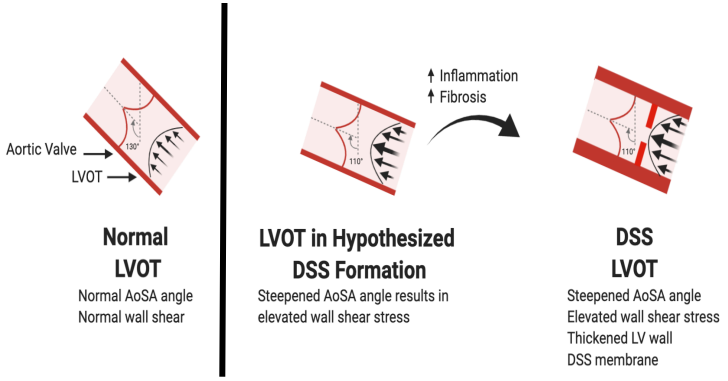
Creating an *In Vitro* Model of the Left Ventricular Outflow Tract

Katie Brown [1], Dr. Sundeep Keswani [2], Dr. Jane Grande-Allen [1]

1. Department of Bioengineering, Rice University
2. Department of Pediatric Surgery, Texas Children's Hospital

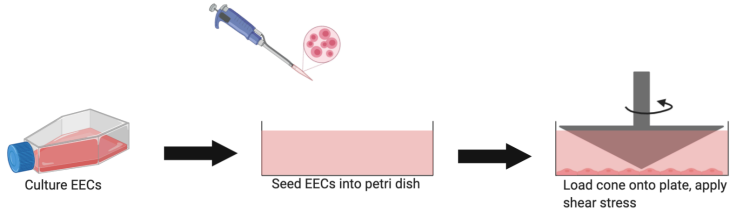
Background

Discrete subaortic stenosis (DSS) is a rare congenital heart disease in which a fibrotic membrane forms in the left ventricular outflow tract (LVOT) of the heart. The cellular mechanisms of this disease are currently unknown. We hypothesize that physical environmental factors contribute to DSS formation by upregulating inflammation and fibrosis. There is a need for an *in vitro* model of DSS to be able to understand how physical environmental factors, like high wall shear stress and tissue stiffening, influence the cells of the LVOT during DSS formation.



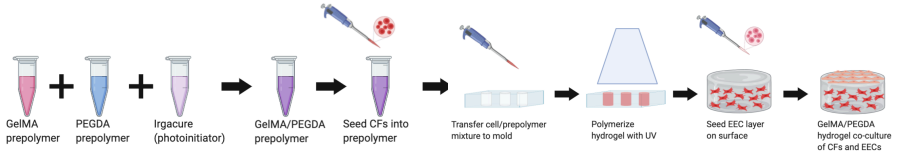
Model Development

Method used to model elevated wall shear stress



We have a developed a system in which we can expose cells isolated from the LVOT to normal and pathological fluid shear stresses, and characterize their response over 1.5-24 hours.

Method used to model tissue stiffening



We are currently working to develop a 3D co-culture hydrogel model composed of gelatin methacryloyl (GelMA) and polyethylene glycol diacrylate (PEGDA) that can be tuned in stiffness to mimic normal and pathological left ventricle stiffnesses.

Results

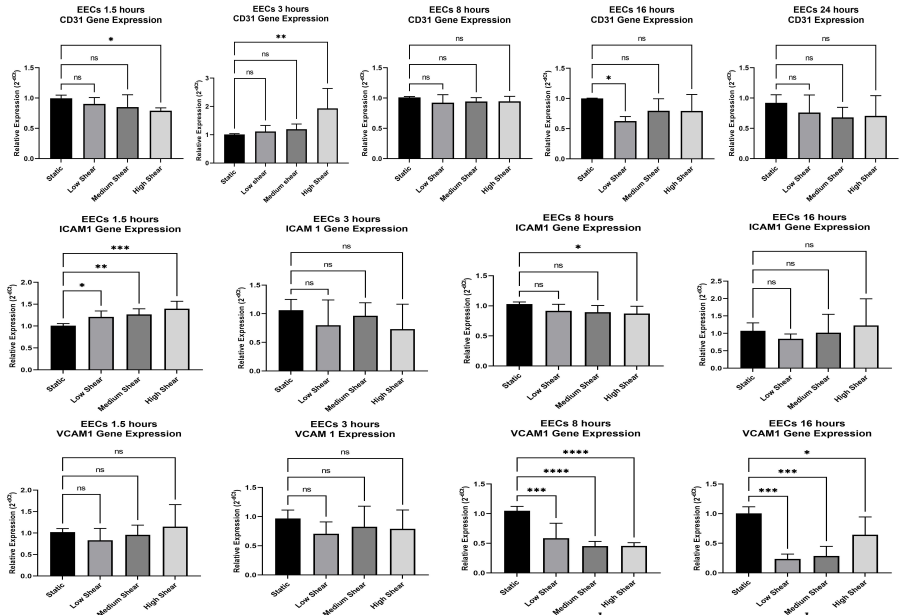


Figure 1. After exposing cells of the LVOT to Low (15 $\frac{dynes}{cm^2}$), Medium (20 $\frac{dynes}{cm^2}$), and High (35 $\frac{dynes}{cm^2}$) shear for 1.5-24 hours, the genetic expression of the cells was evaluated with qPCR.

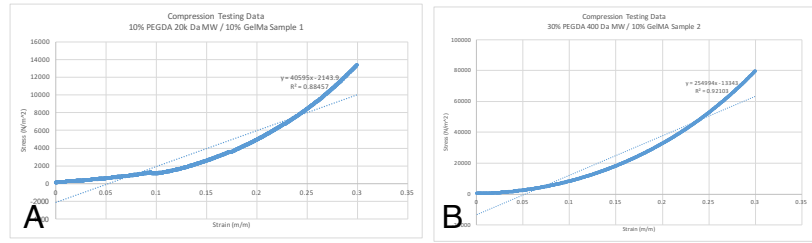


Figure 2. Compression Testing of GelMA/PEGDA hydrogels reveal tunability of the scaffold, ranging from values of A) 40 kPa to B) 250 kPa.

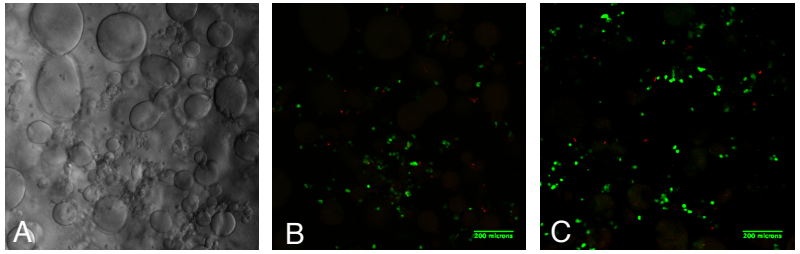


Figure 3. A) Images of hydrogel seeded with co-culture of cells (EECs and CFs) shown at B) 3 days and C) 7 days after seeding with live/dead staining.

Discussion & Next Steps

After exposing cells isolated from the LVOT to a range of shear stresses, we found that the expression profiles of different genes are influenced by time and shear rate. At 1.5 hours, ICAM1 was significantly upregulated in the low, medium, and high shear conditions. At 8 and 16 hours, VCAM1 was significantly downregulated in the low, medium, and high shear conditions. We also have developed a 3D co-culture model of GelMA and PEGDA to vary the mechanical stiffness from 40 kPa to 240 kPa to mimic tissue stiffening observed in cardiac fibrosis. Initial results suggest that the cells we have seeded into this scaffold are viable at 3 and 7 days. Collectively, results we have obtained suggest that the cells of the LVOT respond to the physical environments they experience, which could contribute to DSS. We are working to further characterize the cellular response after being seeded into the hydrogel model. Additionally, we are considering other heart diseases that could benefit from being studied with the model we have developed.

Acknowledgements

This material is based upon work supported by the National Science Foundation Graduate Research Fellowship Program under Grant No. (2019272296). Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. This work was funded by and completed in the Grande-Allen Lab at the Rice University Bioengineering Department.