A Parallel Plate Flow Chamber to Investigate Endothelial Glycocalyx Remodeling After Pneumonectomy

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***Abstract* — As a result of pneumonectomy, pathological changes such as organ and fluid displacement can occur. However, few studies have examined the changes in shear stress magnitude on the endothelial glycocalyx post-operation. A parallel plate flow chamber that can mimic normal pulmonary arteries, a left, and a right pneumonectomy was designed. Fluid flow simulations were performed to compare the shear stress experienced by the control and pneumonectomy models, revealing that the shear stress after either a left or right pneumonectomy is greatly increased. To further validate this, the flow chamber was developed. Human Lung Microvascular Endothelial Cells were seeded in control and pneumonectomy models and flow that mimicked physiological blood flow rates was introduced into the system. After being exposed to the flow, the cells were examined with a confocal microscope to identify damage to the glycocalyx due to increased shear stress post-pneumonectomy. The effect of shear stress on endothelial cells increases post-pneumonectomy and this can damage the endothelial glycocalyx. Understanding how the increase in shear stress affects cells is a critical step in reducing patient mortality after complications post-pneumonectomy.**

I. Introduction

A pneumonectomy is a surgical procedure where an entire lung is removed from a patient and the connective vessels are sealed off [1]. Very few studies have examined the effect of the increased shear stress, post pneumonectomy, on the endothelial glycocalyx. The glycocalyx is a cell coating composed of proteoglycans, glycosaminoglycans, and plasma proteins. The endothelial glycocalyx forms an interface between blood vessels walls and circulating blood[2]. The glycocalyx protects the endothelial cells and in doing so helps maintain cellular homeostasis[3]. The disruption of this layer can lead to cardiovascular disease which can be fatal in many cases[4]. Our study will examine how the glycocalyx is affected after a pneumonectomy by measuring how the endothelial cells are affected after the increases in shear stress by utilizing a parallel plate flow chamber that models the geometry of the pulmonary arteries.

II. Methodology

To mimic the conditions that endothelial cells and the glycocalyx experiences after a pneumonectomy a flow chamber was developed in Solidworks as seen in Figure 1. The flow chamber has a flow path on the bottom plate that was created to accurately mimic the anatomical diameters and angles of the pulmonary arteries[5]. The flow path also has designated spots for cells to be seeded to measure the shear stress at these points. Teflon gaskets and a top plate were also created to complete the flow chamber.

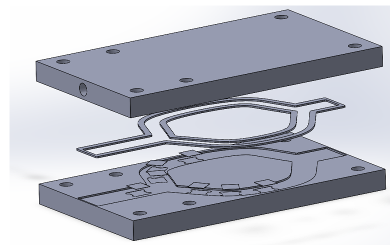

Figure 1: Solidwork model of the 2D flow chamber.

This model can be used to study changes in the shear stress for a no pneumonectomy or a pneumonectomy scenario. Flow simulations were run to study the increase in shear stress that would occur after a pneumonectomy.

For in-vitro experiments, the parallel plate flow chamber was developed. HLMVEC were cultured on glass slides and placed in the flow chamber. The cells were exposed to uniform flow of 171.6 mL/min for 6 hours. Experiments were completed for the no pneumonectomy scenario and for both types of pneumonectomies. Afterwards, the HLMVEC were fixed with a solution of 2% paraformaldehyde (PFA) and 0.1% glutaraldehyde in phosphate-buffered saline (PBS). Following the fixing of the cells, they were stained with wheat germ agglutinin (WGA; Vector Labs) to label the glycocalyx. Secondary detection uses Alexa Fluor 488-conjugate at a concentration of 1:1000. The glycocalyx samples were covered with VECTASHIELD antifade mounting medium (Vector Labs) with DAPI (4′,6-diamidino-2-phenylindole) to stain the cell nuclei. The cells were then imaged using confocal microscopy and cell coverage and thickness was quantified using ImageJ.

III. Results

Solidworks was used to perform virtual flow simulations to compare the shear stress experienced in the pulmonary arteries after a pneumonectomy. Figure 2 shows the shear stress overlays for the 3 different model types.

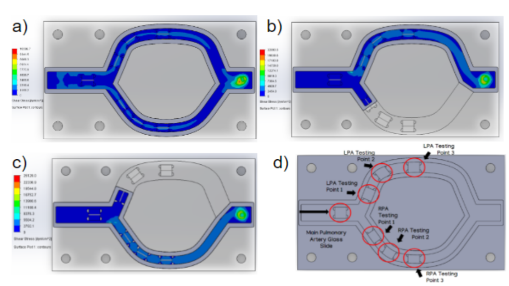

Figure 2: Solidworks flow simulation’s overlays of shear stress. a. No pneumonectomy b. Right pneumonectomy c. Left pneumonectomy d. Seven testing points where shear stress is being measured.

These simulations revealed that the shear stress experienced by the remaining pulmonary artery after a pneumonectomy was significantly higher than the shear stress experienced by that pulmonary artery without undergoing a pneumonectomy.

Quantitative shear stress data was obtained from the simulation at the seven testing locations. Anova analysis and t-tests were used to determine if there was statistical significance in the shear stress increase between the no pneumonectomy model and the two pneumonectomy models. As seen in Figure 3, the increase of shear stress at all seven testing points, due to a pneumonectomy, was determined to be highly significant.

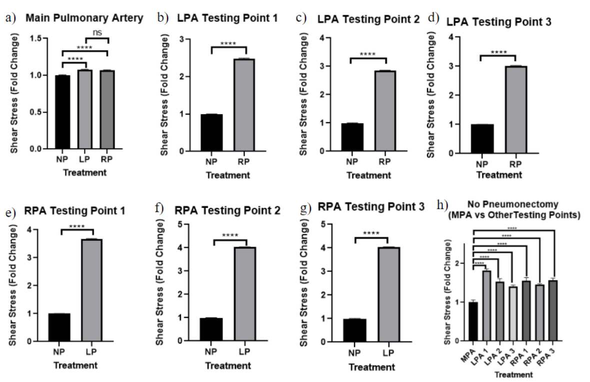

Figure 3: Statistical analysis of increase in shear stress at testing points between a no pneumonectomy (NP), left pneumonectomy (LP), and right pneumonectomy (RP). a. MPA b. LPA testing point 1 c. LPA testing point 2 d. LPA testing point 3 e. RPA testing point 1 f. RPA testing point 2 g. RPA testing point 3 h. Control

In-vitro experiments confirm and validate the parallel plate flow chamber's capabilities. Figure 4 shows the confocal images taken for the 7 different testing points, as well as for an additional control with no flow. The green section observes the glycocalyx, while the blue dots are the cell nuclei.

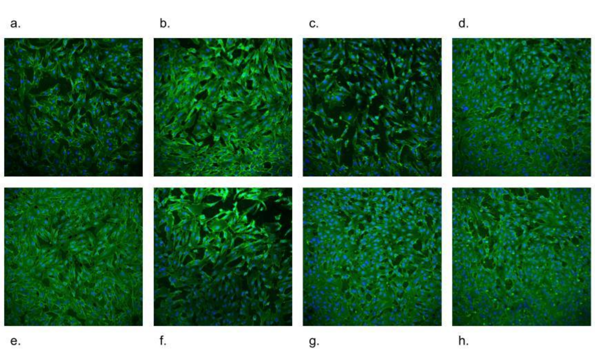

Figure 4: Images of No Pneumonectomy HLMVEC a. Control b. LPA Testing point 1 c. LPA testing point 2 d. LPA testing point 3 e. Main Pulmonary Artery f. RPA Testing Point 1 g. RPA testing point 2 h. RPA testing point 3

As seen in the images, the different locations of the cells display a different coverage. The coverage threshold of these images will be found using Image J, and this will be done for 2 iterations of each different flow situation to see potential damage to the glycocalyx.

IV. Discussion

The flow simulations determined that there is a three-fold increase in the shear stress on the glycocalyx after a right pneumonectomy and a four-fold increase in the shear stress on the glycocalyx after a left pneumonectomy. These increases are statistically highly significant. This data and information can be critical in helping reduce the mortality rate of patients with complications post-pneumonectomy.

The success in the early stages of in-vitro testing with the designed parallel plate flow chamber validates the design and capabilities of the device. On-going testing and imaging of the HLMVEC for the pneumonectomy models will hopefully confirm the degradation of the glycocalyx after a pneumonectomy which will further help in understanding post-pneumonectomy complications.

Other future work for this project includes designing a 3D model that aims to confirm what was found in the current 2D flow chamber. The 3D model will be more anatomically correct and will allow for a more accurate representation of the pulmonary arteries and how the cellular components of the endothelial glycocalyx are affected by increased shear stress resulting from a pneumonectomy.

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