The Design and Application of a Flow Chamber to Study Leukocyte Migration *In Vitro*

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INTRODUCTION

Leukocytes are white blood cells, and the movement of these cells is aided by the laminar flow of blood through blood vessels. The leukocytes possess several surface receptors that bind to ligands located in the endothelial cells that comprise the walls of blood vessels. After binding, the leukocyte diffuses into the tissue. This binding is what triggers the body's immune response to infection or tissue damage. Because modeling this entire system is very difficult *in vivo* (in the body), our client's research will be conducted *in vitro* (out of the body), as this method will provide him with the freedom to alter certain experimental variables that would not be possible in a human test subject.

He will use glass beads in the place of leukocytes and water instead of blood. Many current devices used to study this phenomenon are referred to as flow chambers and require expensive components such as gaskets that need to be replaced routinely throughout the expected lifetime of the design. These chambers can cost upwards of $2,000, and the cost only increases when the monthly gasket purchases are considered. Therefore, our group has designed and 3D printed a cost-effective flow chamber to model the flow of leukocytes and study how the rigidity of the endothelial cell wall affects the velocity of the leukocytes (Sarvestani, n.d.).

METHODS

Because of the 2020 coronavirus pandemic, the group was not able to conduct testing; however, we have laid out extensive test plans that will allow for testing to be conducted by future teams.

The flow chamber assembly will consist of a microscope slide sitting on top of it sealed using a gasket. The experimental set up can be found in **Figure 1**. Using a syringe pump, groups can pump water (which has a similar viscosity as blood) with glass beads that have a comparable diameter to leukocytes (approximately $5-10\,\mu m$) through the flow chamber. Using the software that allows for particle tracking, individual glass beads can be tracked; therefore, the inlet and outlet velocities of the beads can be documented and analyzed.

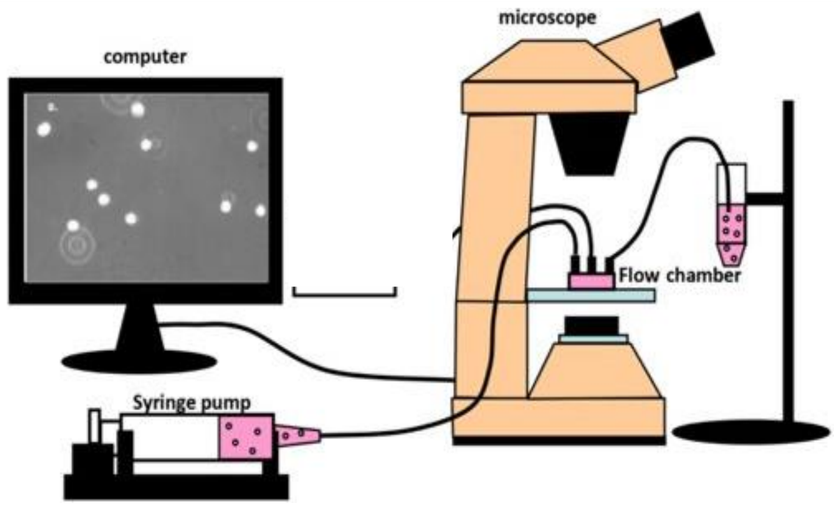

Figure 1. Experimentation Set Up

RESULTS

This project takes place in the spring semester of the year 2020. During this time, a worldwide pandemic called the novel coronavirus spread throughout the world at a rapid rate. This virus is a mutation of the coronavirus that causes flu-like symptoms affects the respiratory system of the affected and is potentially lethal, especially to those with compromised immune systems preexisting conditions.

Due to this virus, Mercer University (along with all other universities and non-essential businesses) were closed beginning in late March. Group meetings across the country were strongly discouraged as they could help further spread the virus. To protect team members, professors, and the family of those mentioned, in-person meetings were discontinued. This severely hindered the progress of this project, and it was unable to be finished.

Using this information, the team can graph the outlet velocity versus the inlet velocity for each stiffness. Once this is done, a curve can be fit through the data according to the approximate shape of the relationship to find an expression for the outlet velocity as a function of the inlet velocity for each stiffness. From here the team can compare the relationships of the varying stiffnesses (including the one from the previous test). Using a 95% confidence interval, the team will analyze the standard deviation of the velocity. A low standard deviation would be optimal, as this would suggest that the glass beads have similar exit velocities at their corresponding flowrates. The resulting expressions for inlet and outlet velocities at different stiffnesses will then be compared to determine if there is a relationship between hydrogel stiffness and bead velocity.

CONCLUSIONS

The primary goal of this project was to provide the client with a cost-effective flow chamber that can be used to conduct important research on leukocyte migration and how the rigidity of the wall affects the flow and velocity of the leukocytes. The modified chamber (**Figure 2**) that combines the rigid surface on one side as well as the hydrogel reservoir on the other reduced the price of the project while fulfilling the requirements set by our client. We are grateful for the opportunity to provide an effective solution to our client's problem, and we look forward to working with him in the future. Although we are disappointed that we could not fully complete the testing phase, we look forward to seeing how future groups can use our work to move the project further.

We hope that future groups will use the work we have completed on this project as a springboard to push the flow chamber to completion. The testing phase can be further expedited by researching the methods and procedures used to create the different hydrogel rigidities and practicing them in the lab beforehand. Additionally, minor design modifications to eliminate the use of gaskets altogether would be ideal.

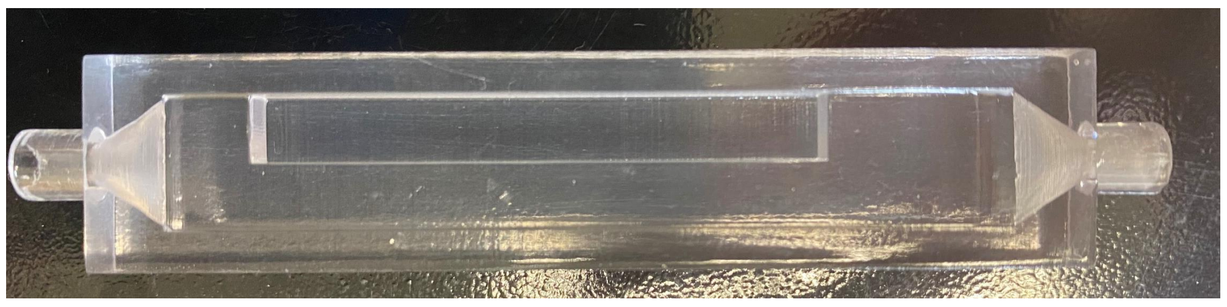

Figure 2. The 3D Printed SLA Flow Chamber with a Hydrogel Reservoir

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Reference: Sarvestani, A. (n.d.).