Capstone Project – Design of a Novel Flow Chamber to Study the Effects of Vascular Stiffness on Migration of Blood-Borne Cells

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Abstract

Cell rolling on vascular endothelium is critical for many physiological and pathological processes. Atherosclerosis is a pathological disorder characterized by stiffening of vascular walls due the growth of lipid-rich lesions. Leukocytes are the mobile keepers of the immune system and are found in abundance within atherosclerosis-prone aortas. Despite numerous studies, understanding the mechanism of leukocyte homing in growing lesions remains a challenge. Leukocyte adhesion begins with rolling on endothelium due to the specific binding between selectin receptors on endothelial cells and their complementary ligands expressed on the surface of leukocytes. Binding to selectin regulates the capture of leukocytes in the face of dislodging hemodynamic forces during their random encounters with endothelium. Progression of atherosclerotic plaques is followed by stiffening of the aortic wall. The effect of substrate stiffness on leukocytes rolling is not particularly well documented in the literature. The primary objective of this Capstone project was to design a novel flow chamber to examine the effect of substrate stiffness on the kinematics of leukocyte motion. The team was formed by students with different majors (Mechanical and Biomedical Engineering) to work on a cross-disciplinary research project. The central hypothesis was that leukocytes roll with a higher velocity over softer substrates. This hypothesis was tested *in vitro* using a flow chamber with soft and rigid substrates. The rigid portion of the substrate was made by 3D printing of a transparent resin and its flexible portion was made of polyacrylamide gel. The design was tested by measuring the migration velocity of flowing particles using video-microscopy. Students benefited from the cross-disciplinary nature of this research. Specifically, they learned about the physics of biological adhesion, the mechanics of leukocyte migration, and how to implement the basic principles of fluid mechanics to design a device with biomedical applications.

Keywords: Capstone project, Leukocytes, Reynolds number; Flow chamber; Substrate stiffness

Introduction

Cell mechanics is an emerging field in biomechanics and biophysics that explores the correlation between cellular behavior and mechanical forces applied on the cells or generated within the cells. Capstone projects that present multi-disciplinary problems bridging cell biology and mechanics are of great importance to help undergraduate students develop qualitative and quantitative understanding of cell mechanobiology. The endothelium is a complex biological organ that regulates blood fluidity, platelet aggregation and vascular tone and is a major

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coordinator of physiological functions such as immunology, inflammation, and angiogenesis. Endothelial dysfunction, on the other hand, plays a crucial role in cardiovascular diseases. Migration, rolling adhesion, and extravasation of leukocytes in face of hemodynamic forces of blood flow are mechanically driven biological functions and could be suitable subject matters for interdisciplinary Capstone projects for engineering students. This Capstone defined a team-oriented research project for undergraduate students majoring in Mechanical and Biomedical Engineering (Figure 1).

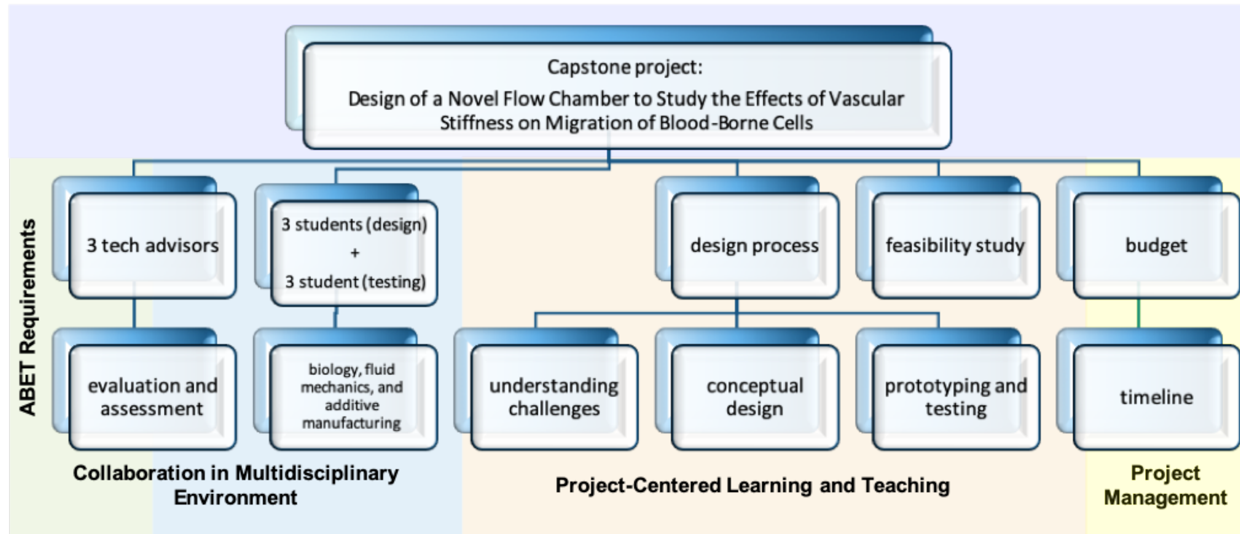

Figure 1. Capstone project overview.

Background

Leukocytes are white blood cells that participate in the body's immune response by travelling to injured areas to fight infection or foreign invaders.[1] Upon initiation of immune response, mediators including histamine are released. On activation by histamine, endothelial cells (ECs) rapidly express selectin at the cell surface.[2] Selectin ligands have specific affinity for the receptors on leukocyte surface (e.g., PSGL-1).[3] Due to the binding between ligand and receptors, leukocytes are captured by ECs and begin to roll on the vessel wall and eventually transmigrate through the endothelium.

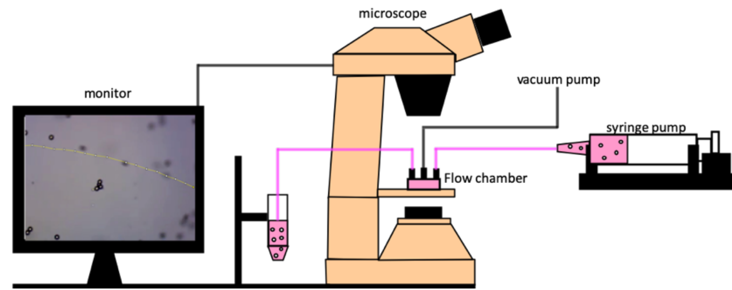

Figure 2. The general experimental setup of a parallel plate flow chamber to study the rolling adhesion of blood-borne cells.

Parallel plate flow chambers have been used extensively to simulate the rolling adhesion of leukocytes *in vitro* (Figure 2).[4] The substrate used in the conventional flow chambers is often a glass coverslip coated with selectin ligands at specific areal density. The glass substrates, however, do not accurately represent the stiffness of inner surface of blood vessels. Measurements by atomic force microscopy show that the average mechanical stiffness of ECs is on the order of 1 kPa.[5] Therefore, unlike the rigid coverslips often used for *in vitro* experiments,

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endothelium presents a soft surface to the circulating leukocytes *in vivo*. The sensitivity of leukocyte migration to the mechanical rigidity of substrate has remained largely illusive.

Understanding how substrate stiffness mediates the kinematics of leukocytes rolling has a broader biological importance and may clarify the mechanism of leukocyte recruitment to atheroscleric lesions. Atherosclerosis is an immuno-inflammatory disease characterized by stiffening of vascular walls due the growth of lipid-rich lesions.[6] It begins by deposition of extracellular lipids on the interior walls of blood vessels followed by inflammatory response of leukocytes into the intimal layer of the arterial walls and proliferation and migration of smooth muscle cells.[7] Formation of atherosclerosis lesions further leads to plaque accumulation through necrosis, fibrosis, and calcification.[8] Continued plaque deposition eventually leads to progressive increase in arterial wall stiffness. Leukocytes are found in abundance within atherosclerosis-prone aortas.[9] Although increased arterial wall rigidity has been known as a major risk factor for cardiovascular pathologies, the direct relationship between arterial rigidity and leukocyte homing in growing lesions is yet to be determined.

Project Objectives and Feasibility Criteria

The objective of this Capstone was to design and assemble a novel parallel plate flow chamber to study the effect of substrate stiffness on rolling kinematics and capturing of leukocytes in a fluid flow. The feasibility criteria were listed as following: (1) the chamber has substrates with different rigidities; one side is a glass (rigid) substrate and the other side is made of a hydrogel with tunable mechanical stiffness, (2) the chamber's wall are transparent, (3) the chamber must fit the stage of an inverted light microscope, (4) the chamber's internal geometry must ensure the laminarity of fluid flow, (5) the chamber must be able to hold up to incubator conditions (37 ºC with 98% humidity), (6) it must be able to withstand sterilization treatment, and (7) the project cost must not exceed $300. The long-term goal of this research is to understand how medial stiffening within atherosclerotic coronary arteries influences the influx and homing of rolling leukocytes.

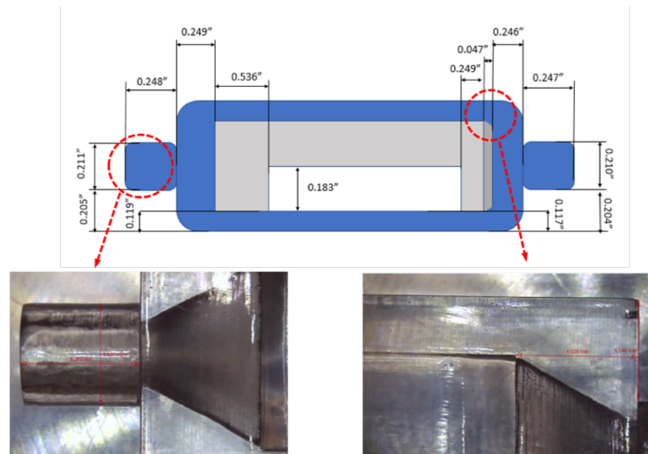

Figure 3. Finalized design with completed dimensions.

Design Process

The created design with its completed dimensions is shown in Figure 3. It is covered by a microscope slide and a gasket (Teflon) to maintain a seal. The chamber was 3D printed with an SLA 3D printer with a transparent resin. The substrate of the designed chamber provides different rigidities for the flowing cells. One side is made of the 3D printed resin which can be considered as a rigid substrate for the flowing cells. The other side, is a small reservoir (width=0.183" and length=1.7") to be filled with a hydrogel with tunable stiffness to mimic the rigidity of endothelium. Microscopic measurements showed that the manufacturing achieved a tolerance of 0.010".

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Internal Dimensions

The interior design dimensions were obtained to ensure the laminarity of fully developed flow and to avoid sedimentation of flowing cells in the parallel plate chamber. The laminarity of flow can be determined by calculation of the Reynold's number, R_e, which represents the ratio of inertial forces to viscous forces. For a fluid to be considered laminar, R_e is typically below 2,300.[10] For the flow chamber, R_e was calculated as $R_e = \rho QD/\mu wh$, where ρ is the density of culture media, Q is the flow rate controlled by a syringe pump (Figure 2), μ is the fluid viscosity, h is the height of the flow pathway (including the thickness of gasket), w is the width of the flow path, and D is the hydraulic diameter of the chamber. The latter can be found as $D = 4A/P_w$, where P_w is the wetted perimeter and A is the cross-sectional area of the chamber.[11] The average R_e of blood flow is 2,000. The Reynold's number in the chamber was targeted to be approximately 200, similar to that in smaller blood vessels where the extravasation of white blood cells occurs.

Fully developed flow occurs when the flow pattern does not change with the distance downstream. To ensure that the flow in the chamber is fully developed, the entrance length, l_e, must be adjusted to be $l_e = 0.06R_eD$.[11] Because cells have a slightly higher density that culture media, there was a concern that when the cells enter the flow chamber, they would sink to the bottom. Assuming all cells are perfectly spherical and $1 < R_e < 1{,}000$, the terminal velocity of cells can be found using the Stokes' law as[12]

$$V_0 = 0.2\left[g\left(\frac{\rho_p-\rho}{\rho}\right)\right]^{0.72}\frac{d^{1.18}}{(\mu/\rho)^{0.45}} \quad (1)$$

where g is the gravity acceleration, and ρ_p and d are the density and diameter of cells, respectively. The sinking is avoided if the terminal velocity of cells does not exceed the settling velocity defined as[12]

$$V_s = \frac{V_0}{1+\gamma\sqrt[3]{c}} \quad (2)$$

where γ is a constant (=1.30±0.24) and c is the volume fraction of cells in the culture media.

Synthesis of Hydrogel

Polyacrylamide (PA) gels coated with (P- or E-) selectin were used as the compliant substrate following the published methods.[13] The main two components of PA gels are acrylamide and bis-acrylamide (Bio-Rad Laboratories), that can be chemically crosslinked to form a polymer network. Different concentrations of acrylamide and bis-acrylamide can be used to generate networks of varied mechanical stiffness. Aqueous solutions of acrylamide and bis-acrylamide with different mass concentrations were used to make gels with stiffness of 1, 5, 25, 85, and 100 kPa. The solutions were photopolymerized by UV (UVP B-100AP). Alternatively, the polymerization can be done chemically by adding ammonium persulfate (APS)

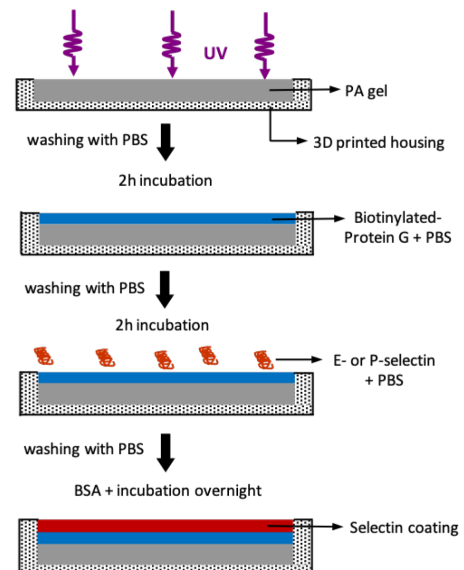

Figure 4. Synthesis and functionalization of hydrogel substrates.

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and TEMED (Bio-Rad Laboratories) at a final concentration of 0.1 % w/v. Protein immobilization on the gel surface was done first by incubation of biotinylated Protein G solution (Thermo Scientific) and subsequently with selectin–Fc chimeras (R&D Systems) (Figure 4).

Preparation of Microspheres

The preliminary studies were conducted using polystyrene beads (OD 10 μm) instead of leukocytes. Protein G-coated beads (Spherotech) were washed in PBS and subsequently incubated with PSGL-1-Fc chimera solution (R&D Systems) for 2 hours at room temperature before experiments.

Device Assembly and Checks

After synthesis and *in situ* crosslinking of the gel inside the reservoir, the excess amount of the gel was brushed off the chamber. A gasket of approximate thickness 300 μm was placed between a microscope slide and top surface of the flow chamber. The whole assembly was securely clamped to avoid leakage. The inlet and outlet of flow chamber were connected to the tubing and the chamber was placed on the stage of an inverted light microscope (Figure 5). The flow rate was adjusted using a syringe pump (Harvard Apparatus) to keep the shear stress, τ, within the range 0.5 –2.0 dynes/cm^2, comparable with the measured shear stresses in the post-capillary venules *in vivo*.[14] The flow rate, Q, needed to develop the desired shear stress was calculated using

$$\tau = \frac{3\mu Q}{2h^2 w} \qquad (3)$$

The fluid velocity can be obtained as $V = Q/hw$ and must be larger than settling velocity shown by Eq. (2). The cells/particles suspended in culture media were perfused over the rigid and soft substrates at room temperature. A 10x phase objective was used to focus on a single view field and continuously monitor and record the cells/particles motion. The trajectories were recorded by video microscopy and the velocity of cells/particles were calculated using a software (ImageJ).

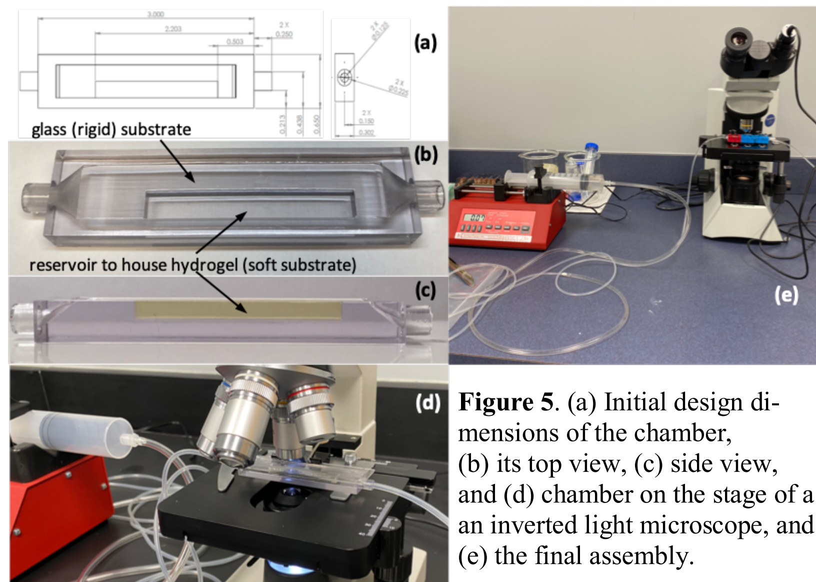

Figure 5. (a) Initial design dimensions of the chamber, (b) its top view, (c) side view, and (d) chamber on the stage of a an inverted light microscope, and (e) the final assembly.

Test Results and Discussion

At the first step, the team decided to use polystyrene microspheres, coated with PSGL-1 receptors, instead of leukocytes. The size of these particles is comparable to that of a typical leukocyte

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(~10 µm). This way, the team could study the particle flow over a compliant surface and elucidate the effect of substrate rigidity on rolling velocity in absence of any internal signaling within the cells.

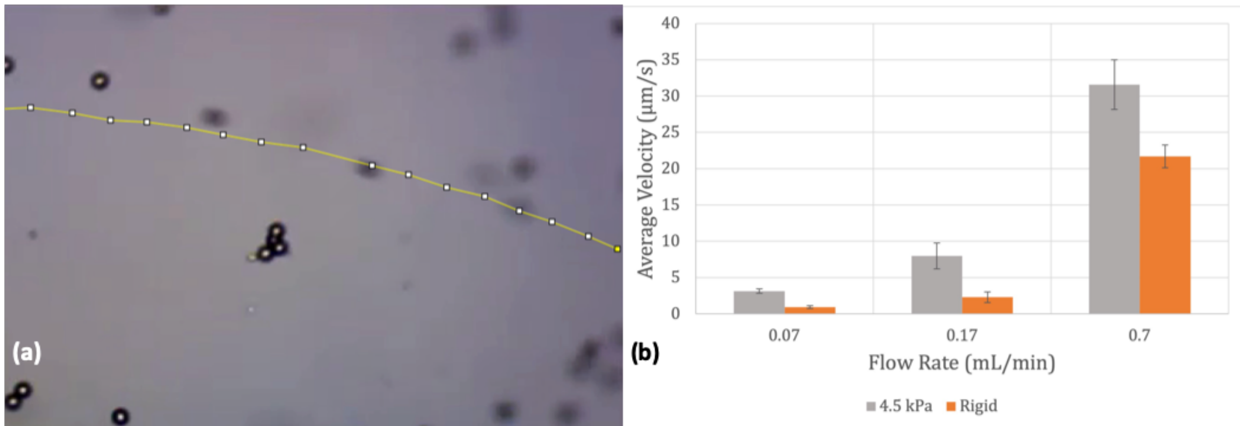

Figure 6. (a) A recording of microsphere flow analyzed in ImageJ, (b) average velocity by substrate stiffness at different flow rates.

The preliminary study shows a significant effect of substrate stiffness on the free flow velocity of the microparticles (Figure 6). Particles consistently showed a larger velocity on softer substrates compared to rigid substrates at different flow rates. This result implies that leukocytes may feel the rigidity of endothelium and roll with smaller velocity on atheroscleric lesions. The mechanism of rigidity sensing, however, could be purely mechanistic and independent from cell internal signaling. The hydrodynamic drag forces applied on a particle can be found by asymptotic solution of Stokes equations.[15] Because Stokes flow is reversible, particles experience no hydrodynamic lift force when flowing on a rigid wall. When the substrate is soft, the particle induces a pressure field that breaks the symmetry of surface profile (Figure 7).[16,17] This asymmetry generates a net elasto-hydrodynamic lift force.[18] This lift force facilitates the long-distance coasting of leukocytes over the deformable endothelium. This is mechanistically similar to the gliding motion of micro-swimmers near a soft interface.[19]

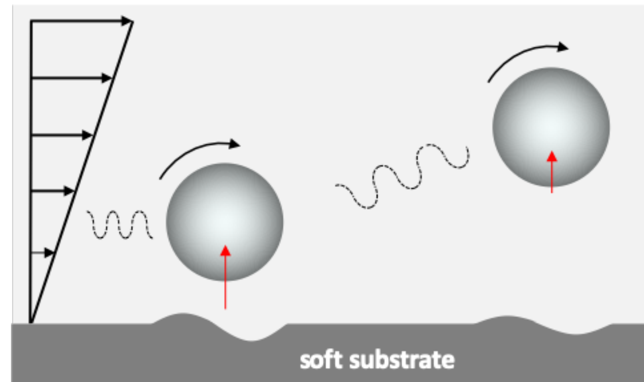

Figure 7. The asymmetric profile of a soft substrate generates a net hydrodynamic lift force.

Benefit to Students

The successful completion of the project required knowledge from different disciplines, including fluid mechanics, additive manufacturing, vascular physiology, cellular biology, and polymer

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chemistry. The problem of substrate rigidity and its possible effects on rolling adhesion and recruitment of leukocytes is not particularly well documented in the literature. Therefore, the design and application of this novel flow chamber was a discovery learning experience for students. They were encouraged to think independently and creatively to provide a simple and affordable design for the chamber. The parts list and their costs are listed in Table 1. In total, the cost to build the chamber was $260.58, less than $300 allocated by the School of Engineering for each Capstone project and just a fraction of a cost compared to the commercially available flow chambers.[21] Although the preliminary studies were conducted on glass microparticle, the chamber is currently being used to study the effects of substrate rigidity on rolling migration of monocytes. In addition, the device will be used as a pedagogical lab module in an elective course (Introduction to Cell Mechanics), taught by one of the technical advisors.

Table 1. Cost of the project.

Item	Cost ($)
SLA 3D Printer Chamber	60.19
Grafco Hoffman screw-compressor clamp	15.40
100 PCS clear transparent blank microscope slides	9.99
3% acrylamide and 0.2% bis-acrylamide solution	64.00
5.5% acrylamide and 0.15% bis-acrylamide solution	64.00
TEMED	19.00
Ammonium persulfate (APS)	14.00
Total	260.58

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