Development of a Single Cell Mechanical Characterization Platform

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Introduction

Sickle Cell Disease (SCD)
SCD is an inherited blood disorder characterized by stiff, sickle-shaped red blood cells (RBC). This disease affects approximately 13 million people worldwide and current treatments for SCD are limited and inefficient in their ability to predict patient response.

Single RBC mechanics
Many studies have turned their focus to the pathological alterations of single RBC mechanical properties, such as deformability, to assess cell state in various diseases such as SCD, malaria, and cancer. These studies focus on changes that independent RBCs go through; however, they don’t connect back to the whole blood rheology where disease complications arise.

Project
The purpose of this project is to develop a single cell mechanical characterization platform that can be used to determine a quantitative link between distributions of single RBC mechanics and whole blood rheology at oxygenated and deoxygenated conditions. This study aims to improve the understanding of blood transfusion therapy by identifying the effect fractions of single RBCs will have on whole blood population.

Methods

Device Design and Fabrication
AutoCAD software was used to design the microfluidic device and standard photolithography methods were used to create master wafers. Soft lithography using polydimethylsiloxane (PDMS) as our elastomer was used to create the device.

Experimental Setup
A syringe pump pushes focusing fluid into the far left entry port while an air pressure regulator pushes blood into the inner left entry port. The gas layer is attached to the gas mixer and a fiber optic O2 sensor to control oxygen tension. A high speed camera is used to capture images of the cell as they go through the constriction channel.

Results

Device Design
Final device platform consists of a PDMS device with a blood and gas channel layer diffusively coupled to each other. The blood channel layer contains two entry ports, three exit ports, resistors, and a constriction channel. The constriction channel measures 14μm wide and 5μm high.

Blood Fixation
Normal (hemoglobin A, genotype AA) blood was fixed using formaldehyde at variable concentrations. The morphology of blood cells fixed with different concentrations of formaldehyde is shown. The upper fixation range for the formaldehyde was found to be 11%, or a 3:7 ratio of formaldehyde to PBS. At this upper range, only a small percentage of cells were lysed.

Image Analysis
A custom MATLAB algorithm was developed to analyze the images of the RBCs before and after they enter the constriction channel. The code turns the cell image into a binary image and removes the border, allowing surface area to be calculated.

Conclusion

• Initial experimental setup shows promising results. The device design and the laminar flow of the blood allows the focusing fluid to focus the RBCs directly into the constriction channel.
• The specific dimensions of the constriction channel induce a wall effect on the cells resulting in a shear force on the outer edges of the cells, causing them to take on a parachute shape.
• The process of fixing the blood samples with different concentrations of formaldehyde serves to create control stiffness steps that will help identify the magnitude of which cell stiffness affects whole blood rheology.

Future Direction

• Our next step is to run the fixed RBCs through the device and measure average deformation. A deformation vs. cell fixation plot can be generated from this data and used as a calibration standard to compare patient samples to.
• Different fractions of cells at different stiffness levels will be tested and assessed for effective viscosity, a dependent variable that depends on single cell mechanics, using the whole blood characterization platform.
• An effective viscosity vs. shear rate plot can be generated and used to compare patient blood samples to. It is expected that as cell stiffness decreases, the plots will collapse and behave similar to an average healthy sample plot.

Future Direction

• From a clinical viewpoint, there are currently no existing biomarkers for disease severity. This study aims to identify quantifiable metrics for patients based on RBC mechanical and membrane properties.
• This approach has the potential to improve patient outcome prediction and allow predetermination of necessary blood transfusion amounts to meet satisfactory treatment levels.