Development of a Microfluidic Mimic of the Human Microvasculature to Study Sickle Cell Disease

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Introduction:

Often characterized by severe pain crises and organ damage due to hypoxia, sickle cell disease (SCD) is a genetic disorder caused by a mutation in the hemoglobin molecule of red blood cells (RBCs) [1]. This mutation leads RBCs to stiffen under hypoxic conditions, in turn making the cells more susceptible to obstructing blood flow. Known as a vaso-occlusive crisis (VOC), this obstruction stands as the major cause of morbidity and mortality in SCD [2]. Currently, hydroxyurea (HU) is the sole FDA-approved drug treatment option available to patients, and yet HU is ineffective in one-third of those treated [3]. Consequently, there is a clear need for more effective drug treatments, as well as a screening platform to evaluate drug efficacy. Thus, we created an *in vitro* microfluidic disease model to screen new drug treatments. A remarkable alternative to *in vivo* techniques, microfluidics provides a convenient and reproducible platform with which to simulate the rheological occurrences of SCD.

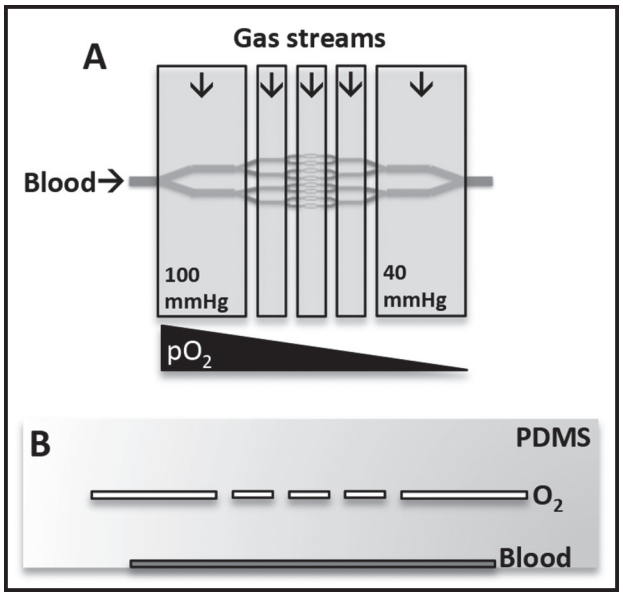

Figure 1: A. Top-down view of device. B. Cross-sectional view of device.

Methods:

The optimal design for the device was to produce a linear oxygen tension gradient across the microvasculature, mimicking physiological conditions. AutoCAD was used to design the device layers.

Design considerations for the microvasculature layer included channel width (vessel diameter), bifurcations, segmental length, total length, and channel height. The most important consideration was channel width, as the range of diameters across vessels composing the microvasculature (small arteries, arterioles, capillaries, venules, small veins) differs according to the anatomical region of interest. To account for these differences, we designed twelve microvasculature layers featuring varied channel width ranges. The design we fabricated for experimental purposes has a range of 10 μm to 60 μm, which is both physiologically accurate and amenable to the dimensional constraints imposed by polydimethylsiloxane (PDMS). In addition, the design we fabricated and tested was 3 mm in total length.

We then designed a layer containing a series of gas channels that, when overlaid onto the microvasculature layer, produced an oxygen tension gradient. The oxygen layer we fabricated consisted of five gas channels, whose dimensions were determined via finite element modeling of oxygen diffusion in COMSOL.

We used soft photolithography with SU-8 2000 series photoresist to fabricate the master for each layer. We then cast these masters with PDMS, and the layers were subsequently cured and bonded to each other and to a glass slide via oxygen plasma treatment.

Specific oxygen tensions were created by cycling an air stream and a N_2 stream via solenoid valves. The duty cycles necessary to produce the desired tensions were specified in a MATLAB/Arduino system. To ensure proper calibration, oxygen tensions were also measured at each gas outlet.

Results and Discussion:

During the modeling phase of this project, we found the most important parameters in obtaining the optimal design to be width of the gas channels, distance between the gas channels, and gap width between the microvasculature and oxygen layers. Furthermore, while a large gap width between layers corresponded to a more linear oxygen gradient, we sought to minimize this gap width for construction purposes. Thus, series of stationary studies were run in COMSOL, each for a specific gap width, and tension data across the microvasculature layer were plotted. After fitting a line to the data and determining variance for each data point, a threshold for the average variance was set (this threshold was applied to all five-channel oxygen layer designs) and an optimal gap width was obtained. Figure 2 displays the modeled tension curve for this final design.

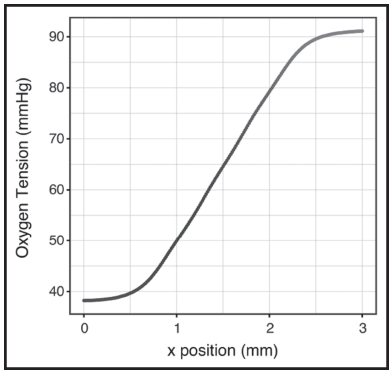

Figure 2: Finite element model of oxygen gradient.

To validate the oxygen gradient experimentally, we used 4 mM tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate (RuBPY), an O_2-sensitive dye whose luminescence is quenched in the presence of O_2. We perfused this solution through channels directly beneath the oxygen layer (i.e., channels analogous to the microvasculature layer), and measured luminescence intensity values using an inverted epifluorescence microscope. Intensity readings were then converted into tensions based on a calibration curve. These tensions are plotted in Figure 3.

Characterization with RuBPY showed that the experimental oxygen tension gradient has a decreasing trend; however, it is not perfectly linear, as the model predicts. Using a luminophore with a higher sensitivity, as well as minimizing the presence of unwanted features/

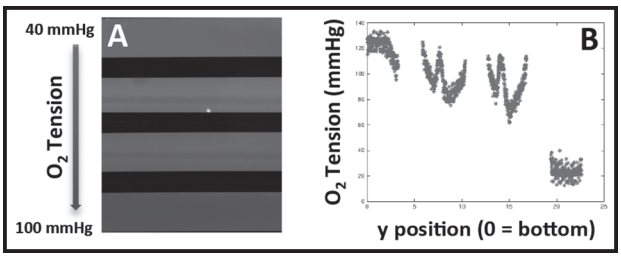

Figure 3: A. Image of RuBPY luminescence intensity gradient across four channels. Light regions correspond to low O_2 tensions. B. Experimental pO_2 vs. position data.

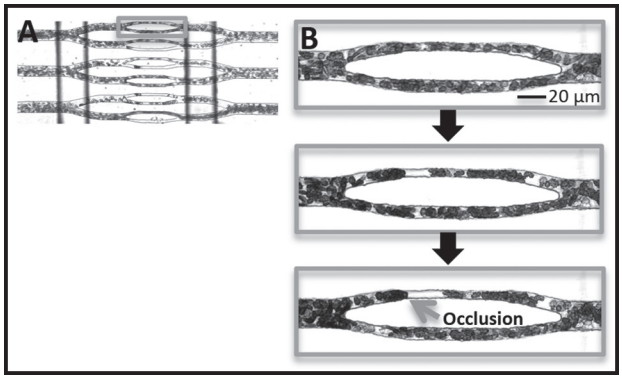

Figure 4: A. Brightfield image of a region of the device, through which sickle cell blood flows. B. Time progression of blood flowing through the 10 µm-wide channels of device as the oxygen gradient develops. In the bottom image, gradient is fully developed.

debris in the device, would likely lead to validation of a more linear gradient.

We experimented with running a sickle cell blood sample through the device under a pressure of 1 psi. Upon gradient development, we observed significant slowdowns in blood flow, as well as occlusions within the capillary-sized channels (Figure 4). Knowing that sickle RBCs respond to these conditions, such experiments lend insight into the mechanical effects on the rheology of SCD.

Conclusions:

We developed a microfluidic device to serve as a SCD model, and determined that the device is capable of reproducing hallmark rheological occurrences of the disease. By capturing even more physiologically relevant parameters in future designs, we hope this device ultimately becomes an accurate, accessible means of screening new drug treatments for SCD.

Acknowledgements:

I would like to thank Dr. David Wood, Daniel Lu, and all other members of the Living Devices Lab and the Minnesota Nano Center for their incredible guidance. I would also like to acknowledge the NNIN REU Program and the NSF for funding this research under Grant No. ECCS-0335765.

References:

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