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SJSU Undergraduate Research Grants

Microfluidic Hemostasis Assay

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Abstract

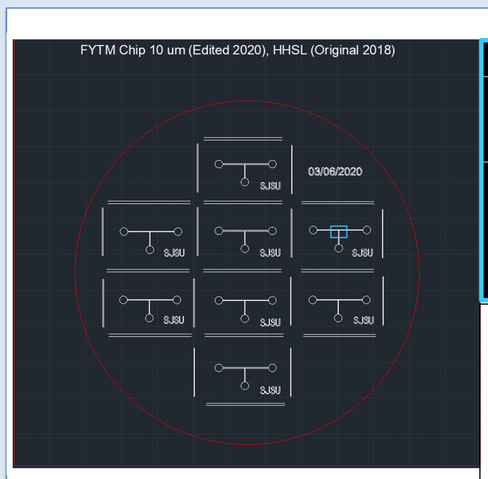
Blood is an extraordinarily complex fluid which the body uses to balance two essential physiological functions: blood flow (hemostasis) and clotting (thrombosis). Despite decades of important research, many of the most basic mechanisms that the body uses to balance thrombosis and hemostasis remain mysterious. The goal of this project is to develop and manufacture a microfluidic chip to study the effects of extracellular matrix proteins on blood coagulation. The chip will be used to test the role of Tissue Factor and the effect of soluble Factor XI inhibition on activation of the extrinsic coagulation pathway.

Project Activities and Project Plan

For the microfluidic chip design, we used Computer-Aided Design (CAD) to create a photomask. The design, shown in Figure 1, consists of one channel that is intersected perpendicularly with another channel. In the area between the two channels, there are three pillars which are spaced 10 microns apart. The pillars were designed to simulate a vessel injury, introduce stagnation points in the flow, and help create surface tension for the extracellular matrix protein solution injected in the perpendicular channel.

For the production, we will manufacture a microfluidic device through the process of photolithography, soft lithography, and plasma-activated bonding.

For the experiments, we will flow re-calcified blood, which is either whole or anti-coagulated, with Hirudin in order to validate the production of a thrombus in the region of interest.



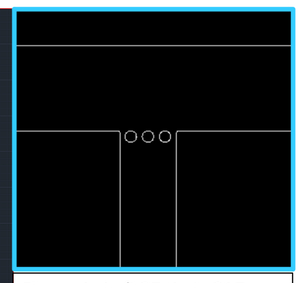


Figure 1. *Left* 2D AutoCAD design of the photomask, top-down view. *Top* Close up of the region of interest. The three pillars are located at the entrance of the intersecting channel.

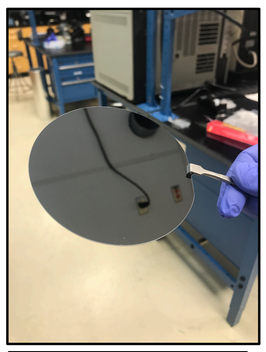


Figure 2. Blank silicon wafer. We will develop our design on a wafer just like this one.

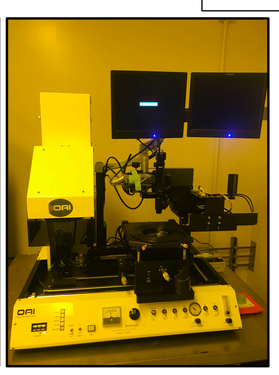


Figure 3. *From Left to Right* Spin coater and MEMS Mask Aligner/ UV Light Source. The spin coater covers the wafer with a negative photoresist layer, which is cured using a hot plate, and then placed in the aligner with the photomask on top. The photoresist is then exposed to UV light through the pattern on the mask. The resulting wafer with the negative photoresist design is then washed, leaving only the cured photoresist.

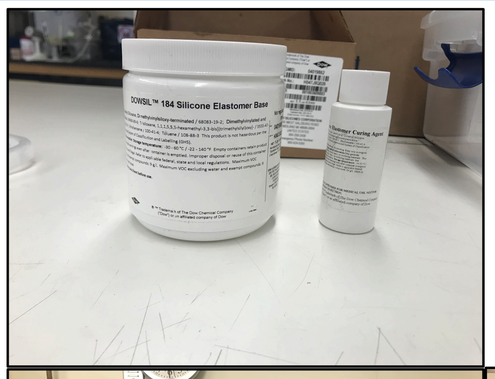


Figure 4. *Top Left* Polydimethylsiloxane PDMS kit. It includes the elastomer base and curing agent. *Bottom Left* PDMS mixing and curing station. It includes a scale to weigh out the base and the curing agent, a desiccator and vacuum pump to degas the PMDS, and an oven to cure it. *Bottom Right* The Plasma Cleaner is used to bond the microfluidic PDMS chips to glass slides. The resulting microfluidic device will have a tight seal between the PDMS and the glass.

Research Questions

- Understand the mechanisms of thrombosis and platelet activation and regeneration
  - Design, manufacture, and validate a microfluidic coagulation assay
  - Characterize the thrombus formation with simulated pathological blood disorders

Citations

- Butenas, S., Orfeo, T., & Mann, K. G. (2009). Tissue factor in coagulation: Which? Where? When?. *Arteriosclerosis, thrombosis, and vascular biology*, 29(12), 1989–1996.
- Schoeman, R. M., Rana, K., Danes, N., Lehmann, M., Paola, J. A. D., Fogelson, A. L., … Neeves, K. B. (2016). A Microfluidic Model of Hemostasis Sensitive to Platelet Function and Coagulation. *Cellular and Molecular Bioengineering*, *10*(1), 3–15.