Shear-Rate Controlled Microfluidic Devices to Examine von Willebrand Factor-Mediated Platelet Deposition

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Primary CNF Tools Used: ABM contact aligner, Class II resist spinners (SU-8), Heidelberg mask writer (DWL 2000), Hamatech mask chrome etch 1, resist hot strip bath, SU-8 hot plates, P10 profilometer

Abstract:

Ventricular Assist Devices (VAD) have been known to cause thrombosis, or the formation of clots in blood vessels due to the pumping of blood at high shear rates. Thrombi are formed when platelets aggregate together as a result of many blood-borne agonists and processes. The purpose of our research is to study how strain rates influence how platelets aggregate and adhere to vessel walls. We will be using microfluidic devices to mimic platelet deposition at clinically relevant shear rates, and use fluorescence microscopy to visualize the thrombi.

Summary of Research:

Our research focus has been to study VAD-related thrombosis. Thrombosis is a process by which platelets aggregate and adhere to blood vessels. This can lead to major health problems, such as nutrient deficiency, higher blood pressure, and stroke [1]. The mechanism through which platelets function is dependent on hemodynamic shear stresses [2,3].

When a ventricular assist device is implanted, it generates supra-physiological shear rates that uncoils a normally globular glycoprotein named von Willebrand Factor. When the protein chain unfolds, it reveals several binding sites that enable tethering to both collagen and platelets [2]. This tether gives platelets sufficient time and contact to collagen to activate via the GP IIb/IIIa integrin and activates the platelets, releasing more prothrombogenic biochemical agonists such as adenosine diphosphate, thromboxane A2, and thrombin [5,6]. Our group's objective is to study this mechanism using microfluidic chips made of polydimethylsiloxane (PDMS) that allow for precise strain rate control.

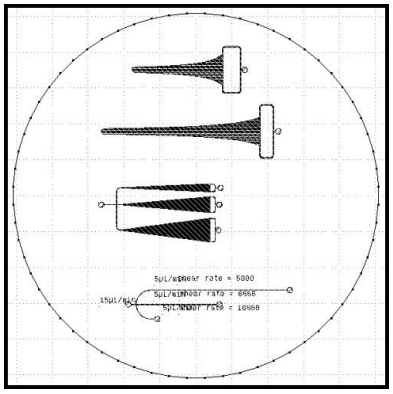

Figure 1: Computer aided design of channel geometries: (from top to bottom) short and long hyperbolic channels, expansion channels (5°, 10°, 15°), standard width channels (40 µm, 30 µm, 20 µm).

The design features three different channel geometries (Figure 1). One channel comprises a single inlet that diverges into three channels, each with a different width (40, 30 and 20 µm) in order to examine platelet deposition at a constant shear rate. Next, there are three channels that expand gradually at angles of 5°, 10°, and 15°. This design helps us study platelet deposition at constantly varying shear rates.

The third design is a hyperbolic expansion channel. We have incorporated hyperbolic channels of two different lengths; however, in both cases, along the central axis, the flow is extensionally dominated. In both the standard and hyperbolic expansions, there are square-shaped microposts that exist serve as a dual purpose of preventing channel collapse and for examining the effects of different obstacle geometries and orientations with respect to the flow direction on platelet deposition.

In order to implement our design into a device, we first used the Heidelberg DWL 2000 mask writer to generate

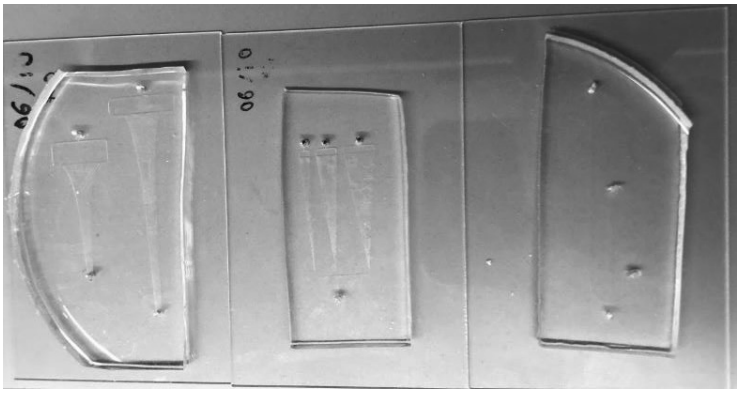

Figure 2: Microfluidic devices made of PDMS plasma bonded to glass surface.

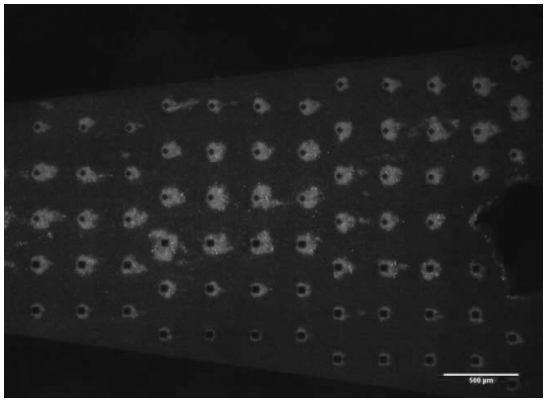

Figure 3: Mepacrine-labelled platelets depositing during perfusion through the 15° expansion channel. Flow rate is 18.8 is μL/min from left to right.

our microfluidic channel design on a chrome-coated glass plate. The chrome sputtered layer was etched on the chrome using the Hamatech Mask Chrome Etch 1, and the resist was stripped using a strip bath. In the class II photolithography room, we spin-coated SU-8 photoresist to a thickness of 50 μm. Bake times and exposure times were calculated based on recommendations provided by experienced CNF personnel [7]. We used the ABM contact aligner with our patterned chrome-coated plate and SU-8 coated wafer to expose the channel features onto the wafer. Following a post-exposure bake and SU-8 developer bath, the features were generated on the silicon wafer. Finally, to check our feature dimensions, we used the P10 profilometer to check the channel thickness.

In order to turn this wafer into a usable device, we poured a 9:1 mixture of Dow Corning Sylgard 184 silicone elastomer and curing agent onto the wafer and used a vacuum pump to create a bubble-free, rigid set of channels. The PDMS was then baked in an oven at 60°C for 150 minutes. Finally, the devices were plasma bonded onto glass slides.

In order to use these devices for imaging platelet deposition, we mix citrated ovine whole blood with mepacrine, a fluorophore taken up by platelets that allows them to be visualized using fluorescent light. We coat the channels with a collagen solution to create a thrombogenic surface so that the platelets can adhere to the glass. In our preliminary work, we have flowed blood through the channels at a constant flow rate and observed platelet deposition over several minutes (Figure 3).

References:

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