Measurement and Analysis of Blood Platelet Activation within a Microfluidic Device

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Abstract:

Cardiovascular disease (CVD) is the leading cause of death in the United States. In CVD, slow accumulation of fat and plaque within blood vessels forms a local constriction, known as a stenosis, whose flow conditions make it prone to clot formation and subsequent occlusion resulting in heart attack or stroke. We have developed an *in vitro* microfluidic device to model arterial flow conditions in stenosis clot formation. Heparinized porcine blood was flowed through the device at shear rates of 500 s^{-1}, 4,000 s^{-1}, and 10,000 s^{-1}, conditions representing normal to pathological patient conditions. *In vitro* clot formation was monitored using simultaneous readings of mass flow and light transmission. Additionally, scanning electron microscopy (SEM) was utilized to visualize the activation-related morphological changes in individual platelets and platelet aggregates within our device. Our device allows for high throughput, simultaneous evaluation of clotting behavior over a large range of shear rates, while SEM provides a visual confirmation of platelet behavior.

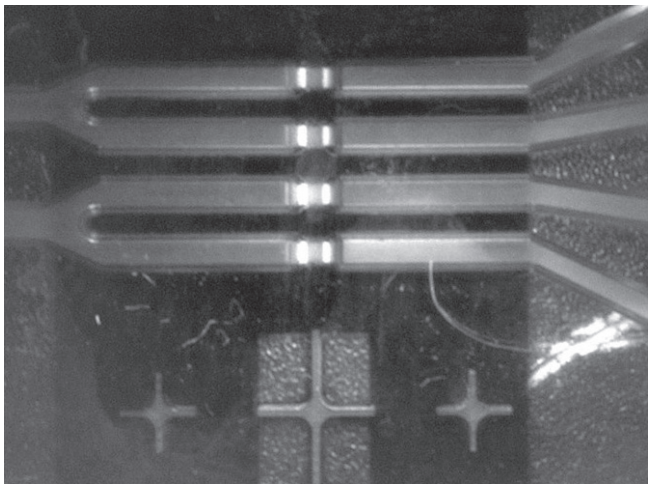

Figure 1: A finished device with applied printed aperture allows for easy alignment and limits channel-channel communication.

Materials and Methods:

Device Manufacture. Microfluidic devices were molded using polydimethylsiloxane (PDMS) on a milled master mold. PDMS was degassed and set at 70°C for 1.5 hours. Devices were bonded to glass slides using corona treatment. After bonding, a printed black aperture was aligned with the device on the back side, to prevent channel-channel communication. A finished device is shown in Figure 1. Devices were filled with collagen and allowed to sit overnight.

Blood Preparation. Porcine blood was obtained each morning of experiments from a local slaughterhouse and heparinized with 3.5 mL heparin per 1000 mL of blood. All experiments were conducted within six hours of slaughter. Blood was filtered through 125 μm polypropylene mesh before trials to remove large aggregates.

Flow Testing. Device, input lines, and output lines were primed with 43.4% glycerol, with a viscosity matched to blood. Blood was flowed through device at shear rates of 500 s^{-1}, 4,000 s^{-1}, and 10,000 s^{-1}. The shear rate was varied by the changing length and diameter of the output lines. Clot formation was monitored using simultaneous mass flow and light transmission measurements. Devices were visualized under light microscope after trial to confirm clot formation.

Scanning Electron Microscopy (SEM). Devices were removed from their glass slide, and the channels were gently washed with distilled water. Clots were fixed using 10% buffered formalin overnight and dehydrated using increasing concentrations of ethanol from 50% to 100% for 15 minutes at each concentration. SEM was performed using a Hitachi S3700 Variable pressure SEM running at 30 Pa and 15kV.

Results and Discussion:

Flow Testing. Light transmittance through thrombus has been previously confirmed using light microscopy (data not shown) [1]. Optical transmission and mass flow rates recorded for a single representative trial are shown in Figure 2. Both mass flow and light transmission were plotted

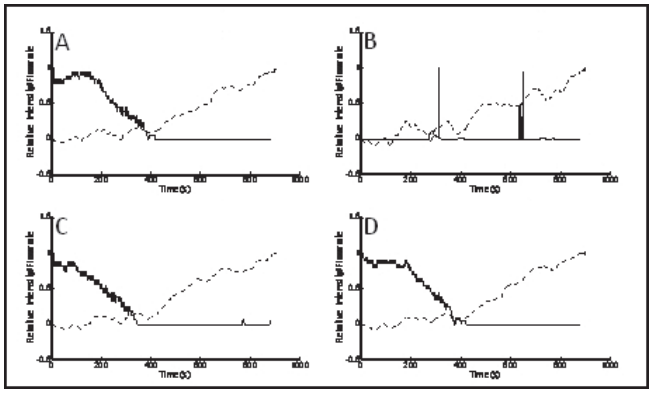

Figure 2: Representative relative mass flow (solid line) and light transmission (dotted line) results. Shear rates are 10,000 s^{-1} (A, D), 4,000 s^{-1} (B), and 500 s^{-1} (C).

relative to their respective maximums. An increase in optical transmittance correlated with occluded mass flow at 409 and 416 seconds for 10,000 s^{-1} and 336 seconds for 4,000 s^{-1}, for this trial. The mass flow and light transmission showed an intersection point. This point will be used to determine when flow has been occluded using only the light transmission data, the ultimate goal of this study. Preliminary data suggests that this point is approximately 0.1 relative. Data (not shown) also suggests that 4,000 s^{-1} clots more quickly than 10,000 s^{-1}, and the time to occlusion increases as the time from slaughter increases. Further study is needed to better characterize the effective range of shear rates for our device and the relationship between time to occlusion and time since slaughter.

Scanning Electron Microscopy. SEM images show increased platelet and decreased fibrin composition as the shear rate increases in platelet aggregates. This agrees with previous results [2]. Figure 3 shows representative micrographs of thrombus run at 10,000 s^{-1}, 4,000 s^{-1}, and 500 s^{-1}. The high shear rate shows a composition of mostly activated (irregularly shaped) platelets, the low shear rates shows a composition of unactivated (spherical) and fibrin, and the middle shear rate shows components of both. The location within the thrombus also affects composition. Regions where flow eddies form have low shear rates and allow fibrin deposition [3]. Figure 3, D and E, show a transition zone between platelet deposition and fibrin deposition. Fibrin deposition is also preferred in the corners where flow is slowed (Figure 2, F). Further histological studies should be performed to confirm results seen here.

Conclusions:

We have created a device that is capable of high throughput, low volume clotting assays of blood at up to four different conditions simultaneously. This device has application as a clinical tool to monitor blood clotting in individuals with unusual clotting response or as a high throughput research tool for cardiovascular applications. Further studies will be performed to study the effectiveness of this device to test pharmaceuticals and biomaterials.

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References:

[1] Li, M., A. Sodemann, et al. (2009). "High throughput formation and measurement of occlusive thrombosis in porcine blood."

[2] Ku, D. N. and C. J. Flannery (2007). "Development of a flow-through system to create occluding thrombus." Biorheology 44: 273-284.

[3] Nesbitt, W. S., E. Westein, et al. (2009). "A shear gradient–dependent platelet aggregation mechanism drives thrombus formation." Nature Medicine 15(6): 665-673.

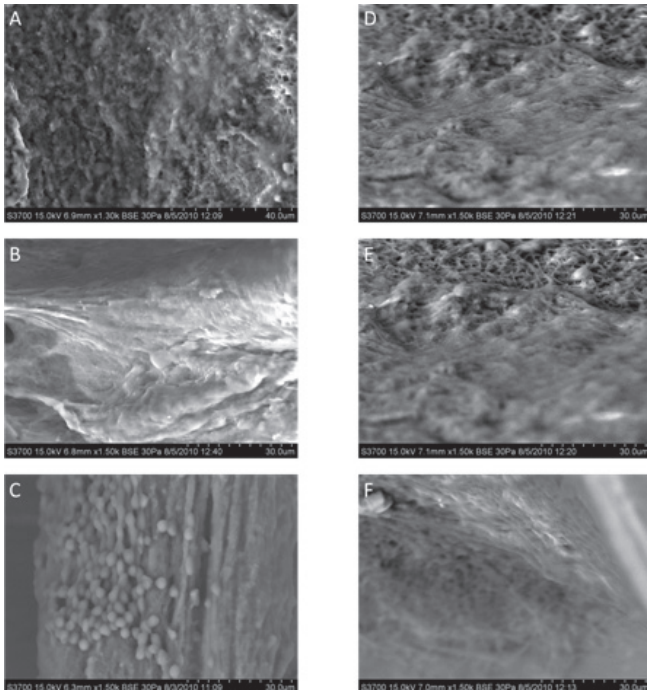

Figure 3: Representative SEMs of thrombus at different shear rates, 10,000 s^{-1} (A), 4,000 s^{-1} (B), 500 s^{-1} (C), and different regions of a 10,000 s^{-1} clot, downstream (D, E), and upstream corner (F). Flow is toward the top of the page in all cases.