Microfluidic Networks for Studying Thrombosis

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Abstract

Since blood is a moving biological fluid *in vivo*, it is important to study blood clot formation *in vitro* under physiological flow conditions. To achieve this objective, we have developed methods that meet the following criteria in an attempt to mimic *in vivo* fluid dynamics, molecular transport, and biochemistry: 1) flow channels with physiologically relevant length scales, 2) the ability to pattern surface molecules with spatial control, and 3) the ability to introduce soluble molecules with spatial and temporal control.

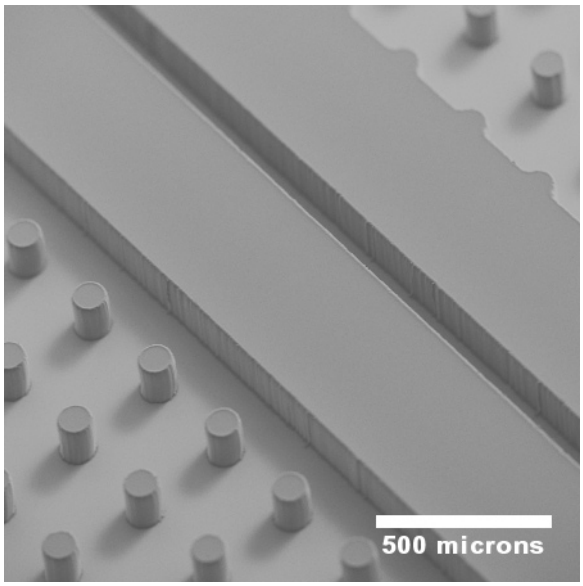

Figure 1: PDMS microfluidic channels and pillars.

Summary of Research

Microfluidic channels are an ideal environment to study blood under flow because they can mimic the size, geometry, and fluid dynamics of arterioles, venuoles and capillaries. In this work, microfluidic devices were fabricated using standard soft-lithography methods [1]. Molding masters consist of KMPR photoresist on a silicon substrate. Our devices use a vacuum assisted bonding method to reversibly seal polydimethylsiloxane (PDMS) devices to glass and polymer substrates [2]. Figure 1 shows a representative microfluidic channel with a cross-sectional area of 100 μm x 100 μm surrounded by a vacuum chamber with an array of cylindrical posts. The posts act as a structural support so that the PDMS does not buckle during application of vacuum.

The recruitment of platelets to injured vessels is facilitated by exposure of surface bound proteins such as collagen, von Willebrand factor, and tissue factor. We adapted a microfluidic method [3] for patterning these proteins onto the surface of glass substrates with high spatial precision (~ 10 μm) within microfluidic channels. The micropatterned proteins mimic a small vascular injury where subendothelial collagen is exposed to flowing blood. Murine whole blood was introduced into microfluidic channels at arterial wall shear rates (1000 1/sec) and platelets were observed to adhere and aggregate only on patches of patterned collagen (Figure 2). Adhesion, rolling, and aggregation of individual platelets have been observed using high-speed fluorescence microscopy.

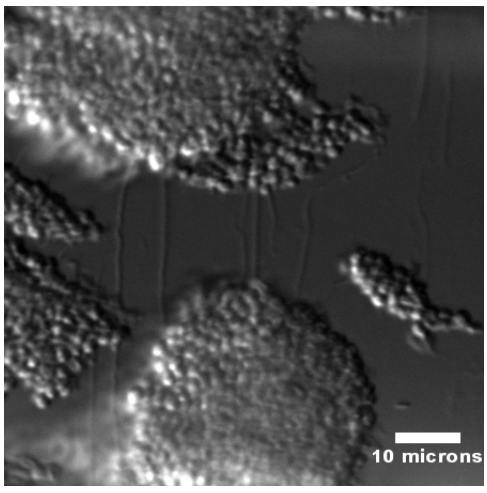

Figure 2: DIC image of platelet adhesion and aggregation on micropatterned collagen.

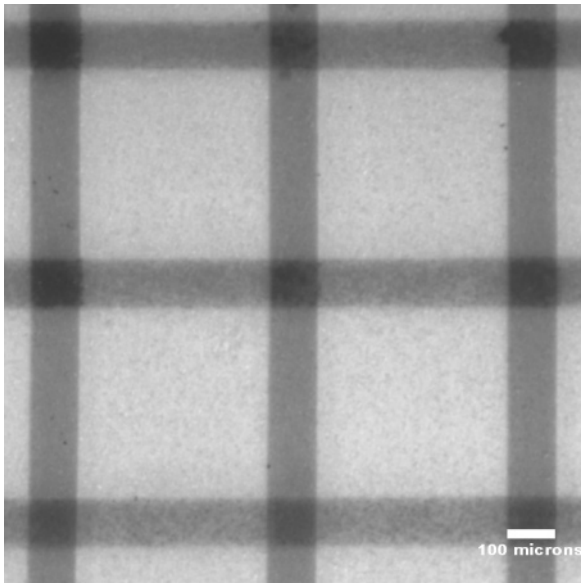

Figure 3: Polycarbonate membrane controls the flux of soluble of molecules between perpendicular channels.

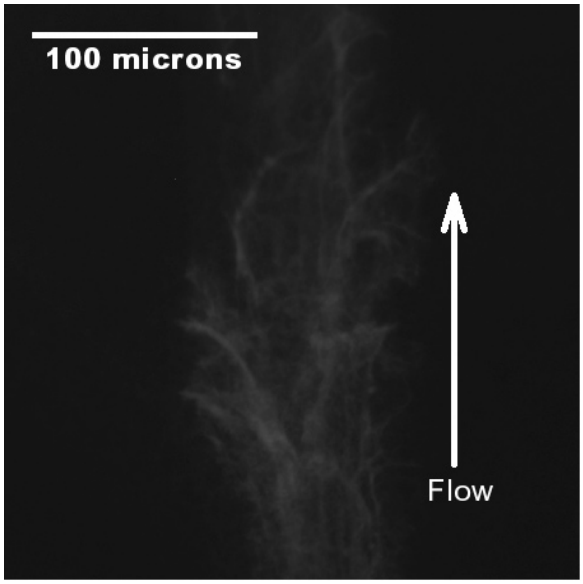

Figure 4: Fibrin gel formed under flow in a membrane based system.

Following adhesion to a vascular injury, soluble procoagulant factors are released from platelets (ADP, thromboxane A2) and generated on the surface of platelets (thrombin). The role of the flux of these procoagulant factors in thrombosis and clot stability is unknown. We have used a ''membrane sandwich'' multilayer technique [4] in conjunction with vacuum assisted bonding to introduce procoagulant factors at a controlled flux into flowing blood. In the device, one set of channels was situated perpendicular to a second set of channels and separated by a membrane which acts as a microfilter between the channels (Figure 3). The flux of the factors between the channels was controlled by concentration gradient, pressure gradient, and pore size. Figure 4 shows a fibrin gel formed by flowing thrombin through the bottom channels and human fibrinogen (3 mg/mL) in the top channel at a wall shear rate of 50 1/sec.

References

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[4] Ismagilov, R.F. et al, "Microfluidic arrays of fluid-fluid diffusional contacts as detection elements and combinatorial tools." Anal. Chem., 73, 5207-5213 (2001).