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HIGH THROUGHPUT FORMATION AND MEASUREMENT OF OCCLUSIVE THROMBOSIS IN PORCINE BLOOD

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ABSTRACT

Understanding and treating cardiovascular disease can benefit greatly from the development of low volume, high throughput tools to study thrombosis. We have designed, fabricated, and validated a microfluidic device to induce shear rates from 4300-7100 s^{-1} using only 10 mL of whole blood for 4 simultaneous assays to measure occlusive thrombosis optically. Shear rates were confirmed by flow rate and particle imaging velocimetry. Thrombosis measurements showed similar acute growth rates for all shear rates while time to acute growth onset and occlusion depend on initial shear rate. Applications range from unravelling the blood clotting cascade to drug dosage response measurement.

KEYWORDS: Blood, shear, platelets, microfluidics, thrombosis

INTRODUCTION

High throughput quantitative tools can be used to study thrombogenic events relevant to diagnosis and treatment of cardiovascular pathologies. Prior work [1] has measured initial platelet adhesion, but was unable to address pathologic high shear rates (>5000 s^{-1}), multiple shear rates, or acute thrombotic growth. Other methods [2] have been low throughput, high volume, and time consuming. Thus there is a need for a low volume, high throughput method for the systematic formation and measurement of thrombotic occlusion.

THEORY

Prior work on thrombosis predicts three distinct phases [4]: adhesion, acute growth, and occlusion. A multi-channel microfluidic device was designed to induce a range of initial shear rates while permitting optical measurement of these phases. Von-Willebrand factor (vWF), activated at 5,000 s^{-1}, has been implicated in thrombosis [3], so the channels are designed with stenoses to induce initial shear rates above and below this threshold. The stenoses' hydraulic diameters of 250-351 μm are large enough to prevent spurious aggregates from causing occlusion.

The shear rate, γ, of each stenosis was modelled with the Poiseuille equation. For hydraulic diameters d=250 and 351 μm, γ=7100 and 4300 s^{-1}, respectively. We focused solely on these channels to expedite analysis and experimentation.

EXPERIMENTAL

A mold was made using a custom micromilling machine (See Fig. 1). Feature sizes ranged from 250-1000 μm, dimensions difficult to achieve using

semiconductor processes. Devices were cast in polydimethyl siloxane (PDMS) and bonded to coverglass after oxygen plasma treatment. Enclosed channels were coated with type I collagen to initiate adhesion. Fig. 2 shows the mold and device.

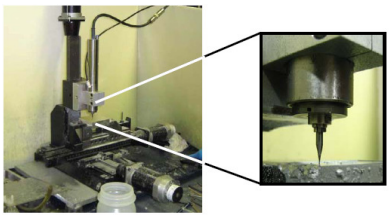


Figure 1. Micromilling machine with 80,000 rpm spindle, 0.05 µm encoder resolution, for mold fabrication. Endmills of size 150, 500 (inset), and 1000 µm cut the mold in 30 min.

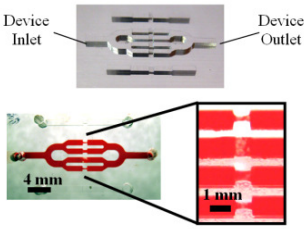
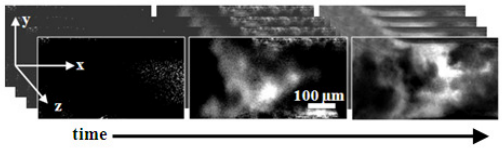


Figure 2. Photographs of aluminum mold (top) and PDMS device (bottom left) showing channel thrombus formation post-assay. Closeup (inset) of stenotic regions hydraulic diameters of 250-351 µm.

Shear rates in the channels were measured using bulk flow rate and particle image velocimetry (PIV). Bulk flow rate measurement was performed at 700 Pa with glycerol (40% w/w) to match the density and viscosity of blood. Mass flow rate was measured to compute average shear rate, $\gamma=6V/d$ [5]. For PIV, average channel velocities were determined by measuring local particle velocities at 20 kHz under identical conditions, averaging them, and computing γ.

After verifying shear rates, we measured thrombotic growth. Heparinized (3.5 U/mL), whole porcine blood was introduced at 700 Pa and images were captured at 12 µm z-axis increments through each stenosis as shown in Fig. 3. We also removed background intensity, compensated for drift, and low-pass filtered.

Figure 3. Images of thrombus, $I(x,y,z,t)$. Occlusion (%) is $\Sigma I(x,y,z,t_i)/\Sigma I(x,y,z,t_f)$ for time t_i.



RESULTS AND DISCUSSION

Shear rates determined from theory and measurement are shown in Table 1.

Table 1. Average velocities and shear rates from modeling and measurement

	theory	**bulk flow rate measurement**		**particle image velocimetry**	
d (µm)	**γ** (s^{-1})	**V** (m/s)	**γ** (s^{-1})	**V** (m/s)	**γ** (s^{-1})
250	7100	0.29	6900	0.22	5400
351	4300	0.26	4500	0.17	2900

The shear rates of 4300 s^{-1} and 7100 s^{-1} adequately span the 5,000 s^{-1} activation shear for vWF. Bulk flow rate shear rate measurements agree well with theory. Shear rates for PIV average 29% lower than those obtained from the bulk flow rate, perhaps due to measurement in a non-centerline image plane.

We measured occlusive thrombus formation in porcine blood at two initial shear rates. One data set is shown in Fig. 4; all measurements displayed in Table 2.

Figure 4. Thrombus growth from whole blood. Similar acute growth rates were seen in all experiments while time to acute growth onset and occlusion depends on shear rate.

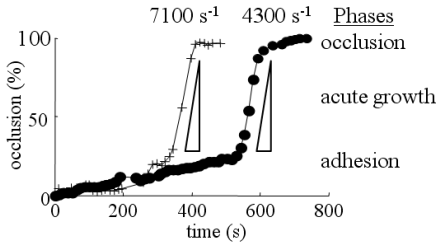


Table 2. Thrombosis formation measurements

Shear rate (s^{-1})	# of trials	Acute growth rate (%/s)	Time to acute growth onset (s)	Time to occlusion (s)
7,100	8	0.0126	160	487
4,300	5	0.0124	302	625

Results from this study show three distinct growth phases [4]. The acute growth rate appears to be identical but time-shifted for the lower shear rate channel. Time to acute growth onset and time to occlusion depend strongly on initial shear rate. This technique is higher throughput and lower volume than other state-of-the-art methods. Only 10 mL of blood are required for four experiments simultaneously in 12 minutes. Previous work [2] requires 250 mL and 20+ minutes for one experiment.

CONCLUSIONS

We have created and tested a device for high throughput formation and measurement of occlusive thrombosis in whole porcine blood. Initial shear rates of 4300 s^{-1} and 7100 s^{-1}, above and below the critical vWF activation rate, were determined theoretically and experimentally via bulk fluid flow and particle imaging velocimetry. The expected phases of occlusive thrombosis were observed; results showed similar acute growth rates in all experiments while time to acute growth onset and occlusion depend on initial shear rate. We aim to develop this technology into a high throughput method for scientific and pharmaceutical applications.

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REFERENCES

[1] Gutierrez, E., *Microfluidic Devices for Studies of Shear Dependent Platelet Adhesion.* Lab on a Chip, 2008.

[2] Ku, D.N. and C.J. Flannery, *Development of a flow-through system to create occluding thrombus.* Biorheology, 2007. **44**(4): p. 273-284.

[3] Schneider, S.W., *et al.*, *Shear-induced unfolding triggers adhesion of von Willebrand factor fibers.* Proc Natl Acad Sci U S A, 2007. **104**(19): p. 7899-903.

[4] Wootton, D.M., *et al.*, *A mechanistic model of acute platelet accumulation in thrombogenic stenoses.* Annals of Biomedical Engineering, 2001. **29**(4): p. 321-329.

[5] Gutierrez, E. and A. Groisman, Quantitative measurements of the strength of adhesion of human neutrophils to a substratum in a microfluidic device. Analytical Chemistry, 2007. 79(6): p. 2249-2258.