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\textsuperscript{-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 \cite{1} has measured initial platelet adhesion, but was unable to address pathologic high shear rates (>5000 s\textsuperscript{-1}), multiple shear rates, or acute thrombotic growth. Other methods \cite{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 \cite{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\textsuperscript{-1}, has been implicated in thrombosis \cite{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, $\gamma$, of each stenosis was modelled with the Poiseuille equation. For hydraulic diameters $d=250$ and 351 µm, $\gamma=7100$ and 4300 s\textsuperscript{-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.

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, $\gamma = \frac{6V}{d}$ [5]. For PIV, average channel velocities were determined by measuring local particle velocities at 20 kHz under identical conditions, averaging them, and computing $\gamma$.

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 $\mu$m $z$-axis increments through each stenosis as shown in Fig. 3. We also removed background intensity, compensated for drift, and low-pass filtered.

**RESULTS AND DISCUSSION**

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

<table>
<thead>
<tr>
<th align="center">$d$ ($\mu$m)</th>
<th align="center">$\gamma$ ($s^{-1}$)</th>
<th align="center">$V$ (m/s)</th>
<th align="center">$\gamma$ ($s^{-1}$)</th>
<th align="center">$V$ (m/s)</th>
<th align="center">$\gamma$ ($s^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td align="center">250</td>
<td align="center">7100</td>
<td align="center">0.29</td>
<td align="center">6900</td>
<td align="center">0.22</td>
<td align="center">5400</td>
</tr>
<tr>
<td align="center">351</td>
<td align="center">4300</td>
<td align="center">0.26</td>
<td align="center">4500</td>
<td align="center">0.17</td>
<td align="center">2900</td>
</tr>
</tbody>
</table>

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.
Table 2. Thrombosis formation measurements

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

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