ABSTRACT
We report on development of a microfluidic device and optical system for measurement of occlusive platelet thrombosis at initial shear rates ranging from physiological to pathological conditions from (500 – 13000 s\(^{-1}\)) within four stenotic channels in unfractionated blood. Real-time, label-free measurement of platelet thrombosis was performed by measuring optical radiation transmitted transversely through flow channels. Occlusive thrombosis occurred at shear rates above 4000 s\(^{-1}\) and measurements showed average differences in of 8.4% and 3.97% compared with traditional flow-rate and white-light microscopy methods, respectively.

KEYWORDS: Blood, Thrombosis, Platelets, Shear, Microfluidic

INTRODUCTION
Thrombosis is the pathological formation of blood clots composed of platelet aggregates that cause stroke and heart attack—the leading causes of death in developed nations. It occurs in three phases: (I) initial adhesion of platelets to a substrate, (II) aggregation of platelets binding other platelets and (III) stabilization of the platelets mass with corresponding flow occlusion [1]. Thrombosis is initiated in flow channels at regions of high shear. These conditions are commonly found in clinical cases of arterial stenosis, or constriction, where local shear rates have been measured in excess of 10000 s\(^{-1}\) [2]. Previous microfluidic assays have demonstrated shear-related platelet activity, but have been limited to the initial adhesion phase, physiological shear values, and non-constricted flow channels [3,4]. Additionally, many of these studies were conducted using bright field and/or fluorescence microscopy—methods that limit measurement area, requires additional cell labeling and/or sample fractionation, and are difficult to scale. Thus, development of a scalable method able to evaluate thrombosis throughout all three phases of platelet aggregation to full occlusion has the potential to supplant these traditional techniques more rapidly, cheaply, and scalably. Large studies enabled by this method could guide studies of platelet behavior and efficacy of clinical anti-platelet therapies including aspirin and Plavix.

EXPERIMENTAL
The microfluidic system design and its operation are shown in Fig. 1. The microfluidic device was designed to address initial shear rates representative of physiological to pathological conditions (500, 1500, 4000, 7000, 10000, and 13000 s\(^{-1}\)) within the stenosis region, from a common pressure source at 1400 Pa. While each of the four channels and stenoses have identical dimensions, the set of different desired shear rates were obtained by using outlet tubing sizes of varying diameters and lengths. The device was designed for these conditions using computational fluid dynamics software (ANSYS Inc., Canondale, PA) and closed-form modeling. Each of the device’s four stenoses have heights of 300 µm to enable observation of all thrombus phases and to simulate the shape of clinical late stage stenosis. High-speed surface milling (Haas, OM-1a, Oxnard, CA) was used to produce a device mold with the requisite feature sizes (125-1000 µm) and the gradual stenotic region transition (Fig. 1). Feature dimensions were verified using microscopy and white-light interferometry. From this mold, we cast the polydimethylsiloxane (PDMS) device layer and bonded it to glass slide following plasma gas exposure (Harrick Plasma, Ithaca, NY) to form enclosed channels. Enclosed channels were filled with collagen I (Sigma Chemicals, St. Louis, MO) to initiate platelet surface adhesion and allowed to incubate overnight.

Measurement of platelet thrombus formation in this system is achieved through measurement of transmitted light intensity through the sample at the stenosis region from a 0.9 mW (CVI Melles Griot, Albuquerque, NM) laser diode, expanded and spatially filtered, to a 3000 pixel 11-bit linear CCD with

![Figure 1: Schematic showing (A) side and (B) top view of the design of the microfluidic device for inducing platelet aggregation at four distinct initial shear rates in whole blood within the high shear stenotic regions. (C) Fabricated device showing stenotic region (dotted box) - Click to zoom.](image-url)

**Figure 1**: Schematic showing (A) side and (B) top view of the design of the microfluidic device for inducing platelet aggregation at four distinct initial shear rates in whole blood within the high shear stenotic regions. (C) Fabricated device showing stenotic region (dotted box).
pixel dimensions 7 µm x 200 µm (Thorlabs, Newton NJ). At 650 nm, platelets show substantially lower light transmission due to decreased scattering and absorption [5]. Thus, during thrombosis, platelets aggregate in the stenotic region, and are therefore measurable by an increase in transmission relative to whole blood, which is comprised of a mixture of red blood cells (40-60%) and a small amount of platelets (~0.1%). Optical cross-talk between channels is mitigated with sufficient channel spacing and the use of an aperture plate from laser-cut Mylar.

Fresh porcine blood was obtained from a local abattoir and treated immediately with 3.5 Units/mL of unfractionated porcine heparin (Elkins-Sinn, Cherry Hill, NJ). For each experiment, intensity from white-light microscopy, $I_{\text{microscope}}(t)$, and laser transmission, $I_{\text{laser}}(t)$, were measured simultaneously (time-multiplexed using a relay) with flow rates, $Q(t)$, using mass balances (Adam Equipment, Danbury, CT). Hardware control and acquisition were sampled at 1 Hz with 20 ms integration time using Labview (National Instruments, Austin TX) and data was analyzed using Matlab (Mathworks, Natick, MA) (Fig. 3C). White-light microscopy images were acquired with a Zeiss Stemi 2000c microscope and Motic 2000 CCD camera. Images were post-processed with background subtraction and low-pass noise filtration to produce relative intensity measurements $I_{\text{microscope}}(t)$.

**RESULTS AND DISCUSSION**

Results comparing the laser optical system developed in this work with simultaneous measurements using flow rate and white light microscopy are summarized in Table 1. We compared both the occlusion times for all three of these methods, as well as the relative intensity measurements for the two optical techniques. We first conducted 6 trials comparing simultaneous measurements of $I_{\text{microscope}}(t)$, $I_{\text{laser}}(t)$, and $Q(t)$ at a shear rate of 10000 s$^{-1}$ within a single flow channel. The results from one of these experiments is shown below in Fig. 2.

![Figure 2](image)

*Figure 2: (A,B) Formation and measurement of platelet aggregation to full occlusion of flow in the microfluidic device using the laser system, microscope, and flow rate simultaneously at 10,000 s$^{-1}$ initial shear rate. Microscope images (A) show aggregation—brighter areas of the images correspond to more platelet mass. (C) Schematic showing time multiplexed white-light microscopy and laser system optics which produced the data for (A,B).*

Next, we conducted 86 trials comparing simultaneous measurement of $I_{\text{laser}}(t)$ and $Q(t)$ within all four channels of the device at shear rates of 500, 1500, 4000, 7000, 10000, and 13000 s$^{-1}$. End-point thrombotic occlusion times were measured as the time that intensity reached steady state local maxima ($t_{\text{occlusion,laser}}$ and $t_{\text{occlusion,microscope}}$), and the time after which the flow rate was below the detection limit of 5µL/s ($t_{\text{occlusion,flow}}$).

<table>
<thead>
<tr>
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<th>$t_{\text{occlusion,microscope}}$</th>
<th>$t_{\text{occlusion,laser}}$</th>
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<tr>
<td>$t_{\text{occlusion,laser}}$</td>
<td>3.97% (N=6)</td>
<td>8.4% (N=86)</td>
<td>16.76% (N=6)</td>
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Occlusion times measured by flow rate were consistently an average of ~117s (N=6) less than $I_{\text{laser}}(t)$ and $I_{\text{microscope}}(t)$. Measurements of light transmission between laser transmission and microscopy methods at 900 time points over the experiment showed an average difference in relative $I(t)/I(0) = 8.51+/−1.25% (N=5)$.

For channels with shear rates greater than 4000 s$^{-1}$, occlusive thrombi formed within 1200 s (N=74, p<0.05). For channels with initial shear rate of 500 s$^{-1}$ and 1500 s$^{-1}$, occlusive thrombi did not form over the same time course of any experi-
Volumes of occlusive thrombi formed within this system are estimated at 0.0324 mm$^3$ (N=7), approximately 55 +/-8% of entire stenosis area within the channel.

CONCLUSIONS

We have developed an optical method for measurement of transmitted light to indicate occlusive thrombosis through simultaneous measurements of flow rate and microscopy imaging. Our system’s optical measurements showed 8.4% average difference in occlusion times compared to flow rate measurements and 3.97% differences compared to microscopy. Measurements of $I(t)$ for laser transmission and microscopy evaluated at multiple timepoints within each experiment showed 8.51% average differences. In contrast to conventional methods, the optical method developed in this work is more amenable to scaling to multiple flow channels, miniaturization, and low-cost manufacturing. For the range of shear rates tested, occlusive platelet aggregation in porcine blood above a threshold initial shear rate of 4000 s$^{-1}$ while shear rates of 500 and 1500 s$^{-1}$ did not show occlusion or detectable thrombus growth. Relative to fractionation and microscopy techniques, use of whole blood is faster, simpler, and more physiologically relevant. Four channel throughput enables direct parallel comparison of the effects of shear rate on thrombosis independent of variations in sample handling, anticoagulant concentration, and channel geometry unlike previous serial studies. We aim to develop this technology towards a point-of-care clinical method evaluation of the effects of anti-thrombotic agents and flow conditions on platelet thrombosis.

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