BENCH TOP OPTICAL DETECTION OF CLOT CONTRACTILITY FOR DIAGNOSTICS

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ABSTRACT

This paper presents a bench-top fluorescence microscope with the ability to track submicron deflections in microposts in order to diagnose platelet dysfunction during clotting. Blood flows through a microfluidic 'wound-in-a-chip' device, in which shear flow triggers platelets to form a clot and deflect a flexible micropost. The microscope is able to track all the microposts in a microchannel to determine the development of platelet forces force over time. The behavior of platelet contraction can be used to determine platelet dysfunction and can be a diagnostic tool for clinicians.

KEYWORDS: Microfluidics, Fluorescence Microscopy, Clot Contractility, Platelet Dysfunction

INTRODUCTION

Platelets activate, aggregate, and contract into a hemostatic plug to close a vascular wound site. Platelet dysfunction, due to severe trauma or anticoagulants, can cause excessive bleeding. Currently, clinicians in the emergency room measure platelet dysfunction using thrombelastography in order to determine the correct transfusion protocols to administer, but thrombelastography takes more than 30 minutes to perform, which can be an excessive amount of time to get results in trauma medicine.

Recently, our lab developed a PDMS microfluidic device with an array of microscale blocks and microposts (Fig 1). Whole blood flows through an area of high shear rate generated by the block, which then activates the platelets to aggregate and form a clot between the block and micropost. The contractile force causes the micropost to deflect over time and the behavior of this curve serves helps to detect platelet dysfunction [1]. Previously, a fluorescence microscope with a high magnification oil lens was used to track the deflections of these microposts. This setup has a limited field-of-view and its throughput but is not ideal in a clinical setting. Thus, we have designed a compact, inexpensive fluorescence microscopy approach that is capable of tracking the deflections of the microposts for rapid diagnosis of platelet dysfunction.

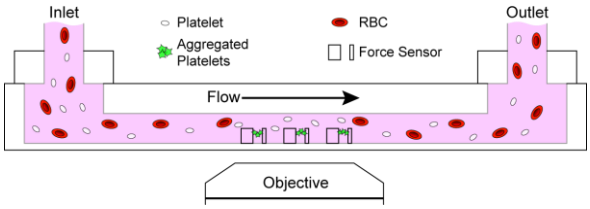

Figure 1: Microfluidic Device Schematic. Whole blood flows into the microfluidic device for one minute. Measurements of micropost deflection are taken every ten seconds. All experiments were conducted at 37°C.

EXPERIMENTAL

The miniature microscope (Fig. 2) contains a reversed webcam lens, which is offset from a Micron MT9P031 CMOS image sensor [2]. The combination of a high-powered LED and an aspheric condenser lens provides illumination on the sample. For fluorescence microscopy, TRITC excitation and emission filters were placed after the LED and webcam lens, respectively. This set of filters is able to excite and capture the DiI-stained features of the microposts. Various parts that make up the frame and the sample holder were either purchased by ThorLabs or 3-D printed.

For the actual experiments, PDMS microfluidic devices were fabricated and coated with rat-tail type I collagen for one hour and stained with Δ^9-DiI. Whole blood is pumped into the microchannel at a

pathological shear rate of 8000 s^{-1}. Images were taken (Fig. 3) and analyzed to plot average force curves for 120 seconds (Fig. 4). Image analysis was done in MATLAB to determine the force generation over time by tracking the centroid between the micropost and block. These curves demonstrate a measurement of clot onset and maximum clot strength, both of which are parameters for guiding clinicians on administering transfusions.

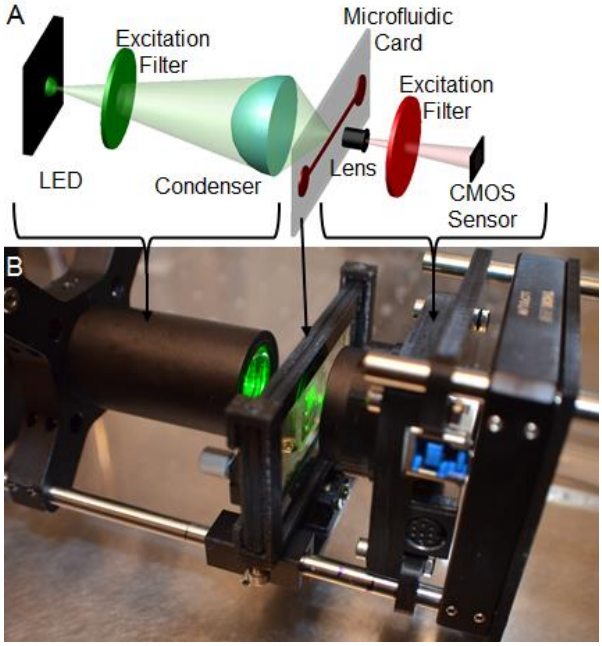

Figure 2: (A) Internal design of the prototype. A high powered LED at the excitation wavelength was focused onto the microfluidic device. A reversed webcam lens and emission filter magnified the fluorescent image to the CMOS sensor. (B) Actual prototype. Whole blood is pumped into the microfluidic device and images were taken 1 fps.

RESULTS AND DISCUSSION

At the current distance between the lens and the CMOS image sensor, the field of view is approximately 0.778 mm by 0.584 mm and has a sensor resolution of 0.3 μm/pixel. This is analogous to the field of view of a 10× objective with a resolution better than 20× object on the previous inverted fluorescent microscope. In comparison, a 40× oil objective produced a field of view 0.223 mm × 0.166 mm with a sensor resolution of 0.16 μm/pixel. While there is a loss in sensor resolution, we now have the ability to track more microposts in a less expensive, compact system.

Figure 3: The raw images are clear enough to track the deflection of the microposts.

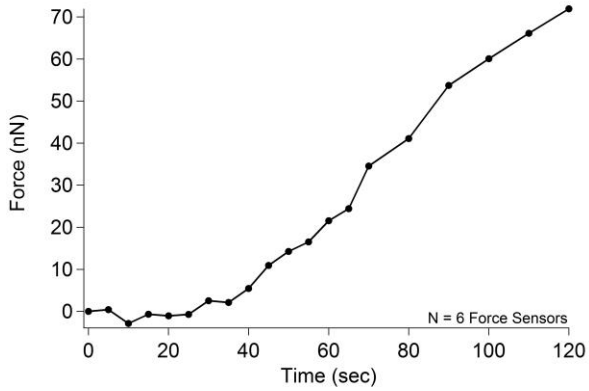

Figure 4: Average Force Curve. This example curve represents the average force for a donor. Because the images are high resolution, the location of the clot onset time is clear.

CONCLUSION

We have developed a compact, diagnostic platform that provides a rapid tool to identify platelet dysfunction in trauma and surgical settings. The system has a large field of view with enough resolution to track submicron movement. This optical system is versatile and can be used for other biological applications.

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