Platelets play a central role in coagulation. Typically, they are separated from other blood cell types through centrifugation, however, because their activation is sensitive to mechanical stresses, they are difficult to extract in an activated state, and have a very short shelf life of 5–7 days. As an alternative to centrifugation, here we investigate the use of label-free, acoustic separation in microfluidic channels to purify platelets from diluted whole blood. At a sample throughput of 3 mL/h per microchannel, we have obtained 95% pure faction of platelets with minimal activation.

**ABSTRACT**

Platelets, a major component of blood that play a central role in clotting mechanisms, are often transfused to patients undergoing a wide range of medical procedures [1]. However, because they are highly mechanically sensitive, they are difficult to extract without activation and have a notoriously short shelf life of 5–7 days [2]. Current plateletpheresis methods involve centrifugation, and the high mechanical stresses involved lead to significant sample losses [2]. Thus, the development of gentle and high-throughput plateletpheresis technology is urgently needed. Previous efforts in microfluidic plateletpheresis have suffered from a number of shortcomings including low throughput and buffer restrictions, with unknown activation levels [3, 4]. In this report, we describe microfluidic acoustic plateletpheresis from diluted whole blood with 95% purity at a throughput of 3 mL/h/microchannel, and present data on platelet activation levels due to our separation method.

**KEYWORDS:** Blood separation, Particle Separation, Ultrasound, Acoustophoresis

**INTRODUCTION**

Platelets, a major component of blood that play a central role in clotting mechanisms, are often transfused to patients undergoing a wide range of medical procedures [1]. However, because they are highly mechanically sensitive, they are difficult to extract without activation and have a notoriously short shelf life of 5–7 days [2]. Current plateletpheresis methods involve centrifugation, and the high mechanical stresses involved lead to significant sample losses [2]. Thus, the development of gentle and high-throughput plateletpheresis technology is urgently needed. Previous efforts in microfluidic plateletpheresis have suffered from a number of shortcomings including low throughput and buffer restrictions, with unknown activation levels [3, 4]. In this report, we describe microfluidic acoustic plateletpheresis from diluted whole blood with 95% purity at a throughput of 3 mL/h/microchannel, and present data on platelet activation levels due to our separation method.

**THEORY**

Our device relies on the microfluidic acoustic separation principles pioneered by Laurell and others [5]. Briefly, a piezotransducer attached to the backside of the device generates a resonant pressure field in the device with a pressure node at the channel half-width. Particles experience an acoustic radiation force \( F_{\text{rad}} \) in the resonant pressure field. In the 1D approximation, the force on a spherical particle of radius \( a \) in the transverse \( y \)-direction across the channel is given by

\[
F_{\text{rad}}^y = 4\pi a^2 (ka) E_{\text{ac}} \Phi \sin(2ky) = 4\pi a^2 (ka) E_{\text{ac}} \left[ \frac{\rho_p \cdot \pi \lambda^2 (\rho_p - \rho_a)}{2 \rho_p \cdot \pi \lambda^2} - \frac{1}{3} \frac{\rho_p c_p^2}{\rho_a c_a^2} \right] \sin(2ky),
\]

where \( 4\pi a^2 \) is the cell surface area, \( ka = 2\pi a/\lambda \) the particle size to wavelength ratio, \( E_{\text{ac}} \) the acoustic energy density, \( \Phi \) the contrast factor, \( \rho_p \) the particle density, \( \rho_a \) the medium density, \( c_p \) the speed of sound in the particle and \( c_a \) the speed of sound in the medium. Particles thus focus towards the acoustic node at a rate given by \( v_y = F_{\text{rad}}^y / 6\pi \eta a \), and the ratio of focusing times for red blood cells and platelets given by

\[
\frac{\tau_{\text{rbc}}}{\tau_{\text{pla}}} = \frac{v_{\text{pla}}}{v_{\text{rbc}}} = \frac{\rho_{\text{pla}} \Phi_{\text{pla}}}{\rho_{\text{rbc}} \Phi_{\text{rbc}}} \approx 6,
\]

thus red blood cells focus approximately 6 times faster than platelets, which enables efficient size-based separation (Figure 1).

**EXPERIMENTAL**

The main feature of the acoustic plateletpheresis device is a separation channel with dimensions 350 µm wide x 50 µm tall x 3 cm long. The outlet channel dimensions were calculated such that the hydraulic resistance of Outlet A was twice that of Outlet B, such that only 1/2 of the total flow exited through Outlet A. The microchannel was etched via DRIE (770 SLR, Plasmatherm) from a silicon wafer. Following etching, fluidic access ports were drilled in the silicon wafer using a diamond drill bit and CNC mill (Flashcut CNC). The wafer was then diced to final chip dimensions of 20 mm x 60 mm, and the microchannel was sealed with borofloat glass cap via anodic bonding (SB6, Suss Microtec AG). A 20 mm diameter piezotransducer (26051, Ferroperm Piezoceramics) was attached onto the Si-side of the device via superglue and driven via custom made amplifier based on the LT1210 op-amp (Linear Technology) attached to a function generator (33120A, Hewlett Packard). Fluidic connectors were attached to the device using epoxy. Sample and buffer were loaded into syringes and pumped volumetrically into the device with syringe pumps (PhD 2000, Harvard Apparatus). To monitor the separation process, the device was mounted on an inverted microscope (TE-2000S, Nikon). Collected fractions at the outlets were subsequently analyzed via flow cytometry (FACSARia, BD Biosciences).
RESULTS AND DISCUSSION

To characterize the device, we first used a model system of 5 μm and 2 μm diameter polystyrene particles (Microgenics Corp.), suspended in ultrapure water at a concentration of ~10^8 particles/mL (Figure 2). The particle mixture was volumetrically pumped into the device at 3 mL/h along with an ultrapure water buffer at 9 mL/h. The piezotransducer was actuated with a sinusoidal signal at 2.044 MHz and 30 Vpp. We achieved high purity separation wherein 93.8% of the fraction in Outlet A were 5 μm diameter particles, and 99.8% of the fraction in Outlet B were 2 μm diameter particles.

Next, we performed plateletpheresis using whole human blood in sodium heparin anticoagulant (Innovative Research), diluted 40-fold with 1×PBS. The dilution was necessary because of the viscosity and high cell count of whole blood: with less diluted samples, the width of the band of focused red blood cells was larger than the width of the Outlet A channel, and so some red blood cells would elute through Outlet B despite proper focusing. The blood sample was pumped into the device at 3 mL/h alongside 1×PBS buffer at 5 mL/h. Collected samples were then directly analyzed via flow cytometry. In a single round of separation, a 14-fold platelet enrichment (95% purity) was achieved in outlet B (Figure 3).

Lastly, we studied the effect of acoustic plateletpheresis on the activation level of platelets. To isolate the contribution of acoustic separation on platelet activation from other effects, the measurements were obtained with the ultrasound both off and on. Briefly, platelets were extracted from fresh refrigerated whole blood by centrifugation at 100×g for 10 min. The topmost platelet-rich plasma layer was then diluted in a 1:10 ratio with HEPES-Tyrode's buffer. A sample of resting platelets was fixed immediately in 2% formaldehyde in 1×PBS for 30 min. Another sample was activated by vortexing at 3000 rpm for 20 min and then by thermal cycling between 4 and 24 °C for 1 h before fixation in the same formaldehyde solution.

Two platelet samples were run through the device at 3 mL/h with a buffer of HEPES-Tyrode's pumped at 6 mL/h. One sample was run with ultrasonic actuation set at 2.044 MHz and 30 Vpp, and the other without ultrasonic actuation.
Figure 3: Flow cytometry analysis- side scatter vs forward scatter of the initial whole blood sample and the two outlet collections. The initial blood sample (a) shows a large population of erythrocytes, gated as 'red' and about 7% platelets, gated as 'pla'. The ungated population contains cell debris, and protein agglomerates. The Outlet A collection (b) consists of 98% erythrocytes, while the Outlet B collection (c) is 95% platelets.

Both samples were fixed right after collection. All samples were then stained with CD62P-PE antibody solution (BD Biosciences), and resuspended in 500 μL of 1×PBS before flow cytometry analysis. CD62P is a membrane protein that is expressed by platelets when they are activated, thus labeling platelets with anti-CD62-PE relates platelet activation level to fluorescence intensity for flow cytometric analysis. As expected, the cytometry results reveal that, compared to initial sample, the sample activated by thermal cycling and high-speed vortexing shows a significant increase in CD62P expression (Fig 4). Importantly, we observe minimal difference in CD62P activation between the samples run through the device with acoustic actuation on or off. We do note a small increase in activation for both samples run through the device compared with the initial sample, which indicates a small amount of activation due to fluidic shear stresses in the device and tubing.

Figure 4: Measurement of platelet activation levels using anti-CD62P-PE. Sample and buffer flowrates were 3 mL/h and 6 mL/h. The piezoelement was actuated at 2.044 MHz and 30 Vpp (Acoustics on). Platelets were activated via vortexing at 3000rpm for 20 min followed by thermal cycling from 4 to 24 °C for 1 h.

CONCLUSION

In conclusion, we demonstrate a novel method of plateletpheresis from whole blood using ultrasonic standing waves in microchannels. Although the current implementation requires significant dilution of sample, alternative device configurations may allow activation-free platelet separation at a high throughput from undiluted whole blood.

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REFERENCES


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