RAPID BLOOD PLASMA SEPARATION WITH AIR-LIQUID CAVITY ACoustIC TRANSDUCERS
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ABSTRACT
Herein we present a fast and low cost separation strategy that utilizes air-liquid cavity acoustic transducers (ALCATs) to extract plasma from human whole blood. ALCATs are oscillating air-liquid interfaces that are acoustically driven and produce microstreaming within liquids in microfluidic channels. With whole blood, the streaming patterns induced by ALCATs include swirling eddies where erythrocytes become aggregated. The device is designed to have a dense array of ALCATs that simultaneously serve to pump fluid forward and to trap red blood cells. Due to the trapping effect, red blood cells have a longer path to be propelled downstream while plasma continues flowing forward resulting in a rapid extraction of plasma. With microsample volumes in one device, 40% of plasma was yielded from a 16 µL sample of EDTA spiked whole blood within minutes of activating the acoustic source. The plasma volumes and plasma extraction rates can be of practical use in many point-of-care applications.

KEYWORDS: point of care, blood plasma separation, sample preparation, air-liquid cavity acoustic transducers, whole blood, red blood cells, erythrocytes, point of care diagnostics

INTRODUCTION
A fast and low cost on-chip sample preparation method is a critical component for effective point-of-care (POC) diagnostics [1]. The rapid extraction of plasma from whole blood is an important sample prep method to prevent blood components from interfering with assay analyses downstream. Specifically, aggregation of erythrocytes in a chip’s optical detection zone can precipitously degrade signal quality. In the absence of a blood-plasma separation method, extra wash steps are required, thus, increasing sample-to-answer times. A lot of effort has been devoted to the separation of components from blood in various microfluidic systems. Separation strategies include the use of mechanical forces (e.g. centrifugation, filtration, sedimentation, and the Zweifach–Fung effect), magnetic interactions, optical interactions, and dielectrophoresis [2][3]. However, many of these strategies have practical limitations in cost, time, and scale up. For example, centrifugation and sedimentation methods can be time-consuming. Many commercial filtration methods in POC utilize glass fiber or membrane filters that require complex and costly processing of filters with multiple reagents in order to reduce non-specific binding of the analyte of interest or to reduce hemolysis effects. Furthermore, most methods have limited control of the quantity of plasma that can be extracted. Many microfluidic separation methods also require diluted blood samples. Hence, there is increasing demand for better plasma separation strategies from whole blood.

THEORY
When liquid fills the main microchannels of hydrophobic devices, pockets of air are trapped within dead-end side channels. Applying an acoustic source will cause the interfaces between air and liquid to oscillate, thereby, creating microstreaming patterns within a localized region of the surrounding liquid (Figure 1). These air liquid cavity acoustic transducers (ALCATs) have been shown to be useful for pumping, mixing, and bead trapping [4]. When whole blood is pumped through an array of activated ALCATs, the red blood cells will agglomerate within streaming eddies. These eddies

![Image](https://example.com/figure1.png)
are steady second-order flows that are induced by a first-order periodic flow from the oscillating air liquid boundary [5] As cells are trapped within the eddies, plasma continues to flow downstream effecting a separation of cells and plasma that is visible at the leading end of the flow (Figure 2).

**Figure 2.** Heparinized whole blood (70 µL) was pumped through a dense ALCAT array that is activated by a 5 cm diameter piezoelectric ceramic at 30 Vpp square wave at 44 kHz. The direction of the flow is indicated by A. The leading edge of the flow (B) has a significantly lower concentration of red blood cells compared to the trailing edge.

**Figure 3.** An eddy of red blood cells is indicated by the arrow (A). Over time, the eddies of red blood cells grow larger and saturate (B). After separation of plasma from blood, the leading edge of the flow has minimal red blood cells (C) while the lagging end of the flow is concentrated with erythrocytes (D). All images are at 100X with the widths of the main channel at 500 µm.

**EXPERIMENTAL**

The microfluidic devices were designed to have a high density of ALCAT arrays that serve both as pumping and cell trapping mechanisms. The devices were fabricated using standard soft lithography where poly(dimethyilsiloxane) (PDMS) was bonded to a glass slide that was cleaned with isopropyl alcohol. The fluidic channels were sealed by simple adhesion where PDMS’s natural adhesive property will bind to glass reversibly. Human whole blood was collected with Ram Scientific SAFE-T-FILL capillary blood systems that contain heparin or EDTA as the anti-coagulant. Devices were filled with varying volumes (16 µL, 70 µL) of whole blood and ALCATs were activated by a piezoelectric ceramic at 20 or 30 Vpp square waves at 44 kHz.

**RESULTS AND DISCUSSION**

Blood plasma separation began within a minute of activating the piezoelectric transducer. From a macroscopic view, the leading edge of the device showed clear reduction of red blood cell concentration (Figure 2). From a microscopic view, the hydrodynamic force generated by the ALCATs formed local eddies where blood cells agglomerated within stationary pressure gradients and oscillating viscous forces. Over time, a cell saturation point is reached in each eddy whereby the mass of blood cells will move to a subsequent eddy downstream. (Figure 3 a,b). Images of post separation show a dense aggregation of blood cells at the trailing end of the flow versus >90% pure plasma at the leading end of the flow (Figure 3 c,d). In another device (Figure 4), within ~3 minutes of activating the acoustic source, approximately 40% percent of plasma (3.8 µL) was yielded from red blood cells. Whole blood (16 µL) with EDTA was pumped on a device that is activated by a piezoelectric ceramic at 20 Vpp square wave at 44 kHz. Based on a 40% hematocrit, plasma yield is approximately 40% of the expected volume of plasma from the original sample.
EDTA-spiked whole blood (16 µL) based on a 40% hematocrit. This volume of plasma is more than enough for what is required to use on many POC tests.

Plasma extraction rates were also characterized as shown in Figure 5. A linear regression for n=16 time points shows a steady increase in plasma extraction over time that is statistically significant (P <0.01). The rate of plasma extraction averages 10.6 nL/s (95% Confidence Interval: 6.9 to 14.3 nL/s). For plasma extraction, the longer the acoustic transducer was activated, the longer the plasma leading end became. This suggests that more efficient blood plasma separation (thus, more plasma volume extracted) can be controlled by the duration of an activated acoustic source. However, it should be noted that plasma extraction slows to a halt toward the end of the extraction period due to the increasing viscosity of the concentrated red blood cells at the trailing end.

CONCLUSION

Our devices utilize a unique method of blood-plasma separation that can replace conventional centrifugation, filtration, and sedimentation strategies. To note, plasma separation has been shown to occur in less than 10 seconds on some of our devices. Furthermore, preliminary observations have shown that plasma separation can be achieved using whole blood without anti-coagulants so long as blood is tested within minutes after being collected from a fingerprick. Our design is easily fabricated and amenable to scale up. Because ALCATs are passive features that can be designed on a single plane of a chip, ALCATs lend themselves to being manufactured inexpensively. Finally, this separation strategy can be integrated on-chip as a sample preparation method that can be suited to many POC diagnostic applications.

ACKNOWLEDGEMENTS

This work was supported in part by the Defense Advanced Research Projects Agency (DARPA) N/MEMS S&T Fundamentals Program under grant no. N66001-1-4003 issued by the Space and Naval Warfare Systems Center Pacific (SPAWAR) to the Micro/nano Fluidics Fundamentals Focus (MF3) Center.

REFERENCES


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