ACOUSTIC MICROCENTRIFUGE ARRAYS FOR RAPID PARTICLE SEPARATION FROM MICROVOLUME BLOOD SAMPLES

Arlene Doria, Maulik Patel, Nicholas E. Martin, and Abraham P. Lee
Department of Biomedical Engineering, University of California - Irvine, USA

ABSTRACT

Technologies for the separation of mixtures are essential tools for chemists and biologists. Research and clinical laboratories use different strategies such as sedimentation, filtration, chromatography, and centrifugation. It is ideal to use smaller sample volumes when reagents and samples are costly and/or limited. However, sample handling can pose a challenge when dealing with microvolumes. Herein we report a microfluidic device that acoustically separates a 15 µL blood mixture into three components: a) 43µm latex beads, b) red blood cells (RBCs), and c) fluorescein-spiked plasma in roughly a minute.

KEYWORDS

Cell separation, acoustic, ultrasound, blood, microfluidic, latex beads

INTRODUCTION

Centrifugation has long been the standard lab technique for separating particles by particle size, density, shape, and viscosity [1]. However, lab researchers encounter difficulties when centrifuging small volumes because samples are easily lost during pipetting and decanting steps. Automated robotic sampling can address the issues with handling small sample volumes. However, these machines are costly. Here we present a novel device that rapidly separates a dilute amount of large latex beads (43 µm) from a dense 15µL blood sample. The device also separates RBCs from plasma (as presented previously [2]). The mechanism of separation is accomplished by air-liquid cavity acoustic transducers (ALCATs). ALCATs are air cavities that form naturally in hydrophobic devices filled with liquids. When activated by ultrasound, the air-liquid interfaces will oscillate and create stable cavitation streaming within a localized region of the surrounding liquid. ALCATs have been shown to be useful for pumping, mixing, and cell/particle switching [3]. Fluid and particle manipulation can be accomplished on a passive, disposable chip that is placed on top of an external acoustic transducer with a coupling medium (Figure 1).

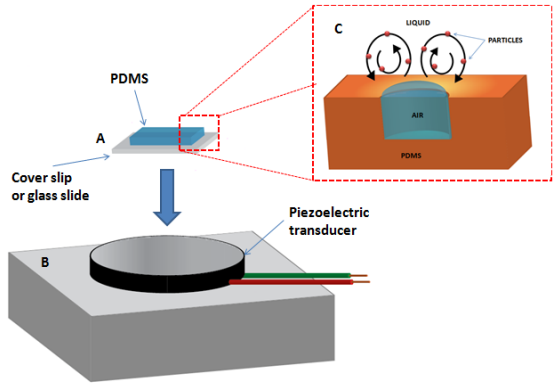

Figure 1. Experimental setup. A passive chip made of Polydymethylsiloxane-on-glass (A) is placed on a piezoelectric transducer (B). A general ALCAT structure is shown in C. No external tubing or syringe pumps are required.

EXPERIMENT

In our device design, arrays of ALCATs line a serpentine channel (Figure 2, left). ALCATs are angled toward the direction of flow (Figure 2, right). When actuated by ultrasound, ALCAT arrays trap particles in microvortices while simultaneously pumping plasma downstream effecting a separation of plasma from cells and beads in roughly a minute.

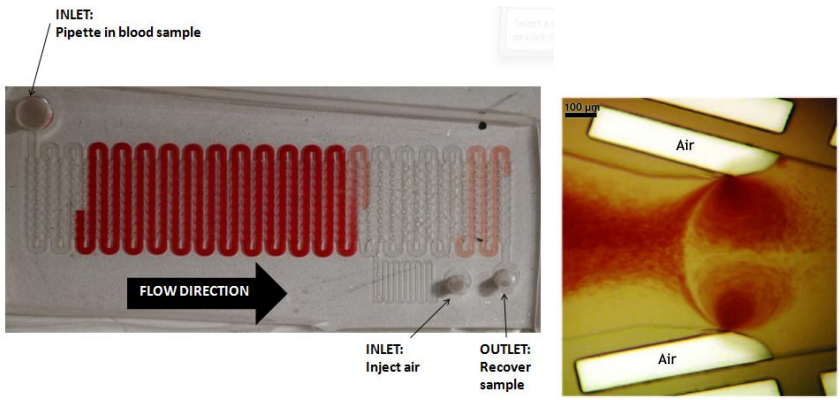

Figure 2. Chip design (left). While blood flows to the outlet, actuated ALCAT arrays trap particles in microvortices effecting a separation of plasma from cells. Microscopic view ALCATs show that ALCATs are angled toward the direction of flow from left to right.

The cumulative effect of thousands of microvortices will separate particles based on size because larger particles experience greater shear gradient lift forces [4] from cavitation streaming which in turn traps them in microvortices. A macroscopic view of the device in action shows the formation of a purple plug of 43 µm beads (~0.200µL) being separated from RBCs (~5um-8um) in the lagging end of the flow (Figure 3). In the leading end of the flow, cell-free and bead-free plasma is separated. A microscopic examination from upstream to downstream locations (Figure 4A-D at 100X magnification) on the device shows successively diluted solution. This preliminary evidence supports that the separation is based on particle size. For the 43µm latex beads, the interaction of the beads with the walls of the 500µm by 100µm channels is enhanced by the acoustic streaming generated by ALCATs. Hence, a combination of the beads interacting with the channel walls and the microvortices result in the concentration of beads in the lagging end of the flow.

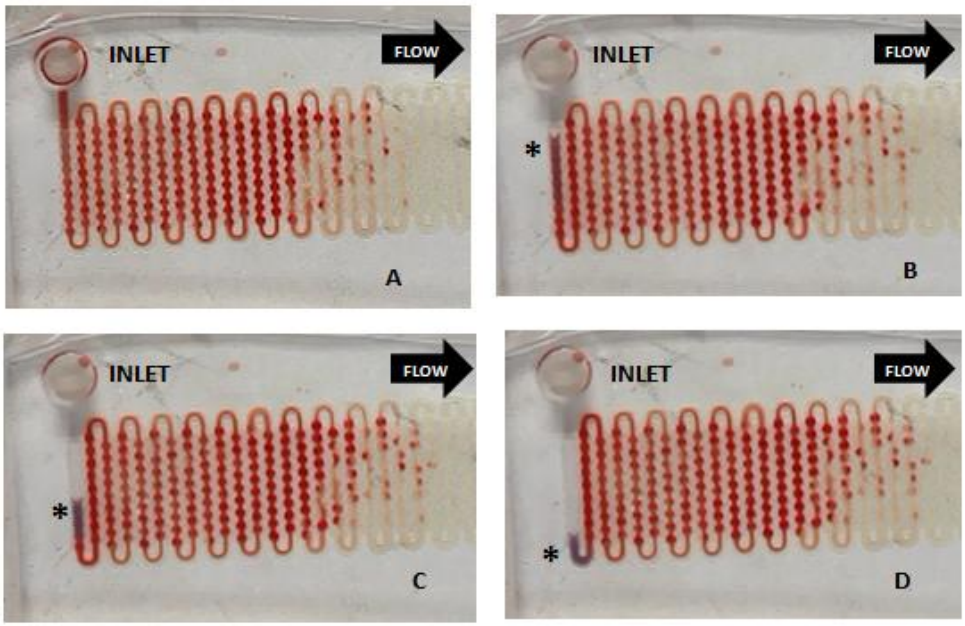

Figure 3. Snapshots of large purple 43 µm beads (asterisk) progressively being separated over a few seconds from red blood cells (~5-8um) while plasma is also being separated downstream.

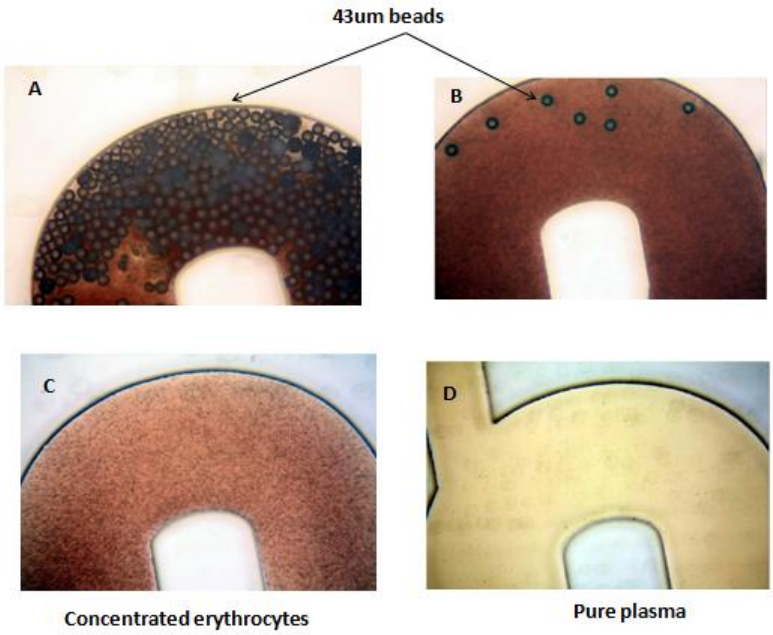

Figure 4. Microscopic examination of the fluid within the channels from upstream to downstream (A-D) locations after component separation has occurred. Upstream is concentrated with large beads(A) while downstream (D) is cell/particle free plasma.

The chip was made of polydimethylsiloxane bonded to a glass cover slip and fabricated using standard soft lithography techniques. A blood mixture was made by mixing equal volumes of whole blood, a fluorescein 1% salt solution, and 1% latex bead solution. The device was activated using 20 voltage peak to peak at 44 kHz. The oscillating air-liquid interfaces create local vortices that trap particles while simultaneously pumping plasma downstream effecting a net separation of cells from plasma. This was modeled on CFD-ACE+ using oscillating velocities that are approximately normal to arc-shaped inlets (Figure 5, top left). A snapshot of the velocity magnitudes from the simulation shows that fluid will be propelled downstream from left to right (Figure 5, top right). Cells were modeled as spray particles. In the presence of the oscillating velocities, transient simulations show particles are trapped near the inner corners of the ALCAT structures (Figure 5, bottom left). Simulation of blood cell trapping showed qualitative agreement with experimental (Figure 5, bottom right).

In summary, this unique method of separation does not require external pumps to flow sample into the device. It is expected that biological and non-biological samples can be used to separate particles of various size from small sample volumes. However, high throughput can potentially be reached by parallel construction of ALCAT arrays. As demonstrated in these blood samples, cell types of different sizes (e.g. large cancer cells or small bacteria) can potentially be separated from solution if the ALCAT arrays and transducer properties are optimized. Because ALCAT microstructures can be fabricated in only a single layer it is amenable to conventional manufacturing processes such as hot embossing and injection molding. Furthermore, piezoelectric transducers as fluidic drivers are durable because no moving parts are required. Finally, the simplicity of the design will allow this separation technology to be integrated into various lab-on-chip assays.

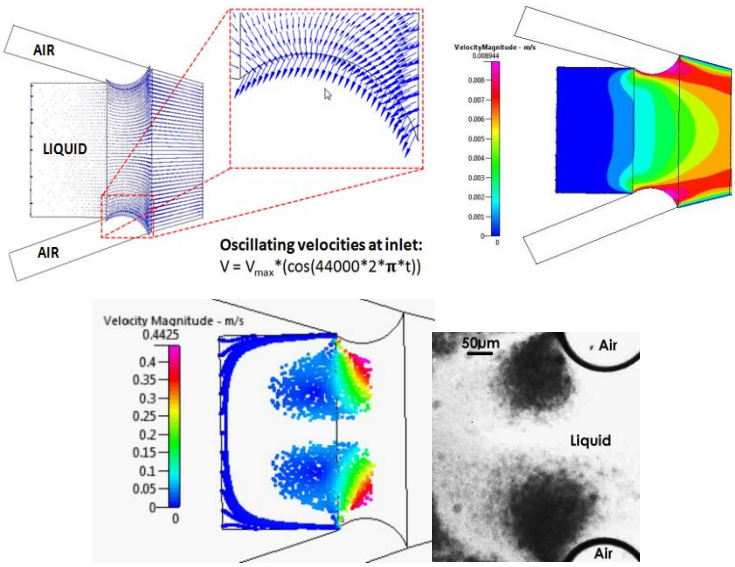

Figure 5. Computational fluid dynamic simulation of trapping using CFD-ACE+ showing qualitative agreement with the video snapshot of dynamic particles trapped in vortices.

REFERENCES

[1] C. A. Burtis, E. R. Ashwood, D. E. Bruns and N. W. Tietz, Tietz fundamentals of clinical chemistry, Saunders Elsevier, St. Louis, Mo., 2008.

[2] A. Doria, M. Patel, and A.P. Lee "Rapid blood plasma separation with air-liquid cavity acoustic transducers." 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences (uTAS 2010)Seattle, October 2011.

[3] A.P. Lee, M.V. Patel, A.R. Tovar and Y. Okabe "Microfluidic Air-Liquid Cavity Acoustic Transducers for On-Chip Integration of Sample Preparation and Sample Detection." Journal of the Association for Laboratory Automation, vol. 15(6) pp. 449-454, 2010.

[4] J. Mach , JH Kim , A Arshi , SC Hur and DD Carlo, Automated cellular sample preparation using a Centrifuge-on-a-Chip, Lab Chip, 2011, 11, 2827-2834

CONTACT

Arlene Doria (arlened@uci.edu)
Abraham P. Lee (aplee@uci.edu)
Website: biomint.eng.uci.edu