RAPID TWO-STEP BLOOD SAMPLE PREPARATION WITH ACOUSTIC MICROFLUIDIC CHIPS

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ABSTRACT

Analysis of proteins in blood such as detecting antibodies in serology tests necessitates that the blood samples be stripped of their cellular components and diluted. The standard method of sample preparation requires a centrifuge and careful pipetting techniques to transfer the sample to the analysis tool. Here we present a microfluidic device that can rapidly perform two-step blood sample preparation using air-liquid cavity acoustic transducers (ALCATs). ALCATs are trapped microbubbles that generate cavitation microstreaming when activated with ultrasound. Using ALCATs, plasma separation from a blood sample followed by plasma dilution is performed wholly on chip in approximately one minute.

KEYWORDS

point-of-care, acoustic, ultrasound, blood, diagnostics, sample preparation, erythrocytes, microfluidic

INTRODUCTION

Blood sample preparation is an important step when developing a point-of-care (POC) diagnostic device. Device errors stem from operators handling samples incorrectly [1]. Rapid plasma extraction from whole blood is required to prevent cellular components from interfering with the detection analysis which is often performed optically in a POC device [2]. The conventional method to separate cells is centrifugation. In rapid diagnostic devices, commercially available filters are integrated into the device to extract plasma. However, manufacturing these filters requires complex and costly processing with multiple reagents. Other microfluidic strategies to extract plasma include the bifurcation law, hydrodynamics, filtration, and magnetophoresis [3]. However, many of these strategies have practical limitations in cost, time, scale-up, and plasma yield. Previously, using ALCATs we reported the rapid extraction of plasma from whole blood in which plasma yields were comparable to some commercially available methods [4]. 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. 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). Here we combine the versatility of ALCATs in cell separation and micropumping of a diluent to achieve a quick two-step sample preparation. Plasma dilution is often required for analyzing immunoassays (e.g. ELISAs of antigen-spotted microarrays) because antibodies in whole blood are present in large amounts [2].

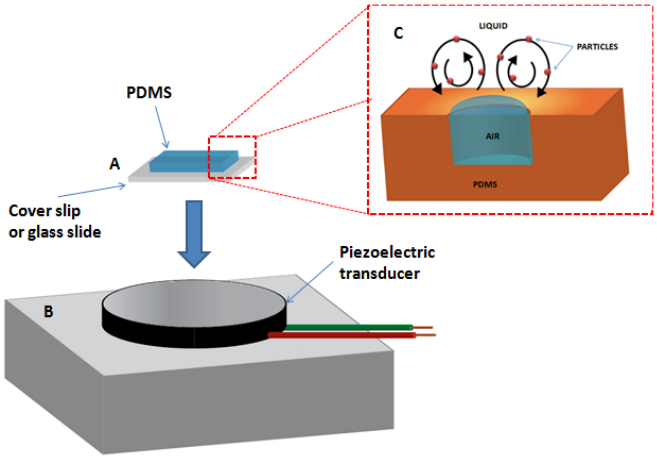

Figure 1. Experimental setup. A passive chip made of Polydymethylsiloxane-on-glass (A) is placed on a piezoelectric transducer (B). A general ALCAT structure is shown in C. No external tubing or syringe pumps are required.

EXPERIMENT

We present a fast, two-step blood sample preparation that is performed wholly on-chip utilizing ALCATs. Figure 2 shows a device with a serpentine channel that is lined with angled ALCAT arrays to separate red blood cells from plasma. To better visualize separation, the blood sample was pre-diluted with an equal volume of fluorescein solution so that the plasma extract is yellow.

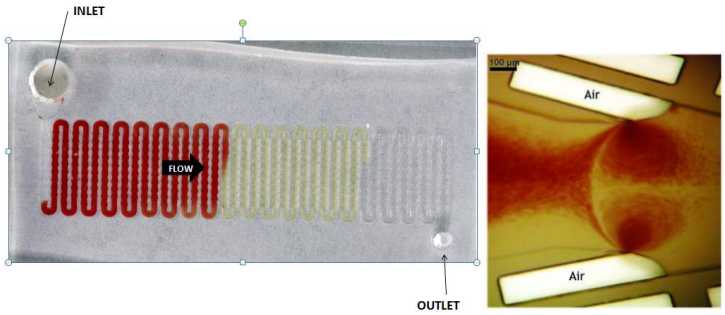

Figure 2. Plasma separation from blood with ALCATs (left figure). Plasma separation is easily visualized with addition of the yellow fluorescein solution to the original blood sample. Microscopic view ALCATs show that ALCATs are angled toward the direction of flow from left to right (right figure). Blood cells are trapped in vortices while plasma is simultaneously pumped downstream effecting a net separation of cells from plasma.

As seen in Figure 3, the device consists of two parts: blood plasma extraction followed by dilution with a blue dye. Two serpentine channels with high density ALCAT arrays were positioned in parallel and joined into one channel (JC) downstream (Figure 3A). First, the top channel is acoustically activated to filtrate red blood cells and deliver extracted plasma to the JC. Roughly 3.5µL of plasma is extracted from 15µL of blood sample. Secondly, the bottom channel containing a 15µL of blue diluent is then activated. Here diluent is pumped into the plasma containing JC to effectively dilute the extracted plasma. With the combination of yellow plasma and blue diluent, the final diluted sample at the outlet is green (Figure 3B). All devices were made of polydimethylsiloxane bonded to glass and fabricated using standard soft lithography techniques. Devices were activated using 20 Vpp square waves at 44 kHz. The oscillating air-liquid interfaces create local vortices that trap red blood cells 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 4, top left). A snapshot of the velocity magnitudes from the simulation shows that fluid will be propelled downstream from left to right (Figure 4, top right). Blood 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 4, bottom left). Simulation of blood cell trapping showed qualitative agreement with experimental (Figure 4, bottom right). Particle counts of ALCAT extracted plasma show that the purity of extracted plasma is comparable to centrifuged

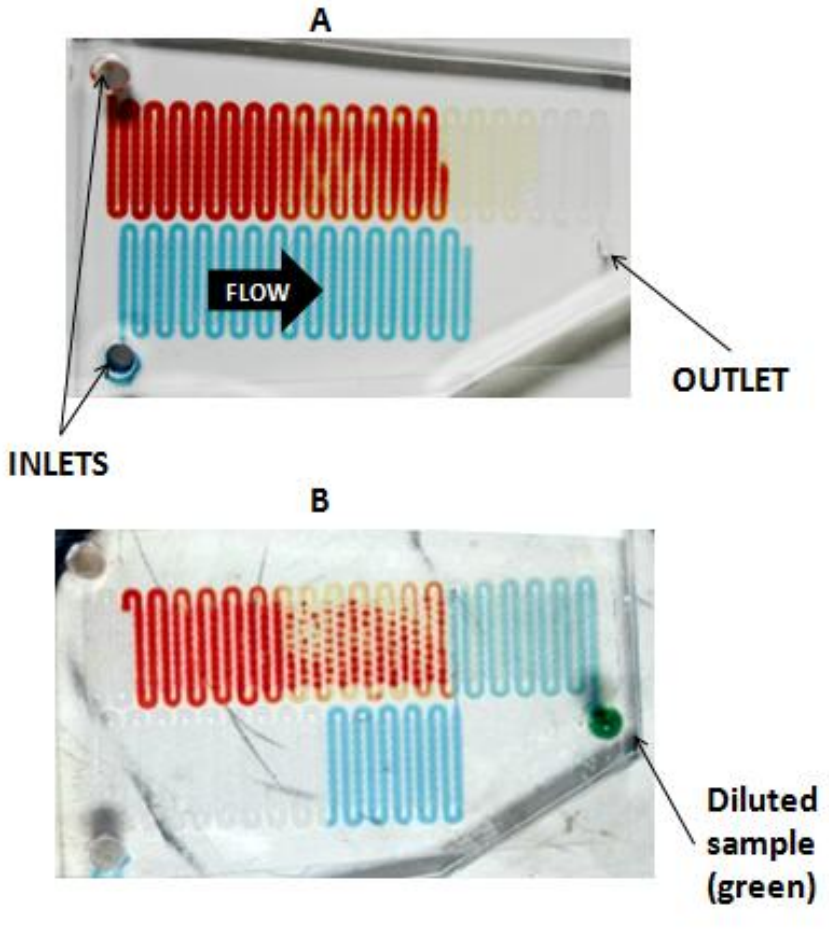

Figure 3. Two step sample preparation. The result of plasma extraction is shown in the top channel (A). A snapshot of on-chip dilution shows micropumping of the blue dye into the yellow plasma resulting in green solution in the outlet (B. The on-chip sample preparation was achieved in roughly one minute.

plasma (Figure 5, top). Using Wright Stains, blood films of the acoustically activated blood show normal cell morphology compared to non-activated blood (Figure 5, bottom images).

In summary, this novel method of microfluidic control is tunable and durable since no moving parts are required for the chip. Because ALCAT microstructures can be fabricated in only a single layer, they are amenable to conventional manufacturing processes that produce inexpensive parts. This sample preparation strategy uses low blood volumes that can be obtained from a fingerstick. Furthermore, the technology can be integrated on-chip to accelerate sample-to-answer times in plasma/serum analyses for various POC diagnostics.

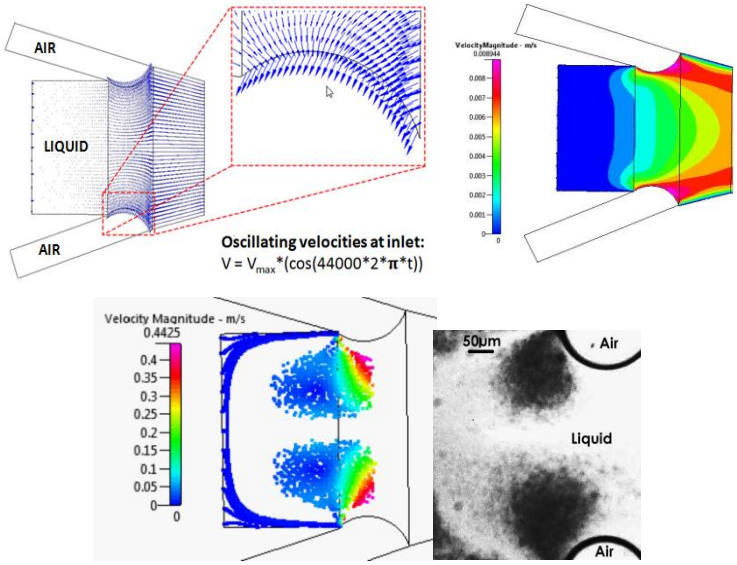

Figure 4. Computational fluid dynamic simulation of trapping using CFD-ACE+ showing qualitative agreement with the video snapshot of dynamic particles trapped in vortices.

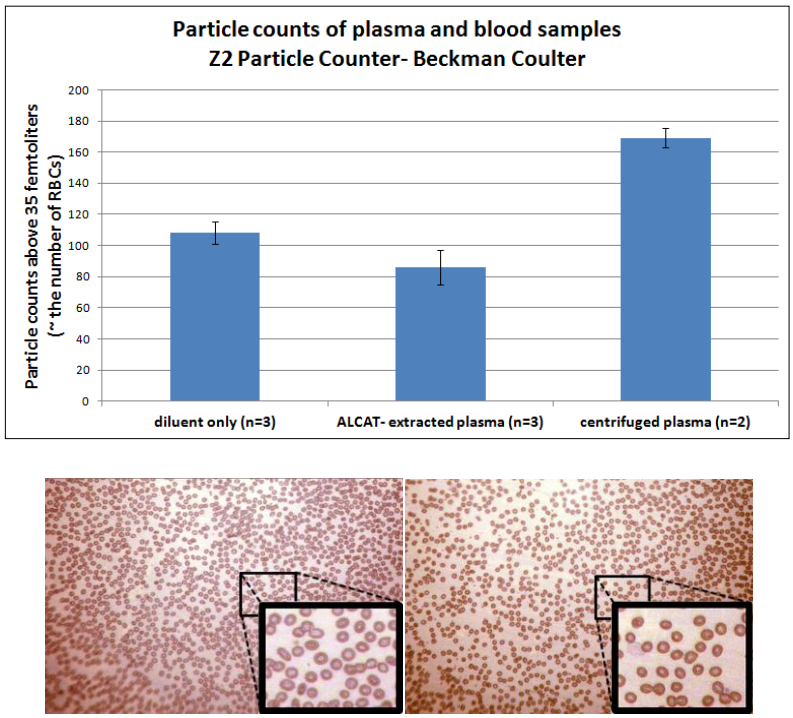

Figure 5. Particle counts of plasma extracted by ALCATs (top) are compared to centrifuged plasma and a pure diluent (top). Normal cell morphology of red blood cells (RBCs) is shown from a blood sample activated by ALCATs (bottom left) and from a blood sample not activated (bottom right).

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