TUNABLE CELL LYSING OF DENSE BLOOD CELL SAMPLES WITH AIR-LIQUID CAVITY ACOUSTIC TRANSDUCERS

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ABSTRACT
The extraction of nucleic acids and cell proteins typically requires the destruction of the cell membrane. Because the cells are suspended in solution, the solution has to be further processed to recover the components of interest after lysing. Herein, we present a device using air-liquid cavity acoustic transducers (ALCATs) with a tunable parameter that will lyse cells and then extract the sample without the remaining cellular debris. Red blood cells (RBCs) are used for this demonstration though this can be applied to other cell types. We demonstrate a fast and chemical-free lysis method for sample preparation.

KEYWORDS
Nucleic acid purification, point-of-care, acoustic, ultrasound, blood, diagnostic, sample preparation, microfluidic

INTRODUCTION
Cell lysis is the first step in nucleic acid and protein purification methods. The conventional ways to lyse cells include physical or chemical means. Cell lysis methods include mild osmosis, sonication, centrifugation with beads, detergents, and nitrogen burst methods [1]. However, these methods require a step to separate the remaining cellular debris. Additional handling steps increase the chances of operator error. Furthermore, the efficiency of lysing is dependent on the density of cells in the solution, the total volume of the solution, and the toughness of the cell (e.g. the cell wall of a plant cell is rigid). By varying the input voltages applied to the acoustic transducer, ALCATs can be tuned to first lyse cells and then separate by components from solution.
ALCATs are air cavities that form naturally in hydrophobic devices filled with liquids. When activated by an acoustic source, 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 [2]. Fluid and particle manipulation can be accomplished on a passive, disposable chip that is placed on top of an external acoustic transducer (Figure 1).

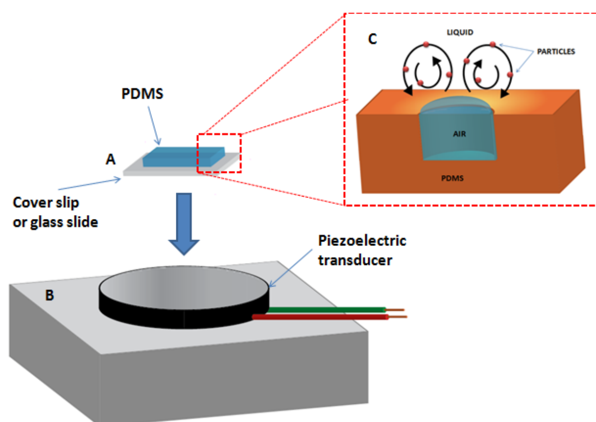

Figure 1. Experimental setup. A passive chip made of Polydymethylsiloxane-on-glass (A) is placed on a piezoelectric transducer (B). A general ALCAT structure is shown in C. No external tubing or syringe pumps are required.

EXPERIMENT
Dense RBC solutions were used in the cell lysis demonstration. When RBCs are lysed, they release hemoglobin into solution making it visible to the naked eye at >200 mg/L. The hemoglobin can be roughly quantified by a spectrophotometer at 415nm, also known as the Soret's peak. In this device, arrays of ALCATs line a serpentine channel. ALCATs are angled toward the direction of flow (Figure 2). The sample undergoes lysis by applying a high input voltage (30 Vpp) to the piezoelectric transducer while inhibiting flow by plugging the outlets (Figure 3, left). Sample recovery is then performed by unplugging the outlets and allowing separation of cellular debris to occur (as described by Doria [3]) at a lower voltage of 15 Vpp (Figure 3, right). Numerical modeling of blood damage is often modeled by the power law approach using empirical coefficients that depend on the shear stress(τ) and the time of exposure (t) that the blood experiences [4]. A hemolysis index has been developed [4]:

$$\text{Hemolysis Index (\%)} = 3.62 \text{ X } 10\text{-}5 * t^{0.785} * \tau^{2.416} \quad (1)$$

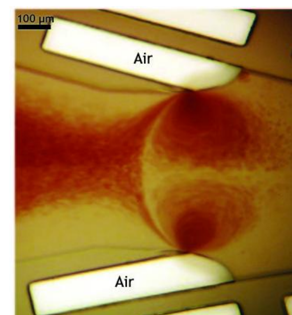

Figure 2. Microscopic view ALCATs show that ALCATs are angled toward the direction of flow from left to right.

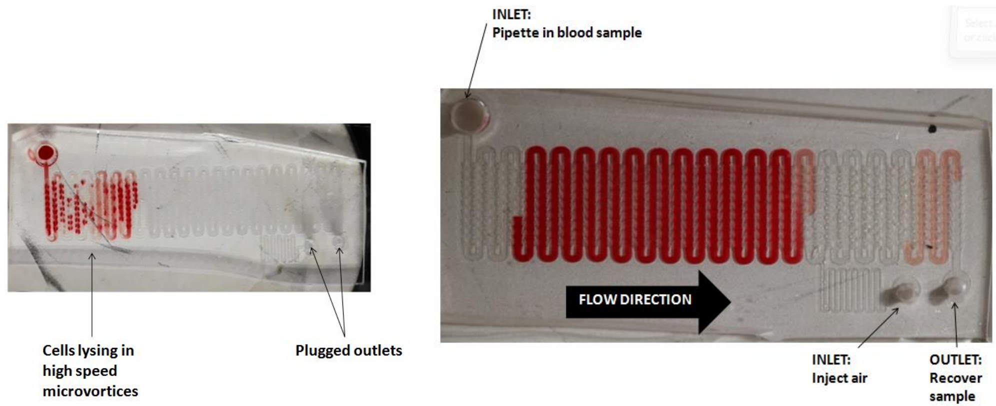

Figure 3. Lysing occurs with increasing input voltages from the piezoelectric transducer while inhibiting flow by plugging the outlets (left). The hemoglobin released in solution is recovered in the extraction step (right).

If the shear stress is constant, the longer the cells are exposed to the stress, the higher the percentage of cells is hemolyzed. Analysis of Soret's peak is seen in Figure 4. Heme-containing groups will absorb strongly at the 415nm wavelength of the visible spectrum. The absorbance can be approximately correlated to the amount of hemoglobin that has been released into the solution after lysing. Increasing absorbencies are observed with increasing lysing times. Specifically, for 0, 20, and 120 seconds of exposure to lysing parameters, the absorbencies increase from 0.102, 0.430, to 0.829, respectively. As a control, the absorbance of plasma supernatant that has been centrifuged was observed at 0.052. This suggests that the method of sample recovery at 15 Vpp may result in limited hemolysis too. Using a Wright Stain, blood film of different lysing times show that longer times of exposure resulted in abnormal, sheared cells (Figure 5).

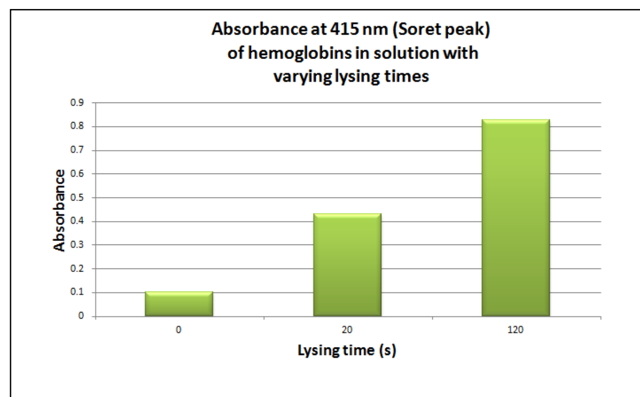

Figure 4. Increasing Soret peaks of sample with higher lysing times.

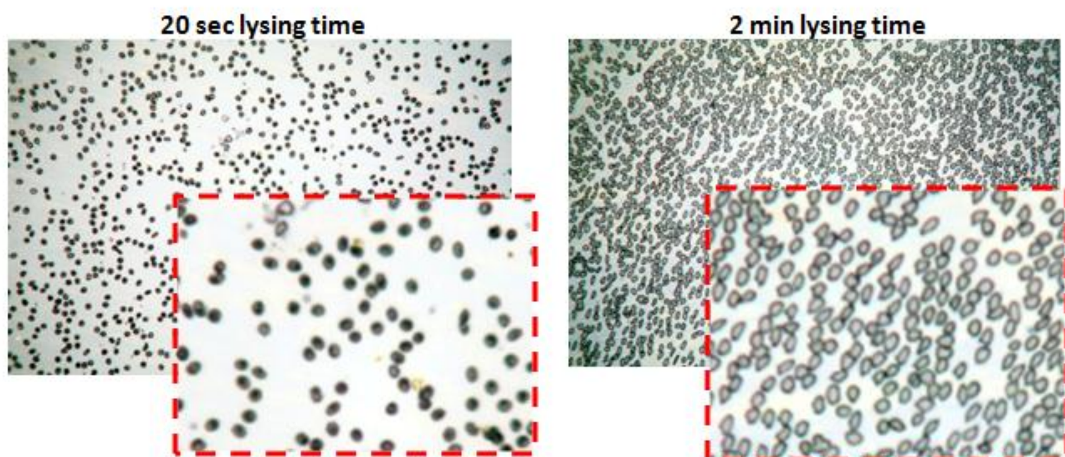

Figure 5. Blood film analysis of varying lysing time at 200X magnification. More shearing occurs with higher lysing time (20 seconds vs. 2 min).

All devices were made of polydimethylsiloxane bonded to a glass cover slip and fabricated using standard soft lithography techniques. The oscillating air-liquid interfaces create local vortices that trap red blood cells while simultaneously pumping plasma downstream effecting a net separation of cell debris from plasma. This was modeled on CFD-ACE+ using oscillating velocities that are approximately normal to arc-shaped inlets (Figure 6, top left). A snapshot of the velocity magnitudes from the simulation shows that fluid will be propelled downstream from left to right (Figure 6, 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 6, bottom left). Simulation of blood cell trapping showed qualitative agreement with experimental (Figure 6, bottom right). Increasing the velocities or increasing the time in the transient simulation increased the hemolysis index in the program.

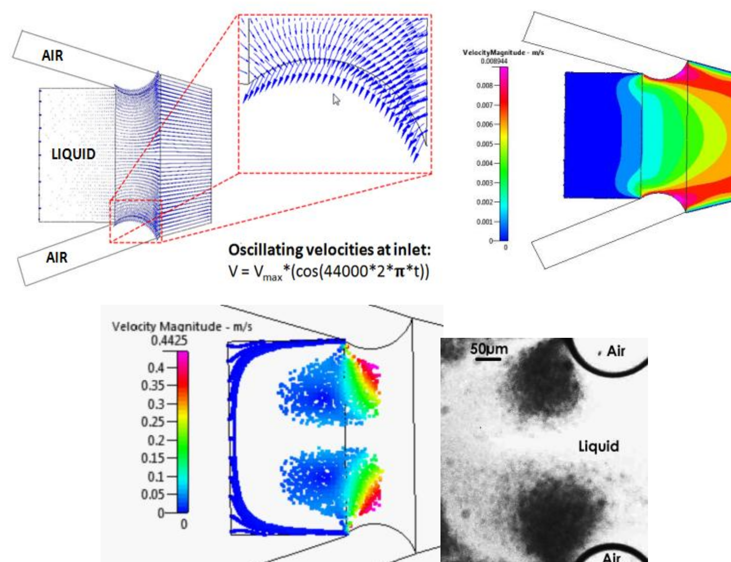

Figure 6. Computational fluid dynamic simulation of trapping using CFD-ACE+ showing qualitative agreement with the video snapshot of dynamic particles trapped in vortices.

In summary, this novel method of cell lysing and fluid flow control is tunable and durable since no moving parts are required for the actuation of the chip. Because ALCAT microstructures can be fabricated in only a single layer it is amenable to conventional manufacturing processes such as hot embossing. Although red blood cells are used in this application for the purposes of modeling, other cell types such as bacteria or white blood cells which contain nucleic acid would be likely candidates for this application. Because this technology is performed quickly and wholly on chip, it can be integrated as a sample preparation component that can be linked to downstream analyses in point-of-care diagnostics or other lab-on-chip applications.

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