RAPID QUANTITATION OF C-REACTIVE PROTEIN AGGLUTINATION WITH ACOUSTIC-ENABLED MICROVORTICES

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ABSTRACT

Agglutination assays have been developed to diagnose a host of different conditions including autoimmune disorders, sexually transmitted diseases, bacterial strain identification, and other infectious diseases. However, agglutination is often limited to qualitative analysis or semi-quantitative detection of high concentrations (mg/L) of an analyte. Here we present a microfluidic device that uses air-liquid cavity acoustic transducers (ALCATs) to quantify concentrations of C-reactive protein (CRP). Using ALCAT microstreaming, agglutination is enhanced through mixing while clumping is contained and measured in microvortices. The setup allowed for the quick detection 500 ug/L CRP which is 12 times more sensitive than the corresponding slide test.

KEYWORDS

Agglutination, C-reactive protein, point-of-care, acoustic, ultrasound, blood, diagnostic , bead assay

INTRODUCTION

In agglutination slide test cards, beads coated with antibodies will clump in the presence of the antigen solution. Conventionally, agglutination assays are qualitative because the amount of clumping is not quantified. However, light scattering (nephelometry) and light transmission (turbidimetry) techniques have been applied to quantify the clumps. The use of microscale geometry or force fields has also been exploited in agglutination assays [1]. Herein, we present a device that allows for easy quantitation of clumping at varying CRP concentrations. CRP is a general marker of inflammation or infection. This unique detection method relies on ALCATs to produce microvortices where particles get trapped.

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,cell/particle switching, and plasma separation [2][3]. Notably, ALCAT chips are passive single layer devices that are placed on top of an external acoustic transducer as the controller (Figure 1). The dynamic vortices produced by geometrically equivalent ALCATs can serve as detection sites of concentrated bead clumping. Specifically, ALCAT vortices can be used as "counters" for metering the amounts of agglutinated beads in solution. The sizes of these counters (or analogously the tick marks on a ruler) are tunable.

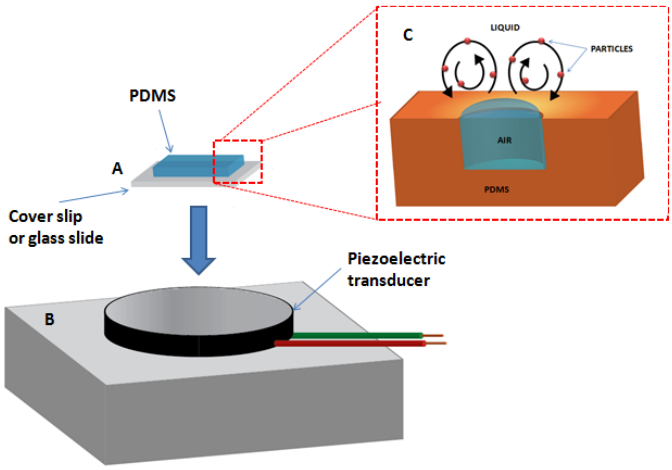

Figure 1. Experimental setup. A passive chip made of Polydymethylsiloxane-on-glass (A) is placed on a piezoelectric transducer (B). A general ALCAT structure is shown in). No external tubing or syringe pumps are required.

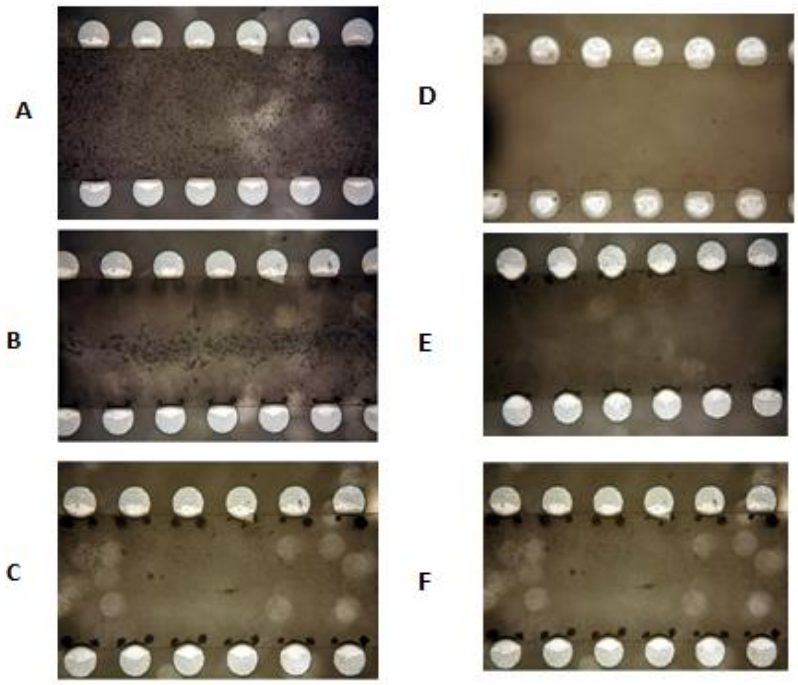

Figure 2. Dynamic formation of agglutination (A-C) for 12 mg/L CRP and comparison of clumping between varying concentrations of CRP (0, 6, 12mg/L, for D,E,F respectively.

EXPERIMENT

Reagents from a commercially available CRP agglutination kit were used. A non-reactive control, 6 mg/L CRP reactive serum, and 12 mg/L CRP of reactive serum were mixed with 1:2 dilution of the latex bead solution (~600nm beads). One microliter of each solution was then immediately (< 2 min) transferred to microfluidic chips. The chips were designed with an array of circular ALCATs lining the sides of the channels(Figure 2). ALCATs were activated at 44 kHz and 500 mV. Clumping was viewed under 50X magnification after a few minutes. Chips were made of polydimethylsiloxane bonded to a glass slides and fabricated using standard soft lithography techniques.

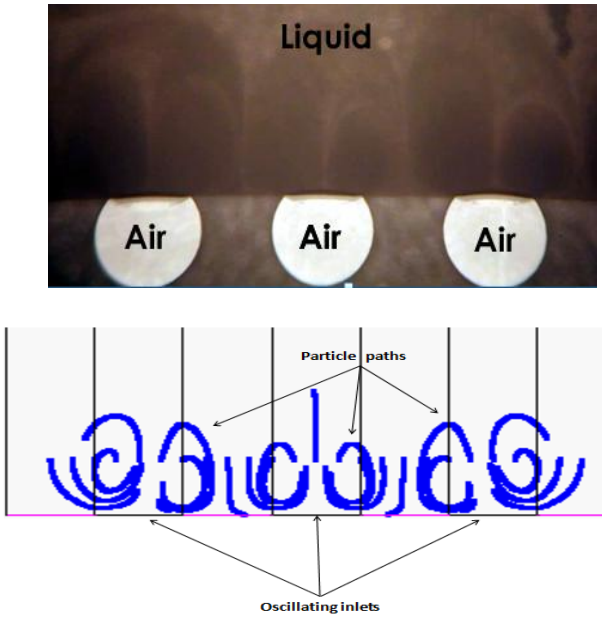

Figure 3. Top image shows a snapshot ~600nm latex beads trapped in microvortices generated by circular ALCATs. Spray particles are used to model the latex beads. Computational fluid dynamic simulation of particles trapped in vortices is shown in the bottom image. Oscillating velocities are applied to three inlets. Qualitative agreement is shown with experimental results.

Figure 2A-C, shows snapshots of the 12 mg/L CRP sample showing disperse agglutinated beads (Figure2A) before ALCAT activation. Subsequent mixing with ALCAT activation is then shown (Figure 2B). After a few minutes, large agglutinated clumps are seen swirling within microvortices (Figure2C). Figure 2 D, E, and F show differences in clumping between the varying concentrations (0, 6mg/L, 12mg/L, respectively). Clumping was visualized with concentration as low as 500 ug/L CRP which is 12 times more sensitive than the corresponding slide test. Computational fluid dynamic simulations of particles in the presence of these vortices show qualitative agreement with experimental results (Figure 3). Figures 4 and 5 show that clumping can easily be quantified with image analysis. Results show significant difference ($p<0.001$) between the average area of clumping of 6mg/L and 12mg/L CRP sera. The sum of the area of clumping per unit area is also shown to be linear in that region (Figure 5).

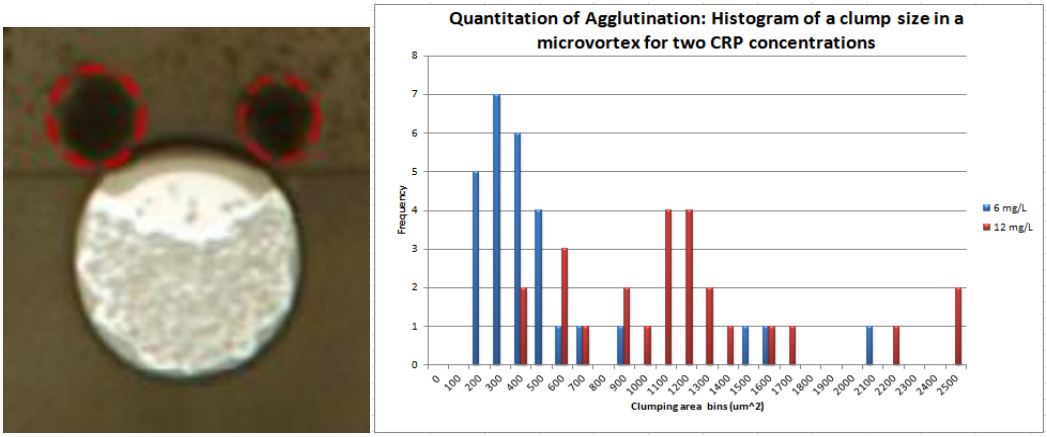

Figure 4. Quantitation of average clump area (red ellipses). The difference of mean area of clumping in a t-test assuming unequal variances is significant ($P<0.001$) where the means are 1134µm^2 (12mg/L CRP) and 482 µm^2 (6 mg/L CRP)

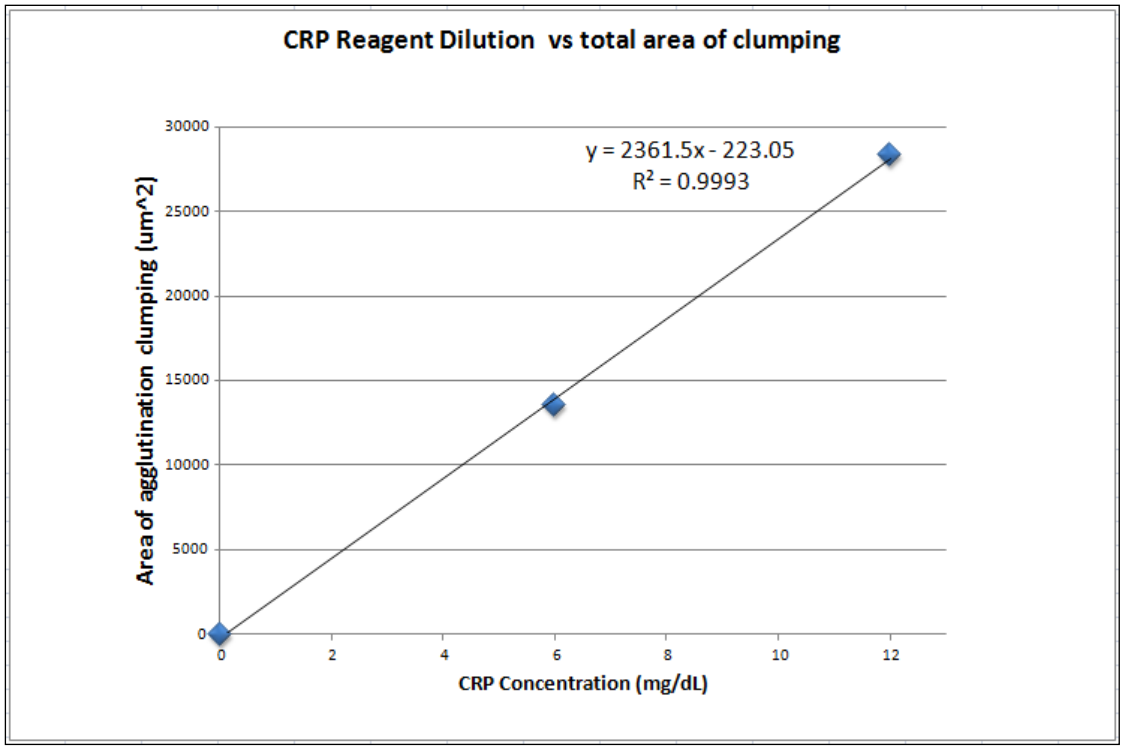

Figure 5. Total sum of clumping for varying concentrations of CRP per unit area. The sum of the area of clumping per unit area is also to be linear with the given CRP concentrations measured.

In summary, leveraging the versatile functions of ALCATs in mixing and particle trapping allowed for the quick detection of CRP at a lower limit of detection (500µg/L) compared to the slide test (6mg/L). Clinically, CRP is now used in predicting patient risk for cardiovascular disease (CVD) [4] and is considered more accurate than cholesterol data. Specifically, CRP concentrations are stratified as such:

Less than 1.0 mg/L = Low Risk for CVD
1.0 – 2.9 mg/L = Intermediate Risk for CVD
Greater than 3.0 mg/L High Risk for CVD

Hence, the CRP agglutination strategy described in this report may be used to assess patient risk to coronary problems. Furthermore, the method of analyte quantitation can be transferred to the detection of other analytes. The geometry of the ALCAT structures can be optimized to increase sensitivity over current methods. 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. This rapid detection technology can easily be integrated into point-of-care diagnostic applications.

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