CONTROL OF BLOOD'S RHEOLOGICAL PROPERTIES USING SURFACE ACOUSTIC WAVES

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ABSTRACT

Blood related diseases and bleeding are responsible for a large number of deaths globally every year. Over 1000 women die as a consequence of pregnancy related complications daily and over 50% of these deaths are related to excessive bleeding [1]. In recent years, there has been a growing interest to study blood coagulation and the development of nano-mechanical diagnostics using acoustic techniques. Here we show the manipulation of blood's rheological properties using surface acoustic waves (SAW). Contrary to the normal shear thinning behavior of blood at high shear rates [2], the application of SAWs results in shear thickening.

KEYWORDS: Blood clotting, Surface acoustic waves, Diagnostics, Rheology

INTRODUCTION

SAW devices have been extensively used in electronic filters, oscillators and transformers. Recently they have shown promise in fluid actuation [3] for microfluidics. SAW are mechanical waves of deformations on a surface, commonly generated when a high frequency signal is applied to electrodes on a piezoelectric surface, using an interdigitated transducer (IDT). When this mechanical wave interacts with a liquid placed on the solid surface, it refracts into the liquid and attenuates exponentially at a specific Rayleigh angle, linked to the speed of sound in both media Equation (1.1) and Figure 1 generating a convective streaming flow [3].

$$\sin\theta_R = \frac{c_f}{c_{SAW}} \tag{1.1}$$

Here θ_R, c_f and c_{SAW} are the Rayleigh angle, speed of sound in the fluid and solid surface (SAW) respectively.
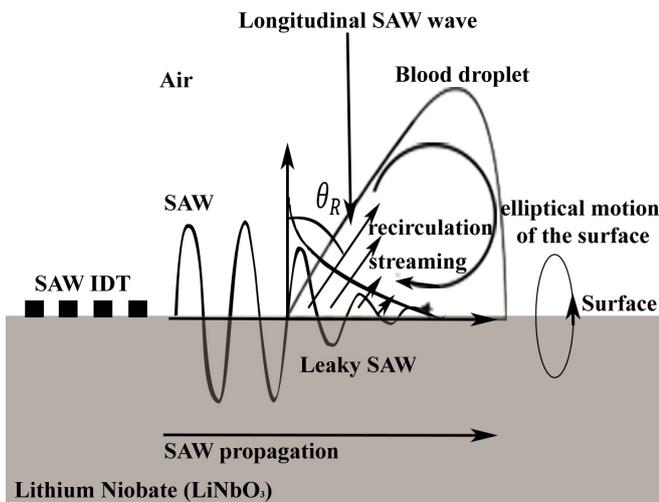

Figure 1: *Interaction of SAW interaction with a fluid leads to acoustic streaming flows and a longitudinal pressure wave in the fluid.*
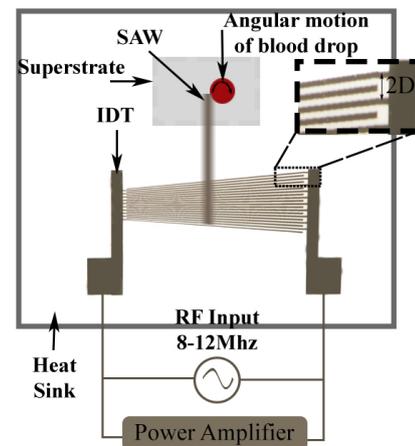

Figure 2: *Schematic of a slanted IDT and the SAW propagating into blood causing streaming. An Agilent Technologies MXG Analog Signal Generator N5181A was used in conjunction with a Mini Circuits ZHL-5W-1, 5–500 MHz amplifier with a gain of 38 dBm to actuate the SAW device. The piezoelectric surface was coupled with a heat sink to control the temperature.*

A range of ultrasonic methods have been used to study blood coagulation *in vitro*, such as high intensity focused ultrasounds (HIFU) [5], acoustic spectroscopy, attenuation, backscattering [6] and elastography. In addition to enabling the investigation of the physical phenomenon of clotting, some of these methods (e.g. HIFU) have been used to clot blood or cauterize blood vessels, using heat. Here we show the controlled solidification of a droplet of blood by the application of high shear rates to alter the physical properties of blood, which can be used to prevent bleeding. We have demonstrated that blood containing anticoagulants can be solidified in a controlled manner onto a disposable microchip by manipulating SAW, showing that this is a physical phenomenon and not one controlled by the biochemical cascade.

EXPERIMENTAL

CHIP FABRICATION AND EXPERIMENTAL SETUP

The blood clotting device comprises a piezoelectric 128° Y-cut LiNbO₃ substrate with a 20 nm Ti and 100 nm Au layer IDT. The fabrication involves spin coating of S1818 photoresist, electrode pattern transfer using UV light exposure, development using Microdev and vapor metallization followed by the acetone. Here we used a tune-able slanted IDT (Figure 2). In this design, which has a 10mm aperture length, the width of an electrode varies linearly from 85 μm to 125 μm, to enable selective and frequency controlled (8-12 Mhz) excitation on the substrate, at specific positions, leading to an asymmetric wave-blood interaction and induced angular movement of the fluid [5] as shown in Figure 1. All SAW devices were characterized using Agilent Technologies E5071C network analyzer. The SAW frequency can be calculated using Equation (1.2) and the corresponding electrode width can be calculated by Equation (1.3).

$$f_{SAW} = \frac{c_{SAW}}{\lambda} \tag{1.2}$$

$$D = \frac{\lambda}{4} \tag{1.3}$$

Parameters f_{SAW}, c_{SAW}, λ and D are the frequency of the SAW wave, speed of sound in substrate, the wavelength and electrode width respectively. A hydrophilic trap was patterned onto the SAW device with hydrophobic regions around them. Standard photolithography was used to form an organosilane film (trichloro(1H,1H,2H,2H-perfluorooctyl)silane (FOTS, Aldrich)) around a protected hydrophilic spot, which pinned the drop of blood in position to provide a controlled local excitation where the location of the SAW with respect to the drop is fixed as shown in Figure 2. The SAW were actuated by applying a sinusoidal signal using Agilent Technologies MXG Analog Signal Generator N5181A, amplified through a Mini Circuits ZHL-5W-1, 5–500 MHz power amplifier to the metallized electrodes of the IDT. To avoid the effect of heating on blood proteins, temperature was controlled by coupling the LiNbO₃ substrate to a heat sink with heat transfer compound and was monitored using an infrared camera (Fluke, Ti25) as shown in Figure 2. The highest average temperature during solidification was observed to be up to 36 °C. Screened blood samples of different blood groups with an anticoagulant agent (Heparin or EDTA) were stored at 4 ºC upon receipt and discarded after a week.

RESULTS AND DISCUSSION

Solidification of the blood causes changes in its mechanical properties, such as viscosity. SAW of 8-12 MHz were applied to whole blood samples of volumes 2-8 μl. Change in the blood viscosity was observed through imaging and an aggregation of RBCs was investigated microscopically. Figure 3a shows the behavior of a 4μl drop of whole blood immediately after the SAW was applied (8.83 Mhz) until a solid layer of aggregated blood was observed, after 7.3 s. We have used a light scattering effect in order to quantify this solidification. A light ring was focused on the blood drop. The blood experienced oscillations of its surface with air with the application of SAW, which are observed as oscillation of the white illumination ring.
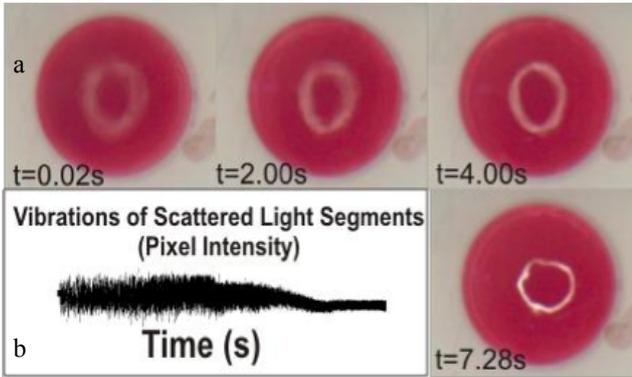

Figure 3: *(a) Effect of SAW on a 4-μl blood drop at various times after the actuation. The streaming induces high shear rates in the drop, causing the red blood cells (RBCs) to aggregate together resulting in the blood having an increased viscosity locally. (b) A cross-section of a line passing through all the 364 images (7.28 s) of solidification process was observed by a YZ orthogonal stack of the scattered light ring in time domain.*
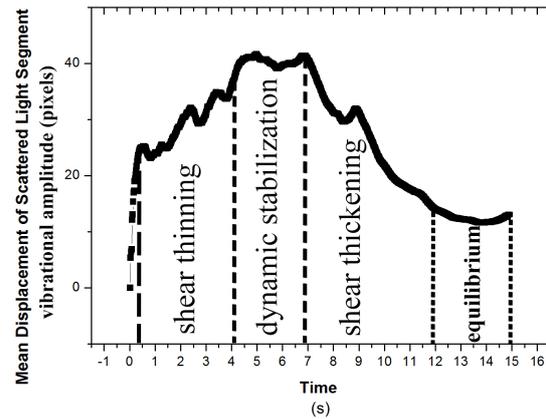

Figure 4: *Shows the mean displacement of scattered light segment that was obtained from calculating the average width of each frame of scattered light segment acquired from Fig 3(b). The vibrations indicate blood viscoelastic behavior changing from initial shear thinning as the displacement becomes larger over a similar period of time, to shear-thickening, when the displacement reduces and finally solidifying. A 4μl droplet at 8.83Mhz, 15.13 mW input power was used.*

With the changes in blood viscosity, the amplitude of these oscillations decreased until a steady state was reached. The images of the whole experiment were captured with a Jenoptik Progres CF camera (50fps) using Leica MZ12 microscope

and Leica CLS 150X optical fiber light ring source. These images were analyzed with an image processing software: Image J. The recorded images were converted into an 8-bit greyscale stack with 256 pixel intensity values. In order to analyze the scattered light displacement and hence the viscosity, the image stack was converted into orthogonal view mode in Image J which gave the scattered light oscillations of a cross-section of a line passing through the image stack, representing the time domain. Figure 3b shows the YZ orthogonal segment of the reflected light from the blood where each vibration corresponds to a mean displacement of the scattered light. As the blood solidifies, the amplitude of this mean displacement reduces. Further analysis of this solid thin film formation leads to Figure 4 where we have categorized the drop behavior into four regimes in time: a shear-thinning phase; dynamic stabilization phase; shear thickening phase; and finally the equilibrium phase. The shear-thickening phase is of interest as it is contrary to the conventional thixotropic behavior of the blood, which is linked to its ability to flow through small capillaries [7]. The blood viscosity is dependent on a series of factors such as the RBC concentration, internal RBC viscosity, or plasma viscosity [7]. Microscopic observations (Figure 5) show that this phenomenon is most likely due to physical RBC aggregation and the effect of internal viscosity of RBCs. This is further evidenced by the fact that no change in viscosity was observed when using plasma with same experimental conditions.
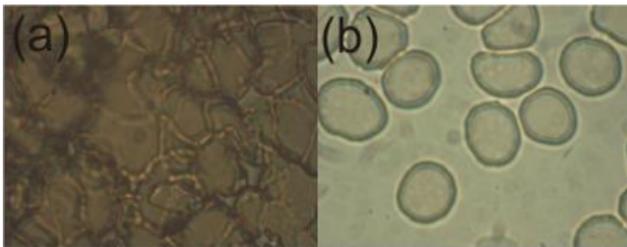

Figure 5: *(a) shows 100x magnification of a film of blood cells. Blood cells collected and dispensed on a glass slide, and spread using a coverslip in a technique akin to a smear. (a) After solidification where it can be seen that RBCs have aggregated together, compared to a control (no SAW) (b).*
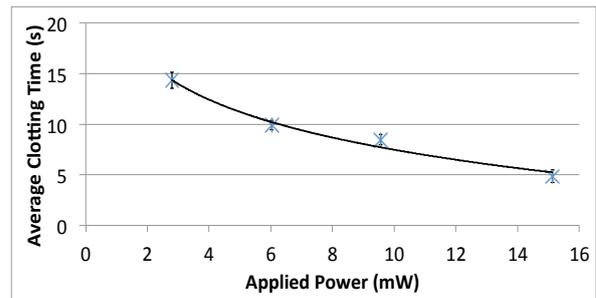

Figure 6: *Time taken for a 4μl droplet at 8.83Mhz to achieve equilibrium (as described in Fig 3&4) for different input powers (2.8-15.1 mW). As the power increases, the solidification becomes faster, reaching ca 5s at 15.1 mW.*

The fact that we only observe a solid film at the interface can possibly be due to the shearing profile of the blood caused by SAW streaming. It means that shear rate is lowest at the middle (vortex center) and highest at the boundary or the wall. This rate of solidification of blood increase with increasing applied power, Figure 6.

CONCLUSION

This device demonstrates the controlled manipulation of blood's physical properties in real-time. Importantly, it could be implemented on a disposable microchip (superstrate) coupled to the piezoelectric surface [8], to provide a low-cost low power technique. This opens up a wide range of opportunities to develop therapeutic devices that can possibly help patients suffering complications during childbirth or for those with blood disorders such as hemophilia.

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