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Using nano-mechanics and surface acoustic wave (SAW) for disease monitoring and diagnostics at a cellular level in red blood cells

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Abstract

A popular approach to monitoring diseases and their diagnosis is through biological, pathological or immunological characterization. However, at a cellular level progression of certain diseases manifests itself through mechanical effects as well. Here, we present a method which exploits localised flow; surface acoustic wave (SAW) induced acoustic streaming in a 9 μ L droplet to characterize the adhesive properties of red blood cells (healthy, glutaraldehyde treated and malaria infected) in approximately 50 seconds. Our results show a 79% difference in cell mobilization between healthy malaria infected RBCs (and a 39% difference between healthy and treated ones), indicating that the method can serve as a platform for rapid clinical diagnosis; where separation of two or more different cell populations in a mixed solution is desirable. It can also act as a key biomarker for monitoring some diseases offering quantitative measures of disease progression and response to therapy.

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1. Introduction and Background

Studies have shown that elastic as well as mechano-transduction properties are significantly different between healthy and diseased cells ?? . Malaria for example, is an infection of human red blood cells (RBCs) that is caused by parasites of Plasmodium species, ?? . Malaria infected cells are known to display a number of adhesion proteins and also experience increased membrane stiffness throughout the various stages of infection ?? , ? . For example, local adhesion sites are formed and marked by the distinct protrusions and bulges developed in the trophozoite and schizont phase ? . These adhesive and structural changes in RBCs could in fact be used to monitor diseases.

Centrifugation, micropipette aspiration and optical tweezers are some techniques that are currently being used to investigate the adhesive properties of cells ??? . The emergence of utilizing a microfluidic approach is becoming increasingly popular in the investigation of the conditions influencing shear-stress dependent RBC adhesion to various cell lines. Earlier work by research groups such as ? , ? , ? and ? , have predominantly focussed on RBC testing under

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static conditions, which successfully provides insight into the molecular mechanisms driving cell adhesion to endothelial cells and vasculature. However, in reality, whether infected or healthy, RBCs are subjected to hydrodynamic blood flow forces ???. Models of these are essential as they enable us to gain an understanding of the physiological impact cell adhesion interactions have in a pathogenic state of a given disease.

Presently the traditional technique of diagnosing malaria involves microscopic examination of a stained blood smear. Although this method is widely used, it is faced with some challenges such as restricted throughput, qualified personnel to perform tests and reduced sensitivity when examining mixed infections or early stages of invasion. Other methods including polymerase chain reaction (PCR), enzyme assays and immunological tests can aid in the previous challenges faced by the gold standard method, however, the later techniques also face limitations such as relatively high costs and well equipped laboratories ? . However, given the remote settings where the disease is prevalent, there is an unmet need for rapid, low cost diagnostic techniques that do not require highly skilled personnel.

Here, we demonstrate a potential method to monitor red blood cell adhesion/de-adhesion using very small fluid samples, delivering results in under one minute, and employing easy-to-use transparent chips that are similar in appearance to conventional microscope slides (Fig.1 a). Specifically, we explore surface acoustic wave (SAW) actuation and SAW induced flows to selectively peel different red blood cell populations off a rigid substrate (Fig 1 a) and separate them based on their adhesive properties. SAWs are analogous to an earthquake loading, though created on an mm-sized chip, and its actuation mechanisms are very well established ?? . SAWs belong to the subset of ultrasonic waves ?? and there mechanisms can be exploited and used for particle manipulation ?, mixing, sorting ?? , droplet generation ? .

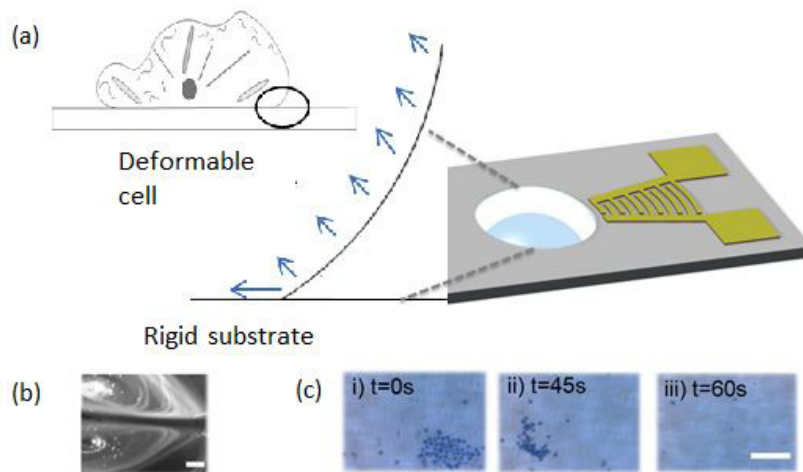


Fig. 1. (a) An acoustic wave propagating along the surface of the substrate, induces acoustic streaming and causes cells to be peeled off. (b) Acoustic streaming effects produced by $7\mu\text{m}$ fluorescent particles on a concentrically focused $30\mu\text{m}$ SAW device at 55 mW. (c) Demonstration of the principle visualising detachment of COS-7 cells (i) Cells at rest having settled for 20 min. (ii)-(iii) Application of a 300 mV AC signal generates acoustic streaming within the droplet, resulting in cell motion from local shear forces acting at the cell/fluid interface. Scale bar is $200\mu\text{m}$.

SAW mechanisms: An electromechanical displacement is induced in the piezoelectric substrate after an AC signal is supplied across the transducers at the resonant frequency of the device. In turn, longitudinal waves (SAW) are created and propagate along the surface of the substrate. If this substrate surface is partially submerged by a droplet of fluid, the generated surface wave interacts with the fluid, and due to spatial variations in the pressure field, gives rise to rapid flow circulation patterns called acoustic streaming. This is illustrated in Fig. 1(b), showing the motion of suspended 7-micron particles in a droplet. The rapidly generated flows impart localised energy (in terms of shear forces) onto the substrate, and where cells are adhered to the surface this fluid motion can be utilized to peel a cell population with particular properties off the surface. This behaviour is demonstrated in a preliminary study (Fig.1c), whereupon Cos-7, monkey kidney cells initially adhered to the piezoelectric surface, was removed after application of a fixed amplitude SAW over time. Here, we exploit SAW induced acoustic streaming further to accurately determine the shear stresses required to peel a population of cells (determining their adhesive strength) and selectively peeling a specific type of cell over another.

2. Methodology and Results

Our prototype SAW device was prepared at Melbourne Centre for Nanofabrication (MCN) cleanrooms, using a combination of photolithography and metal evaporation steps. The device consist of a series of concentrically focussed, gold metal interdigital transducers (IDTs) arrayed on a piezoelectric substrate; lithium niobate (LN).

For experimental characterization, healthy RBCs were retrieved from human donors with consent. A portion of the cells was left untreated, another was treated with a chemical reagent called glutaraldehyde and a third set was infected with Malaria parasites (in 4% haematocrit). To enhance visualisation, all cells were treated with 0.125ml of staining reagent Giemsa. A uniform blood sample of $9\mu\text{L}$ was then placed onto the focal region of the IDT and allowed to sediment for approximately 15 minutes. To validate our proof of concept, experiments were performed for 100 seconds using high-frequency, 132MHz, continuous SAW excitation to study the detachment and rolling behaviour of the healthy, treated and malaria-infected red blood cells. In addition, a Laser Doppler Vibrometer (UHF-120, Polytec, Dexter, MI) was utilized to determine the relationship between power input (W), wave amplitudes and substrate velocities. Amplitudes were measured $100\mu\text{m}$ away from the edge of the last finger of the IDT ($30\mu\text{m}$) and as anticipated, the IDT axis of symmetry generated the greatest wave amplitudes.

Fig.2 illustrates the varying detachment rates for the healthy, treated, and malaria-infected RBCs subjected to SAW- generated acoustic streaming, obtained separately for each group. Initially, with the onset of streaming, a rapid decline in the percentage of RBCs remaining on the substrate is evident. After 30 seconds of excitation the percentage of RBCs (healthy, treated, malaria-infected) remaining on the substrate were 85%, 60%, 9% respectively (Fig.2). The short time scale arises due to the use of focussed SAWs, and an open droplet system. Our open droplet system enables a decrease in the velocity-reducing effect of no-slip boundaries, and in turn, allowing the almost complete removal of healthy cells in approximately 30 seconds.

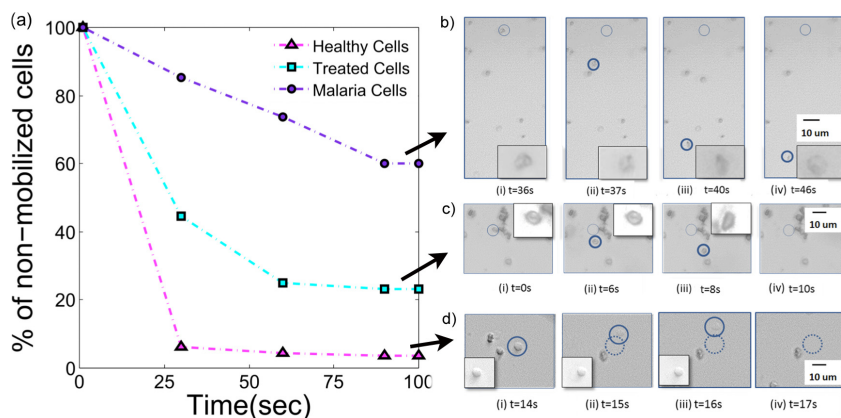


Fig. 2. Percentage of RBCs (malaria-infected, GA-treated, healthy) remaining in a $9\mu\text{L}$ -droplet as a result of a 500 mV AC signal applied for 100 seconds.(b)-(d): Detachment behaviour of various cells (500mV) where a dashed circle indicates cells original position and bold circle tracks the cell over time.

3. Conclusions

In summary, the large percentage differences obtained between same kind of cells treated differently (indicating almost complete removal of healthy cells, versus removal of a low percentage of malaria cells) highlights the potential offered by this technique in rapid diagnostic applications, which require the separation of two or more cell populations. Secondly, the method will enable us to analyse the detachment and migration of individual cells. Though longer exposure times result in increased numbers of detached cells in all cases, unique detachment behaviours were distinct for each of the three cell populations (Fig. 2 b-d). Considering the findings from this study, the proposed method can act as a key biomarker for monitoring some diseases, in addition to measuring cellular mechano-transduction properties.