APPLYING A MICROFLUIDIC ‘DEFORMABILITY CYTOMETRY’ TO MEASURE STIFFNESS OF MALARIA-INFECTED RED BLOOD CELLS AT BODY AND FEBRILE TEMPERATURES

Sha Huang1, Hansen Bow1, Monica Diez-Silva2, Jongyoon Han1,3

1Department of Electrical Engineering and Computer Science, 
2Department of Material Science and Engineering, and 
3Department of Biological Engineering
Massachusetts Institute of Technology, Cambridge, MA, USA

ABSTRACT
The deformability of human Red Blood Cells (RBCs) during thermal transition between room and febrile temperatures is investigated by passing RBCs through microfluidic bottlenecks for deformability cytometry. RBC deformability increases from 23°C to 37°C and decreases slightly from 37°C to 40°C due to alterations in the membrane proteins. Similar experiments conducted for ring-stage malaria-infected RBCs show that infected RBCs are stiffer than healthy RBCs at all temperatures, but the difference in stiffness between healthy and infected cells is maximized around 40°C. This stiffening could be due to the attachment of ring-infected erythrocyte surface antigen (RESA) to spectrin network.

KEYWORDS: microfluidic, malaria, temperature, deformability, red blood cell (RBC).

INTRODUCTION
Decreased red blood cell (RBC) deformability is both a cause of and biomarker for potentially severe diseases such as malaria. Since RBCs have to undergo repeated deformations when they traverse blood capillaries and splenic sinusoids, even minute increase in cell stiffness would lead to severe pathological conditions. The most virulent malaria parasite, Plasmodium falciparum, invades a RBC and exports proteins that stabilize the host spectrin network, stiffening the host cell [1]. While this stiffening is believed to be necessary for the structural integrity of infected RBCs at fever temperature, it also facilitates splenic clearance. Therefore, a quantitative understanding of this mechanical modification would be desirable, both from the perspectives of malaria pathophysiology and drug development.

Previously, characterization of individual RBCs has been accomplished in several ways, including micropipette aspiration, atomic force microscopy [2], optical stretching [3], and microfluidic pores [4]. However, many of these methods were of low throughput and unable to provide population-wide single cell data. In microfluidic pores, cell-to-cell interactions and arbitrary designs obscured quantitative measurements.

Here we present a microfluidic ‘deformability cytometer’ that measures the dynamic mechanical responses of RBCs. The much higher throughput compared to conventional single cell measurement enables us to measure statistically significant differences in deformability among inherently heterogeneous cell populations. In particular, the effect of temperature on cell deformability is investigated.

THEORY
A typical human RBC is of disk shape with 6–8µm in diameter and 2µm in thickness. Since the minimum viable human capillary diameter is around 4µm, RBCs have to deform substantially in order to squeeze through small capillaries. The architecture of our PDMS microfluidic device (Fig. 1) is designed to mimic blood vessel capillaries, with channel depth of 4.2µm and minimum channel gap of 3µm. Controlled by constant pressure gradient, RBCs travel through constrictions under low Reynolds number fluid regime and their dynamic deformability is extracted from their migration velocity [5]. Fluorescence measurements on each RBC are simultaneously acquired, resulting in a population-based correlation between biochemical properties and dynamic mechanical deformability.

The effect of temperature on cell biomechanical properties has often been neglected in many single-cell measurements. However, recent optical tweezers experiment reported that at febrile temperature, malaria-infected RBCs become a few fold stiffer compared to at body temperature [1]. Understanding the specific role of temperature in the context of both malaria-infected RBCs and RBCs in general could be therefore crucial in gaining deeper insights of the malaria pathophysiology and many other disease states. In this presentation, a quantitative analysis is presented to illustrate the temperature-dependency of the dynamic deformability of both healthy and malaria-infected RBCs.

Fig.1.A. Schematic of the set up for live-cell measurements. B. Illustration of the device design. C. Zoomed in view of the dimensions of the constriction.
EXPERIMENTAL

A silicon wafer mold was first made using photolithography and reactive-ion etching techniques. A 5x reduction step-and-repeat projection stepper (Nikon NSR2006i9, Nikon Precision) was used for patterning. The device shown in fig.1.B was molded from the wafer master using standard PDMS casting protocols and bonded to a glass slide.

P.falciparum were cultured in Leukocyte-free human RBCs (Research Blood Components, Brighton, MA) under an atmosphere of 5% O2, 5% CO2 and 95% N2, at 5% hematocrit in RPMI culture medium 1640 (Gibco Life Technologies) supplemented with 25mM HEPES (Sigma), 200mM Hypoxanthine(Sigma), 0.20% MaHCO3 (Sigma) and 0.25% Albumax II (Gibco Life Technologies).

The schematic of experimental set up for live cell measurements is shown in Fig.1.A. Phosphate-buffered saline (PBS) 1x was mixed with 0.2% w/v Pluronic F-108 (BASF, Mount Olive, NJ) as stock solution. Diluted by the stock solution, RBCs enter the inlet of the PDMS device driven by hydraulic pressure difference. Accurate temperature control is achieved by the heating chamber (Olympus).

In experiments involving only healthy RBCs, 1µl of whole blood (Research Blood Components, Brighton, MA) and 1µl of 50µg/ml of Cell Tracker Orange (Invitrogen, Carlsbad, CA) were mixed with 98µl of stock solution and allowed to sit for 30 minutes. All RBCs were seen as fluorescent cells under the microscope. In experiments involving cultured parasites, 1µl of ~50% hematocrit blood and 10µl of 1x10-6 M of thiazole orange dye were mixed with 100µl of stock solution. The malaria-infected cells appear as shadows with a small fluorescent circle inside as shown in Fig. 2 whereas the uninfected cells were seen as black shadows in the background.

RESULTS AND DISCUSSION

The deformability of healthy RBCs measured between room temperature (25°C) and febrile temperature (40°C) is presented in Figure 3. Between 25°C and normal body temperature (37°C), RBCs become statistically more deformable with increasing temperature. This increase can be attributed to factors including the temperature dependent reduction in buffer solution viscosity, elastomeric transition of hemoglobin and membrane proteins, the gradual drop in membrane viscosity, and the decrease in intracellular fluid viscosity [6]. From 37°C to 40°C, the RBCs decrease in deformability, which is probably caused by alterations in membrane proteins, such as spectrin [7]. This deformability shift was reversible, and not due to permanent heat-induced damages on RBCs.

The influence of spectrin network on erythrocyte deformability was further evaluated by repeating the experiment after one hour thermal denaturation of erythrocyte spectrin. The subsequent cell velocity measurements indicate a dramatic drop in the deformability of RBCs at febrile temperature. This result suggests that, while spectrin may not play a dominate role at the temperature range below 37°C, it does influence the deformability of RBCs remarkably especially at febrile temperature. In summary, the current study provides a deeper insight on the temperature dependency of the biome-
mechanical properties of RBCs. It further suggests that the intracellular components of RBCs may be optimized for maximum deformability at body temperature of 37 °C.

Similar experiments are conducted for malaria-infected RBCs (Fig. 4), with ring-stage malaria-infected RBCs being stiffer than uninfected RBCs at all temperatures. Interestingly, the difference in the stiffness between normal and infected RBCs was enhanced around 40°C, which corresponds to human fever temperature. This stiffening may be caused by the attachment of ring-infected erythrocyte surface antigen (RESA) to spectrin [8]. RESA constrains the cytoskeletal structure of infected RBCs by preventing spectrin from heat-induced conformational changes therefore increasing the survivability of infected RBCs at fever conditions. Yet, this stiffening may facilitate the splenic clearance of infected RBCs from the circulation, which suggests a role for fever temperature in combating malaria infection. We believe our microfluidic system may be useful in developing and testing new chemical compounds (anti-malarial drugs), which assist more efficient splenic clearance by artificially stiffen infected RBCs.

**CONCLUSION**

A high-throughput microfluidic deformability cytometry has been developed, which was used to measure the temperature dependent RBC deformability. Current study provides deeper insight on the temperature dependency of RBC biomechanical properties. It suggests that the intracellular components of RBCs may be optimized for maximum deformability at the body temperature of 37 °C. Similar experiments with malaria-infected RBCs at different temperatures indicate that the difference in the stiffness between normal and infected RBCs may be enhanced around febrile temperature. This may be potentially useful in both malaria disease diagnostics and drug efficacy screening.

**ACKNOWLEDGEMENTS**

This work was supported by Interdisciplinary Research Group on Infectious Diseases and BioSYM, which are funded by the Singapore-MIT Alliance for Research and Technology (SMART) and from the National Institutes of Healthy (Grants R01 HL094270-01A1 and 1-R01-GM076689-01). This work was carried out in part through the use of MIT’s Microsystems Technology Laboratories.

**REFERENCES**


**CONTACT**

*Jongyoon Han, tel: +1-617-253-2290; jyhan@mit.edu*