MICROFLUIDIC DEVICE FOR MEASURING THE DEFORMABILITY OF RED CELLS PARASITIZED BY *PLASMODIUM FALCIPARUM*

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ABSTRACT

Central to the pathology of *falciparum* malaria is a reduction in the deformability of the infected red blood cells (RBCs). To enable improved methods to study this disease, we developed a microfluidic device to measure the deformability of infected cells. Individual RBCs are first infused into a series of funnel shaped constrictions. The deformability of individual cells is measured using the pressure required to deform each cell through a constriction. Using this device, we found that parasitized RBCs from ring through schizont stages show distinct deformability distributions with mean values that are 1.5 to 200 times stiffer than uninfected cells.

KEYWORDS

Malaria, *Plasmodium falciparum,* red blood cells (RBCs), deformability/rigidity, and cortical tension

INTRODUCTION

Plasmodium falciparum is the most common species of parasites that cause malaria in humans, and is responsible for ~1 million deaths worldwide every year. A key characteristic of *falciparum* malaria is a decrease in the deformability of infected RBCs, which disrupts normal blood circulation and causes infected cells to accumulate in the microvasculature of vital organs [1]. Consequently, the mechanical deformability of infected RBCs could potentially serve as an important biomarker to investigate the mechanism of infection and patient response to treatments [2].

Recently we developed a microfluidic device to investigate the reduction of RBC deformability in *falciparum* malaria [3]. Individual uninfected or infected RBCs are deformed through micrometer scale tapered constrictions with minimum openings (pore size) ranging from 5 down to 2 µm using precisely controlled hydrodynamic pressure. The threshold pressure required to deform each cell through the funnel constriction is measured and used to determine their intrinsic deformability. Compared with traditional techniques, such as micropipette aspiration [4] and optical tweezers [5], our technique requires significantly less technical skill and specialized equipment, yet yields results with a similar level of precision and sensitivity. Compared with existing microfluidic devices for studying infected RBCs based on capillary obstruction [6], wedging in tapered constrictions [7], and transit time through constrictions [8], our technique is able to produce a more sensitive output with greater discrimination across different stages of infection.

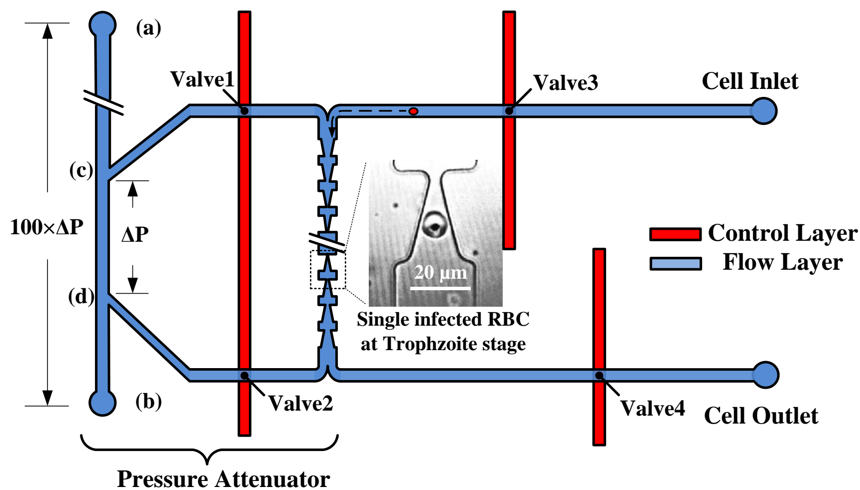

Figure 1 Microfuidic device to measure the rigidity of single infected RBC by P. falciparum using precise and minute pressure and microscopic tapered funnels

DESIGN AND THEORY

Our microfluidic device is composed of two layer PDMS with flow and control layers fabricated using multi-layer soft lithography as illustrated in Figure 1. The device is composed of a long channel spanning from point (a) to (b) with an array of funnels branching out from point (c) and (d). The length of (c) and (d) is 1/100 of the length of the long channel from (a) to (b). This arrangement constitutes a pressure attenuator where the pressure across (c) and (d) is attenuated by a factor of 1/100 of the pressure applied across (a) and (b). Connected to the funnel array is the cell inlet and outlet. The process of infusing test cells into the funnel constrictions is separated from the pressure measurement using membrane microvalves. Specifically, the measurement procedure involves initially introducing single RBCs into the funnel region with valve 1 and 2 closed. Once a cell arrives in the funnel

region, valves 3 and 4 are closed at the same time as valve 1 and 2 are opened. In this configuration, external pressure applied across (a) and (b) is attenuated and applied across the funnel array, which deform individual RBCs through each funnel constriction. Micrographs of the deformation process for a single parasitized cell are shown in Figure 2A. Specifically, each tested RBC is flowed to the mouth of the funnel till it comes in contact with the funnel. The pressure on the cell is then slowly increased until it successfully transits through the constriction.

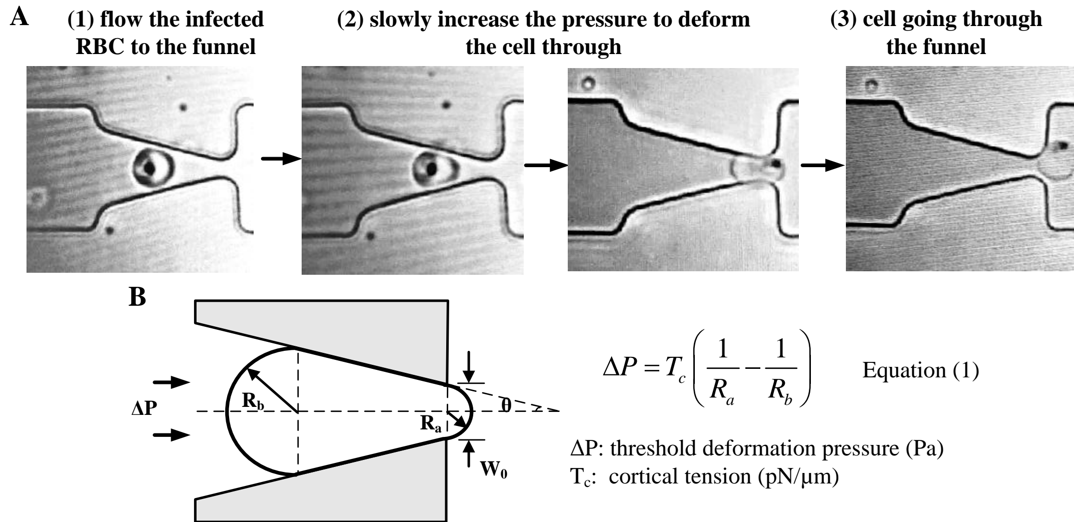

Figure 2 (A) A single parasitized RBCs at the trophozoite stage being deformed through a funnel constriction using precisely controlled pressure; (B) Geometrical model of a single RBC at the critical point before transiting through a funnel constriction, where its intrinsic deformability can be determined using the Young-Laplace Law

Unparasitized RBCs and parasitized RBCs at ring and trophozoite stages are measured using funnel constrictions with a lateral opening (W_0) of 2 to 3 μm constrictions. Parasitized RBCs at the schizont stage are measured using funnels with lateral openings of 3 μm or larger funnel. The height of the funnel constrictions is selected to be 4.1 ± 0.1 μm in order to constrain normal RBCs in the planar configuration while allowing the passage of parasitized cells that can take on a more spherical shape. A device thickness of less than 3.0 μm was found to exclude some infected RBCs from entering the funnel array.

The deformation of single RBCs can be modeled using the liquid drop model, where intrinsic deformability of each cell can be represented using the cortical tension (T_c) and determined using the Young-Laplace law as shown in Figure 2B. As each cell deforms through the funnel with gradually increasing pressure, an instability occurs when the radius of the emerging surface becomes equal to one-half of the lateral opening (*i.e.* $R_a = W_0/2$), and the cell rapidly transits through the funnel without further increases in pressure [9]. At this point, the cortical tension can be determined from the geometrical constraints at this critical point and the threshold pressure using equation (1).

RESULTS AND DISCUSSION

The cortical tension values of various stages of parasitized red blood cells from an *in vitro* culture of *P. falciparum* parasites are shown in Figure 3A and 3B. The results from each stage (except schizont) showed approximately normal distributions with mean and standard deviation of 3.39 ± 0.54 (unexposed), 3.19 ± 0.42 (uninfected, but exposed), 4.66 ± 1.15 (rings), 8.26 ± 2.84 (early trophozoites), and 21.38 ± 5.81 (mid-late trophozoites). The measured cortical tension of schizont stage cells varied from 85 to 1300 pN/μm.

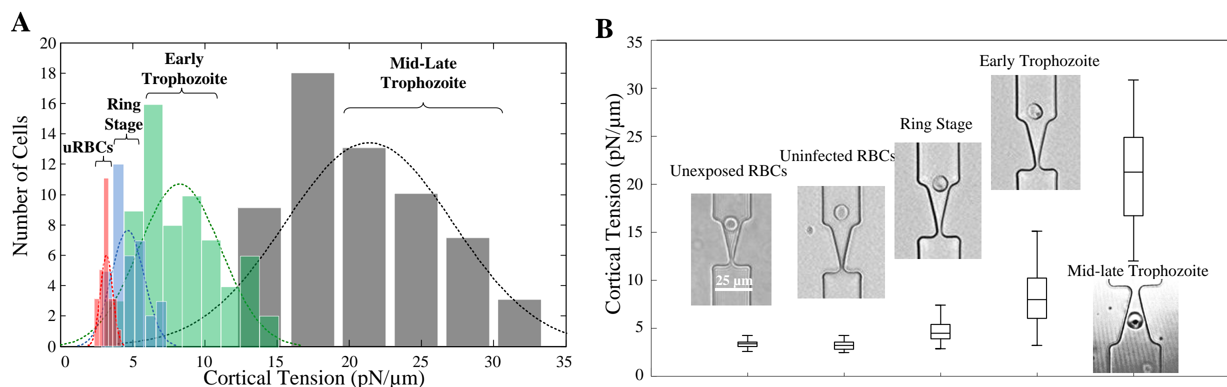

Figure 3 (A) histogram of cortical tension values of parasitized red cells at different stages of infection; (B) boxplot of cortical tension values of red cells at different conditions and stages of infection, with microscopic images of corresponding condition or stages of cell being deformed through the funnel constriction.

Comparing our results with results obtained in optical tweezers studies [5, 10], we measured significantly higher relative stiffness for trophozoite and schizont stage cells, and lower relative stiffness for ring stage cells. Specifically, we found trophozoite and schizont stage parasites to be ~7 and ~200 times more rigid than uninfected cells, whereas optical tweezers studies found these cells to be ~4 and ~10 time stiffer, respectively. On the other hand, we found ring stage parasites to be ~1.5 times stiffer than uninfected cells, whereas optical tweezers studies found these cells to be ~3 times stiffer. These differences can be likely explained based on the nature of the mechanical constraint associated with each measurement method. Optical tweezers experiments measure the resistance to tensile stretching applied to a portion of the cell membrane, while our technique deforms the test cell as a whole consisting of both cytoplasm and membrane. In ring stage cells, the modification of the cell membrane caused by proteins exported by the parasite has been shown to be a dominant stiffening factor [11] and hence, a greater mechanical change can be detected using optical tweezers. In trophozoite and schizont stage cells, the dominant stiffening mechanism is the presence of parasite-derived structures occupying the cytosol. Consequently, a greater mechanical change is measured using our technique. For biophysical studies, our method presents closer resemblance of the mechanics of circulation and blood vessel occlusion. Additionally, it is interesting to note that we observed no difference in deformability between unexposed RBCs and uninfected RBCs as shown in Figure 3B, which contradicts previous findings obtained using optical tweezers. Compared with previous biomechanical measurements of parasitized RBCs performed using micropipette aspiration [4], as well as other microfluidic techniques [6-8], our technique offers significantly enhanced signal, where the mean deformability of uninfected and ring stage parasites are clearly separated.

To further analyze our results, we use the coefficient of variation (CV) to evaluate the variability of the cell deformability measurements. The CV for parasitized cells from ring to trophozoite stages varies from 0.13 to 0.27 while the CV for the schizont stage is 0.94. The greater variation in cell deformability at advanced stages of infection likely results from the unstructured morphological changes associated with the replication of the merozoites from the host cells.

CONCLUSION

In summary, we developed a microfluidic technique to measure the decrease in deformability of parasitized RBCs based on the pressure required to squeeze them through micrometer constrictions. We showed that the deformability distributions of uninfected and infected RBCs at various stages of infection are clearly separated from one another. These results indicate the potential of using this technique to identify malaria infected cells without optical verification, as well as studying the effect of potential drugs.

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