Microfluidic Platform for Detecting Malaria Infected Avian Red Blood Cells

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Summary: A microfluidic platform is designed, fabricated, tested, and demonstrated that the unique surface morphologies of avian red blood cells (aRBCs) infected by the malaria parasite *Plasmodium gallinaceum* enables the cells to interact with the roughened surface and become immobilized inside the microfluidic channels. It is also found that a roughened glass substrate with ten-point-height larger than the depth of surface lesions and furrow-like structures of malaria infected aRBCs (miaRBCs) shows a substantial enhancement of immobilizing infected aRBCs. These findings indicate that surface morphologies, including surface lesions and furrow-like structures, can serve as alternative biomarkers for malaria diagnosis in addition to the elevated stiffness and cyto-adhesion characteristics.

In recent years, advances in research tools of cell mechanics enabled the studies of mechanical differences between normal RBCs and malaria infected ones [1]. It was found that malaria infected human RBCs lose their biconcave shape, become more spherical, and exhibit elevated stiffness by up to ten times that of healthy ones. Furthermore, knob-like protrusions are formed on the cell surfaces starting at the trophozoite stage in the life cycle of the parasite and cause severed sequestration characteristics, especially for the *P. falciparum* stain [2]. These protrusions mediate the cyto-adhesion behavior of infected human RBCs to vascular endothelium, which makes the cells sticky [3]. These findings suggested that at least three mechanical biomarkers could potentially be used to diagnose malaria: (a) elevated stiffness of the cell body, (b) altered cell morphology, and (c) cyto-adhesive characteristics of the cell surface.

In this paper, we report on the preliminary experimental findings that the surface morphological changes of RBCs due to malaria infection could be a biomarker for diagnosing malaria. In particular, it was found that the *P. gallinaceum* infected avian RBCs also lose their oval shape and exhibit modified surface morphology [4]. *P. gallinaceum* parasite leaves a visible lesion on the cell surface after invasion, and it also starts to form furrow-like structures on the cell surface at the early trophozoite stage. These features are uniformly distributed on the cell surface and persist in the remaining cycle with identical density and distribution. The dimensions taken from AFM of this furrow-like structure are around 57.3 nm in width, 7.6 nm in depth, and between 225 nm to 750 nm in length [4]. To verify that the unique surface morphology of infected RBCs can also serve as a biomarker, a microfluidic device that could control wall shear rate and total screen volume were developed and tested for functionality and efficacy.

A microfluidic platform with a long and narrow channel was designed to study the feasibility of using the surface morphology of miaRBCs as the biomarker for malaria diagnostics. The basic design concept is illustrated in Fig. 1a. The diagnostic channel is 11 mm long, 340 μm wide and 50 μm deep, and is flanked by two perfusion channels with 2 μm shallow openings. The square cross section of each of the perfusion channels is 50 μm on a side. This microfluidic platform was made from standard polydimethylsiloxane (PDMS) molded from an SU-8 mold and bounded onto a 170 μm thick glass cover slide with controlled surface roughness. AFM measured ten-point-height surface roughnesses of untreated surface (Fig. 1b), surface after 1-minute treatment with 2% HF [not shown], and surface after 2-minute treatment with 10% HF (Fig. 1c) were around 2.0991 nm, 3.1988 nm, and 14.7238 nm, respectively.

By controlling the wall shear rate at 2.1 s^{-1} and use untreated glass slides as substrate, we found that infection % and the immobilizing efficiency of miaRBCs held a linear relationship, as shown in Fig. 2. The slope of the best-fit line is 0.906, intersecting the y-axis almost at the origin. This is consistent with the assumption that each cell in the diagnostic channel has equal probability to interact with the roughened substrate and is independent of the other cells. The immobilizing sensitivity and efficiency of normal RBCs were always lower than 0.073% and 0.057%. The

specificity increased from 73.33% to 99.18% with 3.2% to 20.1% infection samples.

To verify that the capturing mechanism was based on the nano protrusions of the roughened substrates, identical experiments were also taken with HF treated glass substrates. The experimental results of using 2% HF treated substrate did not show improvement in immobilization sensitivity. The variations were between –5.5% and 5.5%. This could be due to the face that the ten-point-heights of these two substrates were still within the depth of furrow-like structures. However, significant increase in capturing sensitivity were observed on the 10% HF treated glass substrate, which had almost twice the depth of the furrow-like structures. Figure 3 shows two experiments conducted under two different shear rates by using 28% infected blood sample. The total captured miaRBCs increased by 44.35% and 31.37% under shear rate around 3.2 and 2.14 s^{-1}, respectively. This demonstrated that a roughened substrate with nano scale protrusions can interact with the surface lesions and furrow-like structures and provide a separation mechanism to capture miaRBCs.

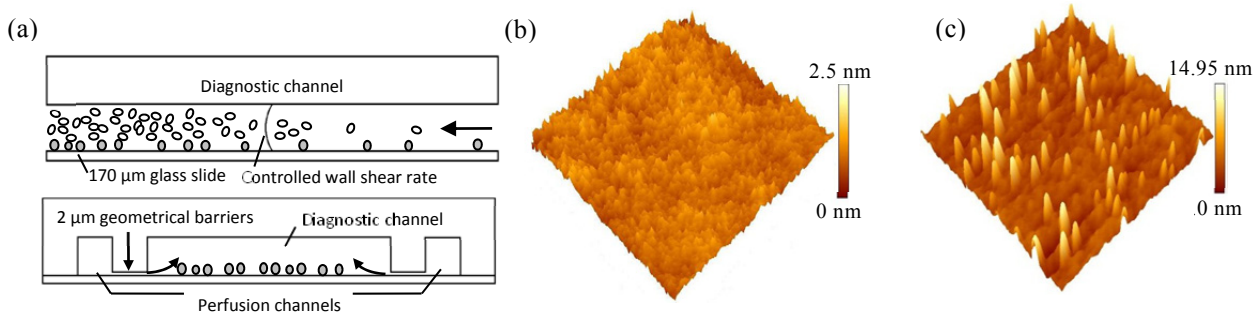

Fig. 1 Conceptual illustrations of the a) longitudinal and lateral side views of the microfluidic platform designed for avian malaria diagnosis, and 3-D AFM images of glass substrates b) at untreated condition and c) treated with 10% HF for 2 minutes, where scan area was 2 µm by 2 µm

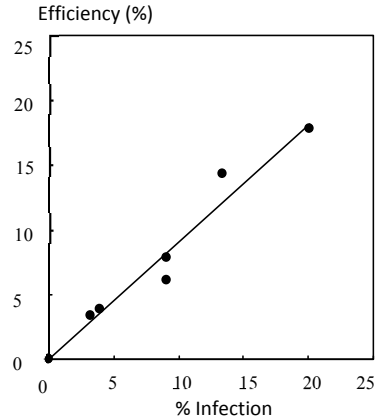

Fig. 2 The relationship between the % infection and immobilization efficiency of miaRBCs.

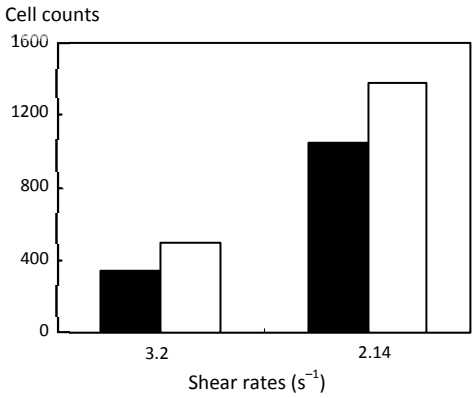

Fig. 3 A Comparison chart of total number of captured miaRBCs by using glass substrates at as is condition (black bar) and treated with 10% HF(white bar) under two different shear rates.

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