ABSTRACT

The loss of red blood cell (RBC) deformability is central to the pathology of falciparum malaria[1]. Deformability-based sorting of RBCs provides a means to enrich for these cells to improve diagnostic sensitivity and to isolate them for further study. Here, we develop a microfluidic device to sort RBCs based on deformability utilizing the microfluidic ratchet effect. We show the device is capable of sorting Plasmodium falciparum infected RBCs (Pf-iRBCs) at different stages of intra-erythrocyte growth and is able to enrich for ring-stage Pf-iRBCs by ~2500-fold, dramatically improving the sensitivity of malaria diagnosis.

KEYWORDS: Cell sorting, Cell deformability, Malaria, Plasmodium falciparum, Giemsa, Rapid diagnostic test

INTRODUCTION

The loss of RBC deformability is associated with the pathology of many diseases including malaria caused by Plasmodium falciparum where the metabolism of hemoglobin generates byproducts that rigidify the RBC membrane[3]. Cell sorting based on deformability provides a means to enrich for the infected RBCs to improve diagnostic sensitivity of the gold standard microscopy-based detection and aid in downstream molecular analysis. Previously, malaria iRBCs have been enriched using magnetic attraction[4], margination[5][6], and dielectrophoresis[7]. A key challenge issue for existing methods is the difficulty in achieving high enrichment of early ring-stage iRBCs at parasitemia observed in clinical samples. Here, we present a microfluidic device to sort RBCs based on deformability and demonstrate its ability to enrich for ring-stage Pf-iRBCs at clinically relevant parasitemia.

THEORY AND DESIGN

Previous physical cell separation based on micropore filtration method have been able to separate nucleated cells from RBCs, but have been unable to sort the highly deformable RBCs. This difficulty arises primarily from the inability to precisely control the pressure applied to each cell during the sorting process, which degrades the selectivity of the separation mechanism. To address this issue, we previously developed...
the microfluidic ratchet mechanism[8], which involves deforming single cells through funnel shape constrictions significantly smaller than the diameter of the cells. The pressure required to deform cells through the constriction along the direction of taper is smaller than against the direction of taper (Figure 1A). Deforming cells through these constrictions using an oscillatory flow causes cells to migrate unidirectionally through the constriction in a way that is selective by deformability. As shown in Figure 1(B), the upward flow acts to filter cells based on deformability, and the downward flow provides the critical function of releasing cells from the constriction to prevent clogging. As a result, the funnel ratchets, together with the oscillatory agitation, sort cells based on deformability without any clogging effect. To extend the ratchet principle, we design the sorting region (Figure 1C) composed of a matrix of funnels with progressively smaller pore sizes, through which the deformability of RBCs is serially tested. The pore size arrangement establishes the sensitivity of the device in discriminating between RBCs of different cellular deformability. Specifically, we design pore sizes ranging from 1.5 to 7.5 µm from bottom to top rows of the funnels.

![Diagram](https://via.placeholder.com/150)

Figure 2: Overview of the device design and operation. (A) RBC population infused through the inlet forms a zig-zag trajectory until reaching (B) a limiting pore size before (C) exiting the outlets.

The overview of the sorting process is shown in Figure 2. The sorting region (Figure 2A) is connected by a left cross-flow inlet and RBC inlet, to nine outlet collectors (Figure 2C) on the rightmost edge of the device. Oscillation channels line the top and bottom of the device. The height of the device sorting region is 4.5 µm, which is sufficient to constrict RBCs in a planar configuration while still allowing them to be freely transported by fluid flow. Under the cross-flow, sample inlet and biased oscillatory flows, cells infused from the bottom left corner of the device follow a diagonal trajectory until they reach a limiting pore size (Figure 2B). The downward flow releases cells that fail to transit the funnel filters, causing them to proceed under cross-flow to the outlets. Using this mechanism, a heterogeneous population of cells can be classified into different subpopulations based on deformability and rare rigid cells such as Pf-iRBCs will be separated from more deformable uiRBCs.

RESULTS AND DISCUSSION

To evaluate the ratchet mechanism to sort based on deformability, we investigate the outlets distributions of Pf-iRBCs at different intra-erythrocyte stages. Here, tightly synchronized malaria culture was used to determine the distributions of the samples at different time points during its 48-hour intra-erythrocyte stages (Figure 3A) post sorting. We show that the distribution for control uiRBC is centered at outlet 2 (1.75 µm). The distribution for Pf-iRBCs is initially centered on outlet 3 (2 µm) and increased to outlet 5 (2.5 µm) over time. The results indicate the stage-dependent shift of the iRBC population to the more rigid fraction of the outlets.

To demonstrate the ability to sort at clinically relevant concentrations, we prepared samples containing 0.0004-0.03% ring-stage Pf-iRBC and showed that our process can increase the parasitemia of these samples to 1-5%, equivalent of 100~2500X enrichment (Figure 3B). This capability enables detection of samples with parasitemia currently below the sensitivity limit of microscopy, and provides a significant improvement over the performance of previous biomechanical enrichment methods[5][6].
CONCLUSION
We developed a microfluidic device to sort RBCs based on deformability and demonstrated the ability to sort RBCs infected with *Plasmodium falciparum* at different stages of growth, as well as enrich for ring-stage infected RBCs ~2500-fold to dramatically improve the sensitivity of current malaria diagnosis.

ACKNOWLEDGEMENTS
Research support provided by the Canadian Institutes of Health Research. Travel support provided by the Institute for Computing, Information and Cognitive Systems (University of British Columbia).

REFERENCES

CONTACT: Hongshen Ma (hongma@mech.ubc.ca)