QUANTITATIVE ANALYSIS OF DEFORMABILITY-BASED CELL SEPARATION USING DETERMINISTIC LATERAL DISPLACEMENT AND OPTICAL STRETCHING

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ABSTRACT

In this work we present data showing deformability-based cell separation in a deterministic lateral displacement (DLD) device. We use cells of defined stiffness (glutaraldehyde cross-linked erythrocytes) to test the performance of the device across a range of cell stiffness and applied shear rates. Optical stretching is used as an independent method of quantifying the stiffness of the cells, thus allowing better understanding of the DLD system for deformability based cell separation. Displacement is shown to correlate with cell stiffness as measured across a range of flow rates. Data showing how the isolation of leukocytes from whole blood varies with shear rate is also presented.

KEYWORDS: deterministic lateral displacement, cell deformability, blood, optical stretching, shear rate

INTRODUCTION

Deterministic lateral displacement (DLD) is a powerful technique for high resolution continuous cell sorting, based on size [1-5]. DLD devices consist of arrays of pillars arranged at an angle with respect to the direction of fluid flow. Objects smaller than a critical size move in the direction of flow (i.e. along the channel axis) and objects larger than the critical size move in a direction defined by the pillar arrangement (i.e. they are laterally displaced). For hard spherical objects the operation of the device is straightforward. However, biological objects (e.g. cells) are often soft and non-spherical and their deformability and shape are known to influence the trajectories in DLD devices [3,5]. Our group and others have recently developed microfluidic DLD devices capable of sorting cells according to both size and stiffness.

Cell stiffness is an important emerging bio-marker for a number of disease states. For example, metastatic cancer cells are softer than healthy cells; malaria infected cells get progressively stiffer with parasite growth. The ability to sort bulk samples of cells based on both size an inherent cellular mechanical properties has a number of applications in diagnostics and clinical medicine.

EXPERIMENT

Deterministic lateral displacement devices were cast in PDMS from DRIE processed silicon wafer masters. PDMS and glass cover slides were treated to an O_2 plasma and bonded to form the microfluidic channels. Prior to bonding inlet and outlet holes were punched for fluidic access. Depending on the experimental sample devices were fabricated with either 4 micron tall pillars (erythrocyte work) or 15 micron tall pillars (leukocyte separation). Figure 1 shows the multi-stage DLD device used in this work, as well as photomicrographs showing the trajectories of latex beads (rigid spheres) at different positions along the device and at the outlet. Size based fractionation is clearly seen for a mixture of bead sizes run simultaneously. For the applied pressures used in this work (0 – 1100 mBar) there was no appreciable variation in the bead displacement as observed at the outlet of the device.

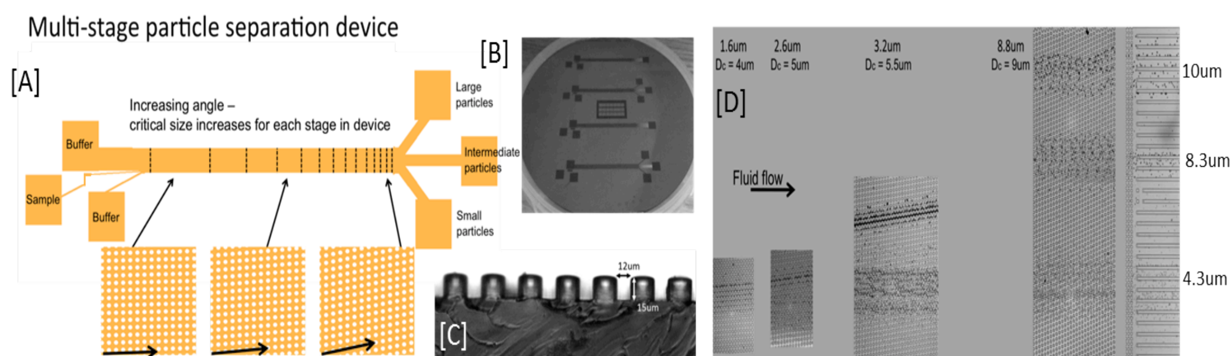

Figure 1: [A] Schematic of DLD separation device showing multiple sections each with a characteristic critical particle size. [B] Silicon master for casting PDMS devices. [C] Section through a PDMS device showing pillars. Depending on the cell type under study the height of the pillars was varied: ~4 microns tall pillars for erythrocytes work and ~15 microns for whole blood. [D] Micrographs showing bead trajectories at different points along the device.

Prior to sample introduction the devices were primed with PBS containing 0.1% Pluronic F108. Blood samples were obtained via finger prick. 50μL of whole blood was collected and the cells were washed 3 times in PBS

containing 0.1mM EDTA. Two experimental systems were investigated: (i) glutaraldehyde treated erythrocytes: cell samples were resuspended at $1x10^6$ cells per mL in the required concentrations of glutaraldehyde (0 – 0.01% in PBS) and incubated at room temperature for 45 minutes to allow crosslinking of the cells. The samples were then washed 3 times in PBS and resuspended in PBS. Glutaraldehyde treatment leads to the cross-linking of membrane associated proteins in the erythrocyte and a resulting stiffening of the cell. (ii) Leukocyte isolation from whole blood: whole blood was dilute 1:20 in PBS containing 0.1mM EDTA and incubated with CellTraker dye (1µM CMFDA, Invitrogen) for 15 minutes. Samples were introduced into the device and imaged either under brightfield or epi-fluorescence illumination using a cooled CCD video camera (ORCA-ER, Hamamatsu). Images were recorded and particle trajectories were analysed using bespoke image analysis software written in Mathematica.

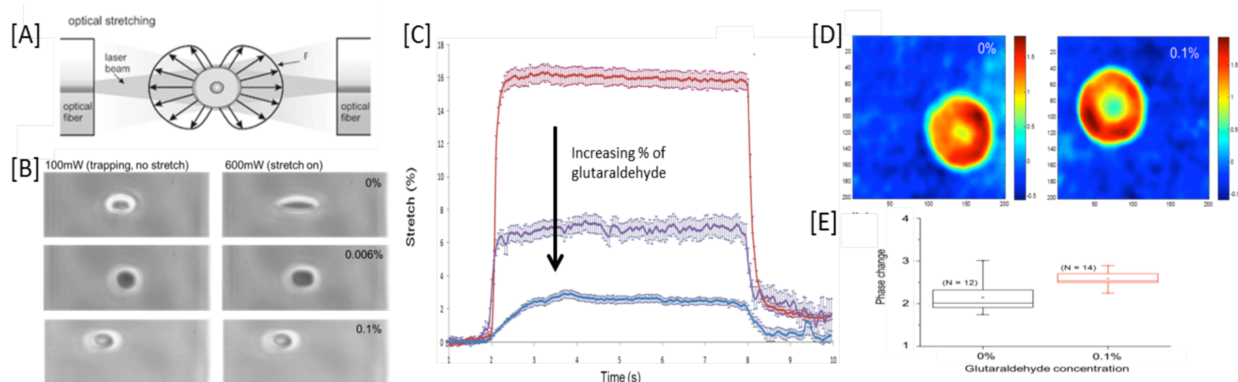

Figure 2. [A] Schematic of optical stretching setup. [B] Microscope images of trapped and stretched erythrocytes with different levels of glutaraldehyde crosslinking. [C] Stretching curves for erythrocytes with various levels of glutaraldehyde crosslinking. [D] Digital holography images showing quantitative phase change of untreated (0%) and "highly" treated (0.1%) erythrocytes. [E] Treated cells show a slight increase in refractive index – corresponding to <1% change in stretch measurement across the concentration range.

For optical stretcher experiments sample were treated as described above. The optical stretcher consists of a two-beam arrangement of counter propagating laser beams to capture and stretch single cells. The light power in both beams was held at 100mW to trap individual cells. When transiently increased to 600mW, the increased radiation pressure deforms the cell. Figure 2[A-B] shows a schematic of the optical stretcher setup and images of erythrocytes undergoing stretching. Treatment with different concentrations of glutaraldehyde leads to a well-define increase in cell stiffness. Figure 2[C] shows data from the cell stretching experiments for a range of different concentrations of glutaraldehyde (45 minutes incubation at room temperature). Quantitative phase measurements, using digital holographic microscopy (figure 2[D-E]) showed a negligible increase in refractive index of cells treated with glutaraldehyde; with even extremely high levels (0.1%) of glutaraldehyde treatment introducing <1% error into the stretch measurement.

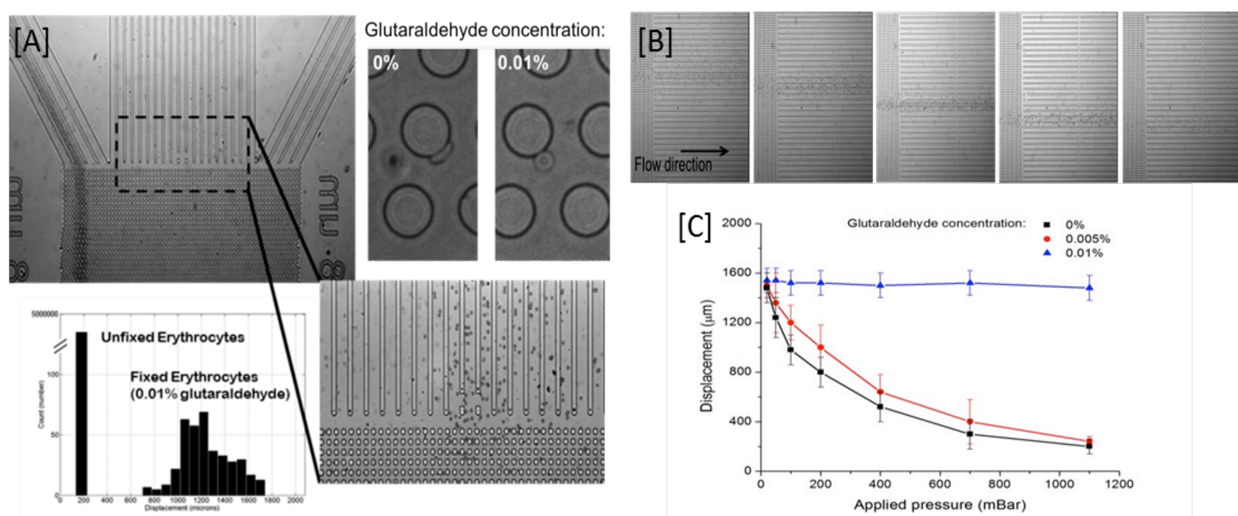

Figure 3: [A] Photograph of the outlet of the DLD device showing separation of RBCs of different stiffness (mixture of 0% and 0.01% glutaraldehyde fixed erythrocytes). Histogram shows cell distribution at outlet for a mix of untreated and glutaraldehyde treated RBCs (displacement direction reversed with respect to figure 1). [B] Photographs of the outlet of the DLD device showing displacement of untreated erythrocytes at different flow rates (increasing flow rate from left to right). [C] Variation in lateral displacement versus flow rate for erythrocytes of different stiffness.

Figure 3[A] shows the outlet of the DLD device showing the continuous separation of RBCs of different stiffness. The histogram shows the distribution at outlet for a mixture of untreated and 0.01% glutaraldehyde treated cells. Figure 3[B] shows how the distribution (lateral displacement) of cells at the outlet decreases with increasing shear rate for unfixed erythrocytes. Figure 3[C] shows characteristic data for the displacement of erythrocytes with different levels of fixation and thus stiffness. High resolution microscopic imaging of treated cells under no flow conditions showed no difference in size between treated and untreated cells.

Separation and washing of leukocytes from whole blood is an important application of DLD devices. Figure 4 shows how separation of leukocytes from other blood constituents (plasma, platelets, erythrocytyes, etc.) is effected by shear rate.

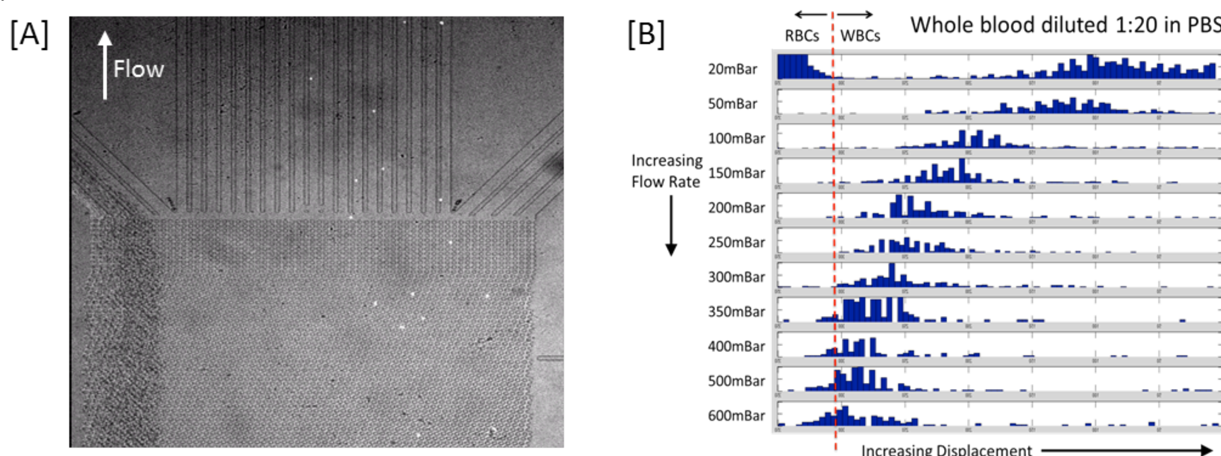

Figure 4: [A] Microscope image showing separation of leukocytes from whole blood using DLD. Leukocytes are labeled with CellTraker dye (bright cells on the right of the image). Erythrocytes are not displaced and run straight through to the leftmost outlet. [B] Lateral displacement of leukocytes as a function of flow rate. Displacement decreases as flow rate increases, due to cells deforming under shear

DISCUSSION AND CONCLUSION

Cell stiffness is an important emerging bio-marker for a number of disease states (e.g. metastatic cancer cells are softer than healthy cells; malaria infected cells get progressively stiffer with parasite growth stage). The stiffness of the chemically modified RBCs cells used in this work is similar to that of erythrocytes infected with the malaria parasite *P. falciparum* (also measured via optical stretching [6,7]), thus demonstrating the potential of using the DLD technique as a method for isolating malaria infected cells from blood and promise for development of diagnostics.

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