DEFORMATION ANALYSIS OF INDIVIDUAL RED BLOOD CELLS IN LARGE POPULATIONS USING A SINGLE CELL MICROCHAMBER ARRAY (SiCMA) CHIP

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ABSTRACT

We analyze the deformability of individual red blood cells (RBCs) using Single Cell Microchamber Array (SiCMA) technology. Our approach is adequate to quickly measure large numbers of individual cells in heterogeneous populations. Individual cells are trapped in a large-scale array of micro-wells, and dielectrophoretic (DEP) force is applied to deform the cells. The simple structures of microchambers and DEP electrodes facilitate the analysis of thousands of RBCs in parallel. This unique method allows the correlation of red cell deformation with cell surface and cytosolic characteristics to define the distribution of individual cellular characteristics in heterogeneous populations.

KEYWORDS

Single Cell Microchamber Array, Sub-population Analysis, RBC Deformation

INTRODUCTION

The ability to deform is essential for red blood cell (RBC) function in the circulation of capillary vessels. Cellular deformability in general is an important characteristic that defines the physiology of cells.[1] In malaria-infected RBCs, the deformation was reported to be different depending on the intracellular developmental stage of the parasite. Benign and malignant cancer cells were also reported to be significantly different in their deformability.

Ektacytometry [2] and slit rheometry [3] have been used to expose the entire RBC population to a defined shear stress and correlate the average morphology to measure elongation of RBC. These well-established measurements provide information on the deformability of the average RBC in a population, but are poorly able to define sub-populations with an altered deformability. Several methods have been developed to measure deformability of individual RBCs, including pipette aspiration [4], optical tweezers [5], dielectrophoresis [6], and atomic force microscopy [7]. While powerful to characterize deformation of small numbers of individual cells, these approaches are inadequate to quickly measure large numbers of individual cells in heterogeneous populations. Moreover, the ability to correlate RBC deformability with other cellular measurements, either in cytosol or membrane, is important to better define the underlying mechanisms for altered deformability. While flow cytometry or (automated) microscopy can provide data for cell surface markers, correlation with deformation is not available.

In this work, we present a novel approach using Single Cell Microchamber Array (SiCMA) technology [8] to analyze deformability of individual RBCs in a large heterogeneous population, allowing correlation of red cell deformation with cell surface, and cytosolic characteristics. Individual RBCs are trapped in a large-scale array of thousands of microchambers. Dielectrophoresis (DEP) is applied to deform RBCs, and the shape changes are analyzed. The simple structures of microchambers and DEP electrodes facilitate the analysis of thousands of RBCs in parallel. In addition, microscopic analysis using fluorescent dye can be used to measure cell surface and cytosolic markers.

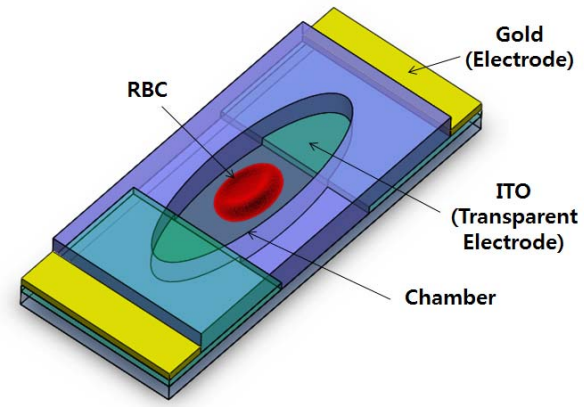


Figure 1. A schematic view of a chamber unit for deformability measurement in the single cell microchamber array.

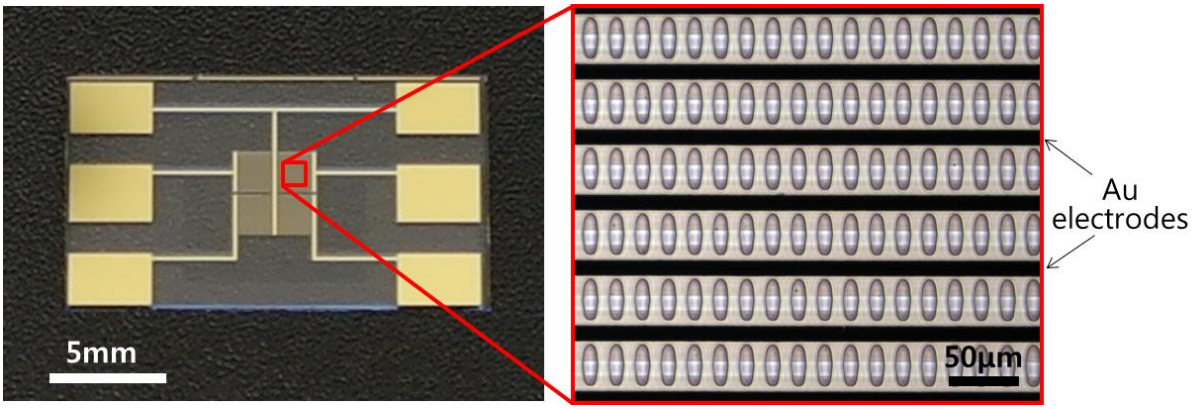


Figure 2: Fabricated chip with an enlarged view of chamber array.

DESIGN AND FABRICATION

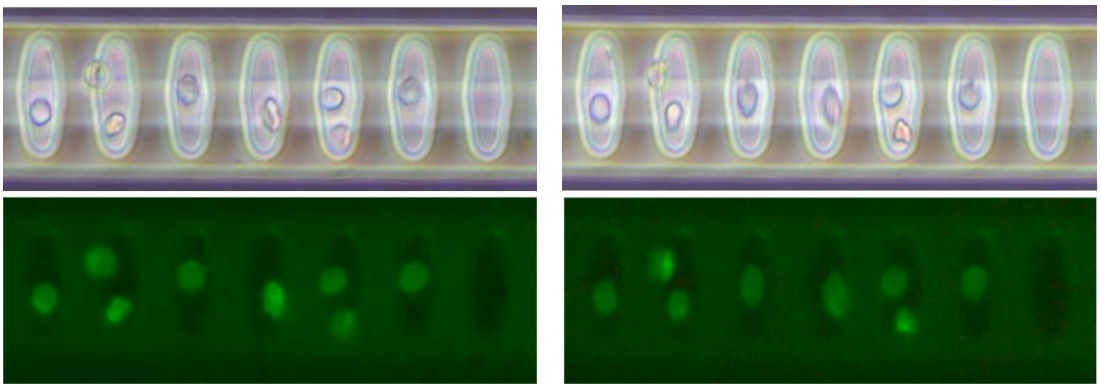
A schematic view of the chamber unit is shown in Figure 1. An electrical field to each microchamber is provided by a combination of transparent indium tin oxide (ITO) and gold electrodes. ITO allows image analysis from the bottom of the chip by using visible light on the microscope. A low resistance gold backbone delivers an identical electrical potential to the ITO in each microchamber. A dielectrophoretic force, aligns the cells in the chambers, and stretches each single cell. The length of the major (x) and the minor (y) axes, are measured and elongation index (EI=(x-y)/(x+y)), is calculated to evaluate the deformation of each cell.

The chip is fabricated by using surface micromachining process. The electrodes in the device were fabricated by deposition of 20/100 nm thick Cr/Au and 120 nm thick ITO layers on a pyrex glass wafer. Over the electrodes, the microchamber array was defined by a 10 µm thick SU8 layer. Figure 2 shows the fabricated device with an enlarged view of the microwell array.

EXPERIMENTS

RBC deformation was measured from two different samples: normal RBC and RBC from patients with hereditary spherocytosis. The spherocytosis is disease originated from a molecular defect in one or more of the proteins of the red blood cell cytoskeleton. Before the elongation, the RBCs were labeled with sulfo-NHS-LC-biotin and streptavidin-FITC conjugate for the visualization of deformed shape. The chips were loaded by applying a drop of fluorescently labeled RBC suspension in Hepes-buffered saline on the fabricated devices attached to a petri dish. A 20MHz AC signal from a function generator was applied to the device and cell deformation was observed by a fluorescent microscope. Figure 3 compare the deformation of RBCs depending on the applied voltage. A custom, MATLAB based, software program was used to process the images, recognize microchambers containing single cells, and measure the EI of each cell relative to voltage applied.

Figure 4 shows the distribution of deformabilities of a typical normal and spherocytosis sample. The EI at 8V in the normal cell population showed a distribution with an average of 36.9±9.4%. The RBCs from the spherocytosis patient showed an EI of 25.6%±10.4%. Low deformability of RBCs in hereditary spherocytosis is a well-recognized characteristic of this cytoskeletal disorder [9]. In addition, the distribution of EI is much larger in spherocytosis, as compared to normal cells at higher electric fields reflecting the heterogeneous cell population as seen by microscopy.



(a) *(b)*

Figure 3: RBC deformation depending on the applied voltage: (a) 0 $V_{p\text{-}p}$; (b) 6 $V_{p\text{-}p}$.

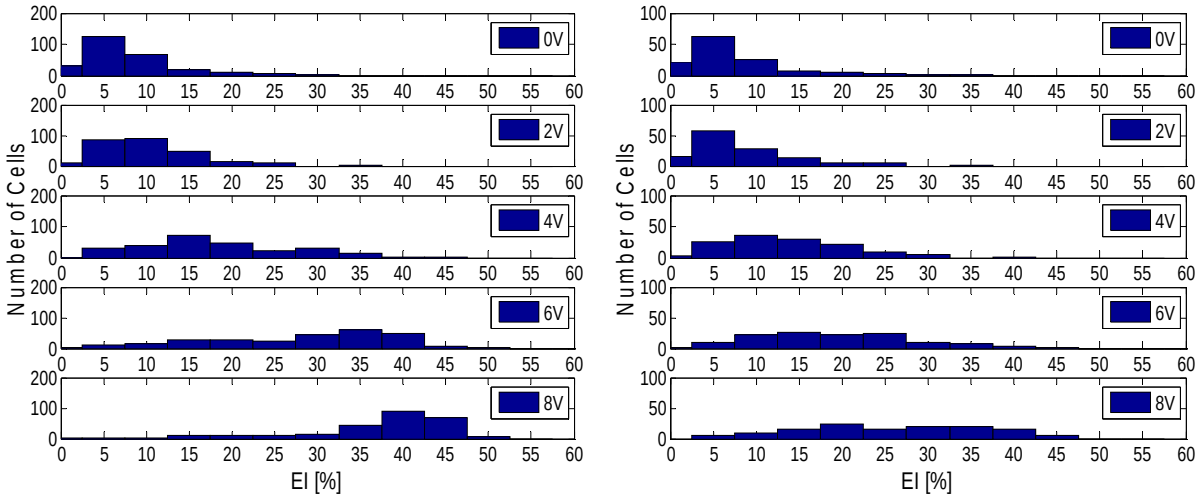


Figure 4: Measured deformability distribution of individual cells depending on applying voltage (Vp-p): (a) normal RBCs from healthy volunteer; (b) abnormal RBCs from patient with spherocytosis.

CONCLUSION

We present a novel approach to analyze the deformability of single cells in heterogeneous cell populations. We envision that the SiCMA technology can be used in a variety of applications that would benefit from the ability to measure the distribution of cellular characteristics in complex RBC populations, important to define hematologic disorders.

ACKNOWLEDGEMENT

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