LEUKOCYTE MECHANOPHENOTYPING BY DEFORMABILITY CYTOMETRY

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ABSTRACT

Immune activation and hematological malignancy are two disease states for which clinical monitoring has been revolutionized by flow cytometry. Numerous structural changes as well as changes in patterns of molecular biomarkers are associated with these states. While biomarkers related to these structural changes are attractive due to the fact that they can be measured without time- and labor-intensive sample preparation, in the clinic only molecular markers can be assayed with the requisite throughput to be used in diagnostics and monitoring. We previously developed a technology capable of high-throughput assays of cell mechanical properties. This deformability cytometer, which possesses a high ease-of-use and requires no labeling and minimal sample preparation, will make label-free biomarkers accessible in a clinical setting. However, a barrier to use remains in validation of mechanical phenotypes against accepted molecular biomarkers for those phenotypes. In this work, we have developed a hybrid fluorescence-deformability cytometer which is capable of acquiring both a fluorescence and a deformability measurement of a single-cell at a high throughput and explore mechanical phenotypes of leukocyte populations of interest in several diseases.

KEYWORDS

Cell Mechanics, High-Throughput, Flow Cytometry, Single-Cell, Mechanophenotype

INTRODUCTION

The mechanical properties of cells have been shown to possess significant promise as powerful label-free and inexpensive biophysical markers for cell phenotype. We previously reported a system for high-throughput (>1000 cells/s) deformability cytometry [1] with clinical applications in immune monitoring, stem cell therapy, and cancer diagnostics [2,3]. The system employs hydrodynamic forces to position cells in flow and stretch them, high-speed imaging to record deformations, and automated image analysis to quantify single-cell deformability (Figure 1).

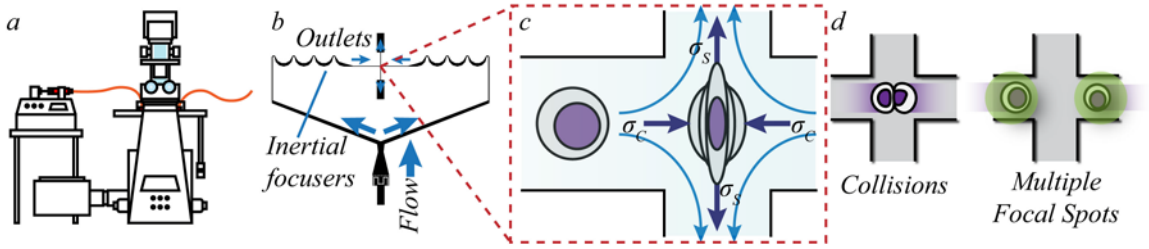

Figure 1. Deformability cytometry (DC) method and device. a. System setup. b. Top view of 1st generation device to scale (blue arrows indicate flow direction). c. Mechanism of deformation (σ_C and σ_S are compressive and stretching stresses). d. Primary limitations to throughput for the hybrid deformability and fluorescence flow cytometer.

Now we are working toward replacing label-based assays by establishing correlations between cell mechanics and traditional affinity biomarkers. In this work we explore new applications in blood diagnostics and report the development of a hybrid fluorescence-deformability cytometer. Using this new capability we will provide the first data that directly correlates surface biomarkers of phenotype with mechanical properties at the single-cell level.

EXPERIMENT

At a population level, we have measured an increase in the activation biomarker, HLA-DR, after *in vitro* stimulation (with anti-CD3, 12F6, and phytohemagglutinin, PHA) of peripheral blood mononuclear cells (PBMCs) while, in parallel, measuring increased deformability (Figure 2a). Increased deformability in lymphoma cell lines (Figure 2b-c) is also expected to correlate well with clinical biomarkers for transformation as well as structural changes (Figure 2d), such as in cytoskeletal composition and nuclear size, shape, and organization. We have also observed a drastic decrease in the deformability of promyelocytic leukemic cells, HL60, upon differentiation toward neutrophils with all trans retinoic acid (ATRA). The differentiated cells resemble granulocytes isolated from human donors. Activation of granulocytes from human donors with 12-O-Tetradecanoylphorbol-13-acetate (TPA) for short periods of time induces an increase in deformability.

We have now built a system which can perform fluorescence flow cytometry and deformability cytometry on the same cell, while maintaining the high-throughput of the two systems, which will allow us to validate deformability cytometry as a low cost reliable replacement for numerous assays in immune activation and leukemia monitoring.

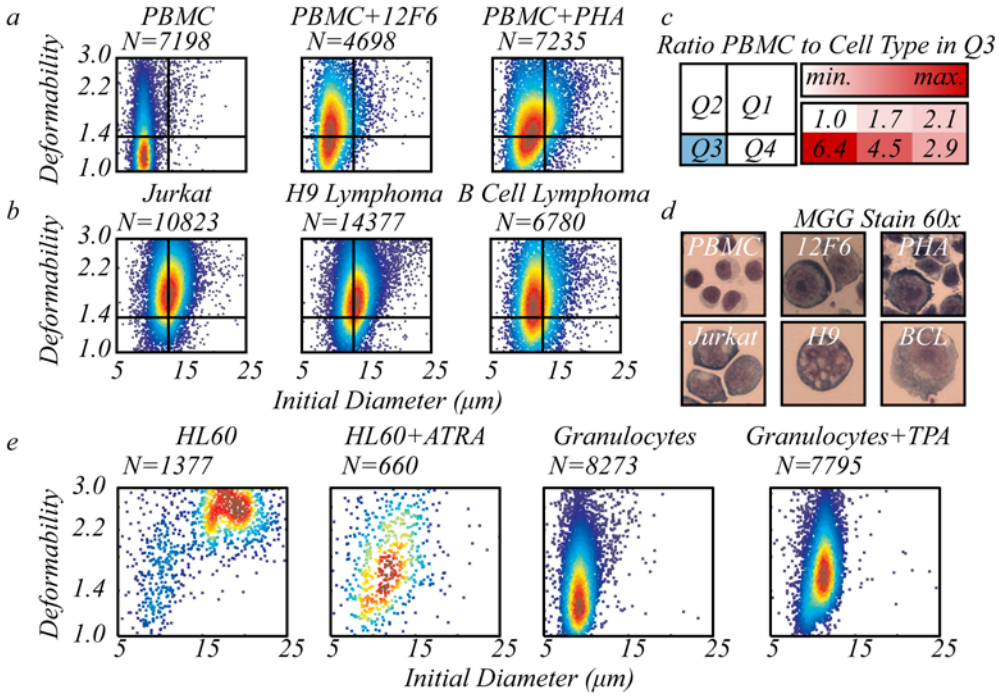

Figure 2. DC of leukocyte disease models. a. Activation increases PBMC deformability. b. Lymphoma cell lines are larger and highly deformable. c. Gating by size and deformability will be an effective gating strategy for monitoring immune activation or residual leukemias. d. Population level changes in cell and nucleus shape and size, and the presence of loosely packed chromatin can be correlated with deformability as well. e. Cells of the promyelocytic leukemic cell line, HL60, are large and deformable. Upon differentiation toward neutrophils with ATRA, the cells become smaller and less deformable, resembling granulocytes isolated from human donors. TPA activation of granulocytes from human donors for short periods of time induces an increase in deformability.

The hybrid deformability and fluorescence cytometer required innovation in the device design as well as design of an optical system capable of performing both high-speed imaging and fluorescence measurements in close proximity. While the 1st generation design enabled interrogation of several thousand cells per second it limits the implementation of fluorescence detection in that cells arrive from two directions requiring two beam spots and occasionally has coincident cells which must be thrown out, limiting throughput. We designed a new inertial focusing channel to position cells at the center of flow and siphon off a controlled volume of cell-free suspending fluid (Figure 3) by tuning the fluidic resistance of the branch channels. This siphoned fluid is then rejoined at a high velocity with the fluid containing the cell stream, creating the extensional flow and deforming cells. We evaluated several geometries for the trifurcation and several resistance ratios before selecting a channel in which nearly all cells were delivered from one direction and deformation most resembled that in the 1st generation device.

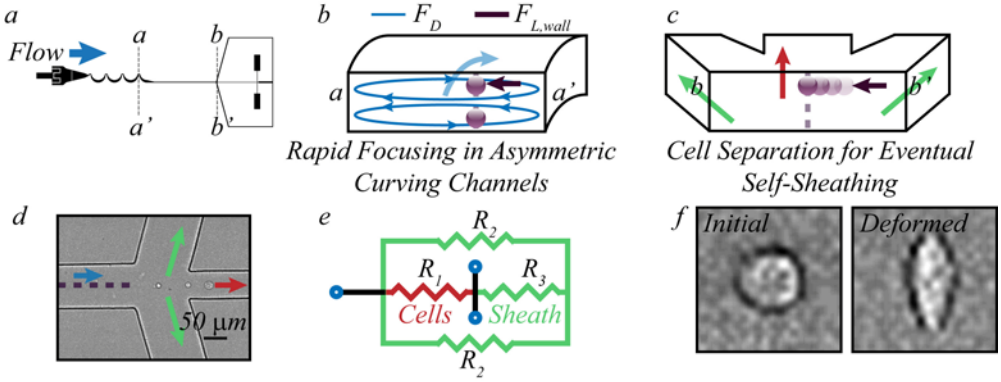

Figure 3. Modified DC channel design for hybrid measurements. a . Top view of 2nd generation device to scale. b. Dean drag and inertial lift forces focus cells rapidly in asymmetric curving channels. c. Focused cells enter the cell branch (red arrow) when the channel trifurcates. d. High speed image of cell focusing and fluid siphoning. e. Fluidic circuit diagram of the 2nd generation device (the resistance of the sheath branch network must equal the resistance of the cell branch: $R_1=R_2/2+R_3$) to replicate the extensional flow of the 1st generation device. f. High speed images of a cell before and during deformation by hydrodynamic forces in the 2nd generation device.

To realize the hybrid system we also built an optical setup which was capable of performing high SNR fluorescence measurements on cells immediately upstream of the deformability cytometry measurement (Figure 4). The optical setup allows both measurements to be performed in close proximity without saturating the photodetector with the

imaging light. The single-cell biomarker correlations this system will provide will enable wider adoption and greater utility of mechanophenotyping in research and medical labs.

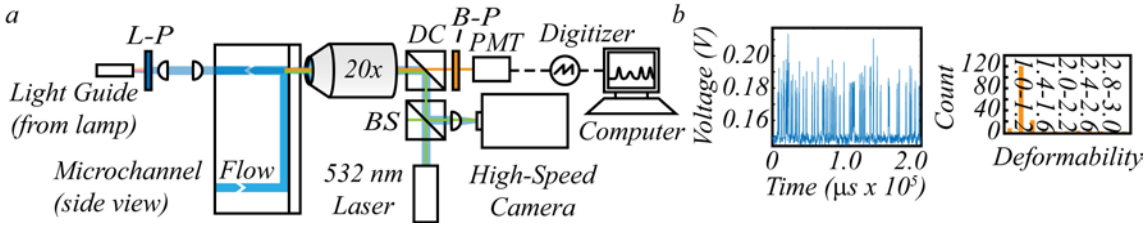

Figure 4. a. Optical setup for parallel high-speed imaging and fluorescence flow cytometry. Light from a xenon lamp is filtered (Low-pass, LP) and focused onto the microchannel for imaging. Simultaneously, a 532 nm laser is focused onto the channel through a 20x objective lens. Emitted light is collected through the objective lens, filtered, and detected by a photomultiplier tube. The signal is digitized and processed offline. Transmitted light is collected through the same objective but is reflected by a series of dichroic and beam-splitting mirrors to a high-speed camera which collects images at 500,000 frames per second with an exposure time of 300 ns. b. Representative data sets acquired simultaneously. Automated image analysis is performed as previously reported.

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