CELL DEFORMABILITY CLASSIFICATION BY CENTRIFUGAL STOPPED-FLOW SEDIMENTATION THROUGH NARROW GAPS

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ABSTRACT

In this report we measure the deformability distribution across populations of specific cell types by their centrifugally driven passage through vertical openings in an array of scale-matched obstacles. Each element in the array consists of two identical structures exhibiting a gap smaller than the diameter of the target cells and allowing collection of deformable cells in a downstream micro-cup. We demonstrate the separation of unfixed from fixed HL60 cells from the same cell line on a simple PDMS device.

KEYWORDS: Centrifugal Microfluidics, Cell Deformability, Rare Cell Differentiation

INTRODUCTION

Cancer cells are distinguished from normal cells by a repertoire of biophysical and biochemical characteristics [1]. In particular cell deformability measurement has recently attracted significant attention for which a wide spectrum of techniques, ranging from atomic force microscopy to micropipette aspiration, have been employed [2]. In microfluidic systems, such biomechanical separation is often based on the penetration of the cells through dynamic sieve structures featuring sequentially decreasing openings [3].

The study outlined in this report aims to combine microfluidic deformability filters with geometrical V-cup structures [4] on a centrifugal microfluidic platform to give quantitative and qualitative information about stiff and deformable cell content in a mixed population.

EXPERIMENTAL

To determine their stiffness distribution, we create an array of 3D structures composed of an entry gap followed by a geometrical V-cup barrier [4]. The cell population is centrifugally driven through the stagnant bulk liquid residing in the dead-end chamber. Only cells exceeding a deformability threshold mainly governed by the width of an entrance gap will penetrate and thus become trapped while stiffer (and larger) cells are deflected to downstream structures. Based on a mean cell diameter of 12.8 μm, with a standard deviation of 1.3 μm, we chose a gap size of 10 μm. The disc-shaped substrate consisted of a PMMA base supporting a PDMS layer patterned with microfeatures, 30 μm high, molded from an SU8 master prepared using UV photolithography (Fig. 1).

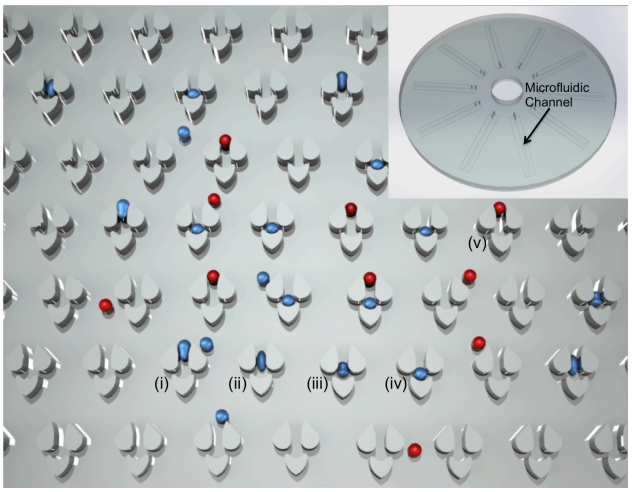

Figure 1: *CAD design representation of microstructure array, situated in the microfluidic channel of the PDMS disc (inset), with unmodified cells (blue) in various stages of deformability and capture (i-iv) and fixed cells (red) (v) unable to traverse through the 10-µm gaps.*

In our experiments two populations of unmodified and fixed, i.e. stiffer HL60 cells (human promyelocytic leukemia) were studied independently. Unmodified cells were treated with two drops per ml of NucBlue® blue fluorescent counterstain; fixation and red fluorescence were induced with 250 µg ml glutaraldehyde. A 3 µl aliquot of each preparation was loaded in separate microchannels and the cells were introduced to the array by spinning the disc at 20 Hz for 30 mins.

RESULTS AND DISCUSSION

We analyzed the behavior of the fluorescently stained cells in the microstructure array (Fig. 2) using image analysis software (ImageJ). Figure 3 shows that 50.9% of the unmodified HL60 cells were trapped and can thus easily be discerned from randomly distributed, free cells while only 1.9% of the fixed, i.e. less deformable cells were captured (Fig. 4). This residual trapping of fixed cells might be linked to the lower-end tail of the size distribution of the HL60 cells and may eventually be eliminated by prior size filtration of the population which is planned to be implemented in the near future.

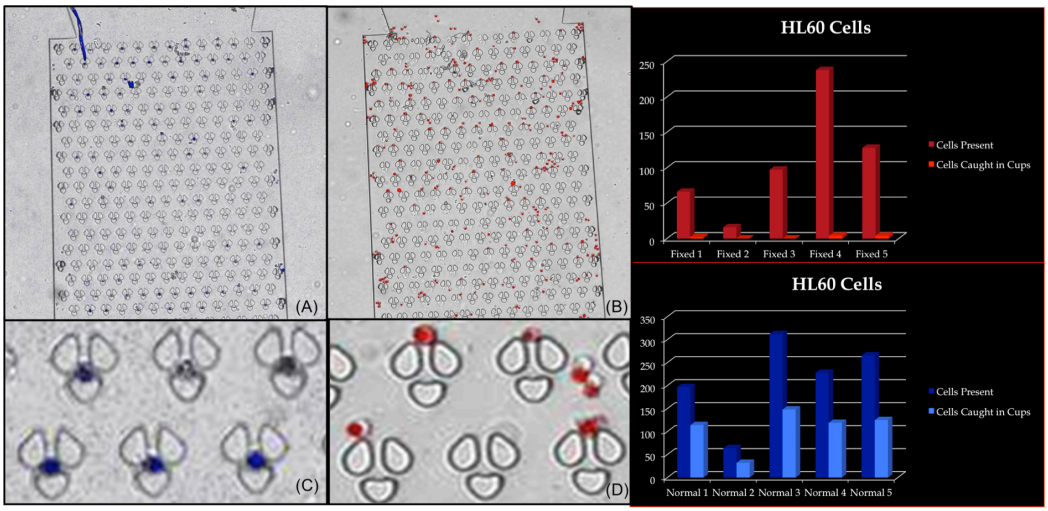

Figure 2: (Left) *Fluorescent microscopic images of unmodified (A) and fixed (B) cells in the microstructure array and magnified views with detail of the respective capture structures in (C) and (D) and (Right) Graphs representing number of cells present in microstructure array vs cells captured for fixed and unmodified HL60 studies.*

CONCLUSION

We have for the first time shown a centrifugal microfluidic platform capable of separating and capturing cells based on their deformability. More than half of the normal cell population was trapped in well-defined spots at the center of the arrays microstructures while less than 2% of the fixed cells were unspecifically captured. Our technique will be further improved by upstream size separation to enhance specificity. The longer-range objective is to relate deformability to the condition of a specific target cell population and eventually to diagnose the health status of a patient.

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