MICROFLUIDIC DEVICES FOR RAPID LABEL-FREE SEPARATION AND SENSING OF CELLS

Suman Bose[*], Chia-Hua Lee[**], Jeffery M Karp[***], and Rohit Karnik[*]

[*] *Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

[**] *Department of Materiel Science and Engineering,Massachusetts Institute of Technology, Cambridge, MA 02139, USA*

[***] *HST Center for Biomedical Engineering and Harvard Stem Cell Institute, Brigham and Women's Hospital and Harvard Medical School, Cambridge, MA 02139, USA*

ABSTRACT

We present a new method for label-free separation of cells by rolling on patterned P-selectin receptors. P-selectin edges patterned on gold substrate using microcontact printing could direct trajectories of HL60 cells along the the edges. The deflection of the cells strongly depended on the angle of the pattern to the flow. We incorporated these patterns inside microfluidic devices and demonstrated separation of a mixture of HL60 from passive microspheres

KEYWORDS: Cell separation, Leukocyte rolling, Point of care, Microfluidics.

INTRODUCTION

Cell separation technologies based on receptor-ligand interactions typically involve extensive processing of the sample due to the necessity for labeling and label removal. We have recently demonstrated that transient receptor-ligand interactions that result in cell rolling [1] on a surface under fluid flow can be used to control the flow of cells on a receptor-patterned substrate [2], which paves the way for a new technique for label-free cell separation. Specifically, we found that when a rolling cell encounters a patterned P-selectin receptor edge, it is deflected from its direction of flow and follows the edge [2]. We envision a microfluidic device that would perform label-free separation of cells by rolling on receptor patterned surfaces (Figure 1). Such a device would need a method for injecting a stream of cells, multiple edges and reduced channel height to ensure separation, and multiple outlets to collect separated cells.

The technique relies on cell rolling- a physiological phenomenon exhibited by several types of cells including leukocytes, hematopoietic stem cells and cancer cells, which is mediated by glycoproteins known as selectins [3]. In this paper we use microcontact printing to indirectly create high resolution patterns of P-selectin on gold substrates. Human myeloid leukemia cells (HL60) were used as model leukocytes as they express PSGL-1 (a ligand for P-selectin). We studied and characterized rolling behavior of HL60 cells on P-selectin patterns. The results were used to build a predictive model for simulating rolling of HL60 cells inside a microfluidic device. Finally we incorporated these devices inside microfluidic channels and demonstrated separation of cells from a mixture of HL60 cells and passive microspheres.

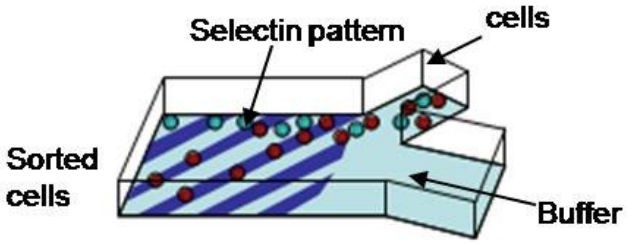

Figure 1. Schematicdiagram of the cell separation device. Cells are introduced from one side in a buffer flow. The cells that interact with the receptor patterns (red) roll on the edge and migrate to the buffer stream while the non-interacting (blue) cells does not get deflected . The sorted stream of rolling cells can be collected in a different channel

EXPERIMENTAL

Fabrication of patterned substrates: Microcontact printing stamps that defined the pattern were fabricated in polydimethylsiloxane (PDMS) by SU-8 molding process. The stamp was inked with a 5mM solution of (1-Mercaptoundec-11-yl)tetra(ethylene glycol) (PEG alkanethiol; Sigma-Aldrich), dried, and pressed onto a gold coated slides (EMF corp) to be patterned. After selective deposition of PEG molecules, the slides were incubated in 15 µg/mL P-selectin solution (R&D Systems) for 3 hours, washed with buffer and used for experiments.

Lines of P-selectin were patterned at different angles on a substrate. The patterned substrate was incorporated inside a commercial flow chamber (Glycotech Inc) and a solution of HL60 cells (10^5cell/mL) was perfused over the substrate at a particular shear stress. Images of cells were captured using a CCD camera (Andor Xion) under 4x magnification. Images were processed using a custom program developed in Matlab. The code employed an algorithm that tracked the cells and identified sections of the tracks that represented cells rolling on an edge (*Le)*.

Prediction of rolling behavior of HL60 cells: For a particular edge angle, the cumulative distribution of Le from the experiment was calculated and fitted to that of a Poisson distribution given by $CDF(Le) = 1 - \exp(-\lambda / Le)$ where the parameter λ predicted the mean value of Le. In order to predict paths of cells inside a microfluidic device, all cells were assumed to start from a single location. Trajectories of cells were then predicted through a Monte Carlo simulation assuming that detachment on every edge followed a Poisson process. The net deflection suffered for a travel length of 1 cm through the device was calculated for 10^5 cells.

Separation experiment: A particular pattern with alignment markers was designed for use in a microfluidic channel and was subsequently used for preparing P-selectin patterned substrate. A device consisting of a microfluidic channel (100µm×1mm), two inlets and two outlets was fabricated in PDMS using SU8 molding process. The device was aligned (to the receptor pattern) and attached to the substrate using a vacuum manifold (see Figure 2). HL60 cells were mixed with fluorescent polystyrene microbeads of similar size (diameter ~10µm) to the cells, to make a final concentration of 10^5 particles/mL and injected into the assembled device from one of the inlet ports, while buffer (DPBS) was flowed through the other inlet port. The cells were collected from the two outlet ports into separate collection vials. Concentration of cells and microbeads were measured using a hemacytometer.

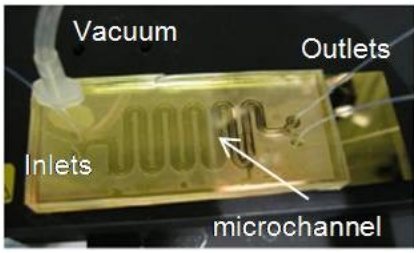

Figure 2. Typical microfluidic device assembly for separation experiment.

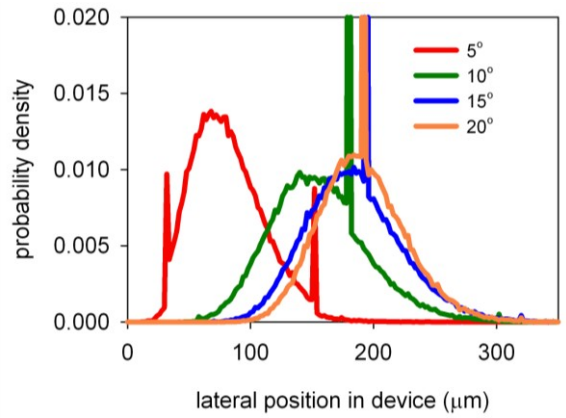

Figure 3. Simulation results showing probability density of net lateral deflection of cells after flowing a total distance of 1 cm through a microfluidic device patterned with receptors at particular angles (shown in colors). Shear stress is 0.5 dyn/cm^2.

RESULTS AND DISCUSSION

The method for fabrication of asymmetric patterns have been reported by us earlier [4], where we observed that HL60 cells specifically interacted and rolled on the P-selectin patterns. Furthermore, we also observed that the average length travelled on the edge (*Le*) decreased sharply as the edge angle to the flow increased (data not shown). However, based on the data from the above experiments we created a predictive model which could simulate the behavior of cells inside a microfluidic device. We analyzed the distribution of length travelled along the edge (*Le*) and found that the data resembled a Poisson distribution , which was then used in a Monte Carlo simulation to predict the distribution of cells inside a microfluidic channel. We assumed that all the cells started from a single location and we plotted the probability distribution of the cells after they travelled a distance of 1 cm through the device (see figure 3). We found that the mean displacement of the cells increased with increase in edge angle reaching maximum at ~15^o after which notable increase in net lateral displacement was not observed. Next, we incorporated these patterns inside a microfluidic channel using alignment (figure 4a) of the patterning stamp and the microfluidic device to the substrate. On injecting a mixtute of HL60 cells and microspheres in a ratio ~1:5 we found than HL60 cells preferentially migrated to the buffer stream and eluted out through the 'sorted' outlet of the device. A few microspheres also appeared to have migrated into the buffer stream probably due to interactions with the rolling cells. Nevertheless, the final ratio of the cells to microspheres in the outlet stream was 1.24:1, which is almost 6 fold increase in concentration of HL60 cells relative to microbeads (figure 4b).

CONCLUSION

In this paper we demonstrated separation of HL-60 cells from microspheres by rolling on surfaces patterned with P-selectin in a prototype device. This is the first demonstration of the concept that receptor patterning can be used for label free separation of cells. Further development of this technology could lead to separation and detection of different cell types based on their differential rolling behavior on receptor edges.

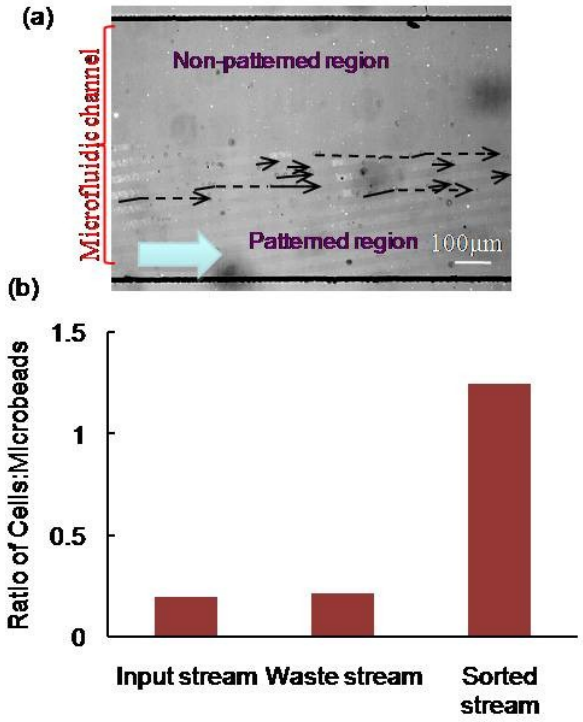

Figure 4. (a) Optical micrograph showing the P-selectin pattern along with the microchannel walls. Cells can be seen rolling along the receptor edges toward the buffer stream. Arrows indicate trajectories of rolling cells. (b) Ratio of concentration of HL60 cells to microspheres in input stream, waste stream and sorted stream.

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CONTACT

*R. Karnik: Tel: 001.617.324.1155 email: karnik@mit.edu