

# Microfluidic Devices for Rapid Separation and Sensing of Cells

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## 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 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.

## 1 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 pattern 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.

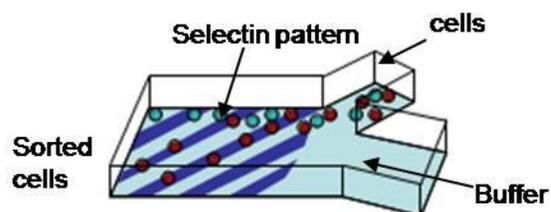


Figure 1. Schematic of the proposed cell separation device. Cells are introduced from one side in a buffer flow. Rolling of cells on patterned receptors causes them to separate laterally from where they can be collected in different channels.

## 2 EXPERIMENTAL METHODS

### 2.1 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-Mercapoundec-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  $\mu\text{g}/\text{mL}$  P-selectin solution (R&D Systems) for 3 hours, washed with buffer and used for experiments. (Figure 2).

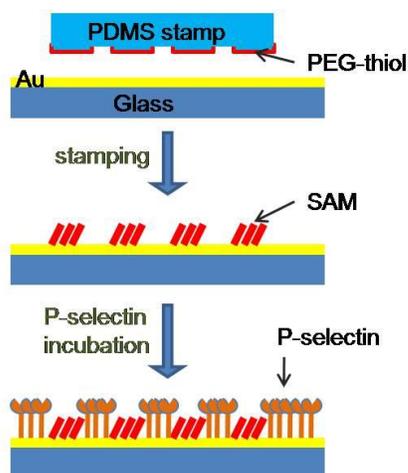


Figure 2. Fabrication of P-Selectin patterns using microcontact printing

## 2.2 Characterization of rolling on the edge

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 ( $10^5$  cell/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 ( $Le$ ).

## 2.3 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:

$$CDF(Le) = 1 - \exp(-\lambda / Le) \quad (1)$$

where the parameter  $\lambda$  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.

## 2.4 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 ( $100\mu\text{m} \times 1\text{mm}$ ), 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 3). HL60 cells were mixed with fluorescent polystyrene microbeads of similar size (diameter  $\sim 10\mu\text{m}$ ) 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.

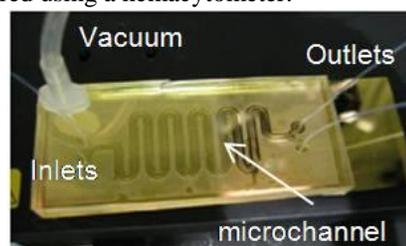


Figure 3. Typical microfluidic device assembly for separation experiment.

## 3 RESULTS

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 (See figure 4a). However, based on the data from the above experiments we wanted to make 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 and hence we fitted a Poisson curve to the cumulative distribution of  $Le$ . This allowed us to calculate the mean for the Poisson process which was then used in a Monte Carlo simulation to predict the path 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 4b). We found that the mean displacement of the cells increased with increase in edge angle reaching saturation at  $\sim 15^\circ$  after notable increase in net lateral displacement was not observed. Next, we incorporated these patterns inside a microfluidic channel using co-alignment of the patterning stamp and the microfluidic device to the substrate. On injecting a mixture of HL60 cells and microspheres in a ratio  $\sim 1:5$  we found that 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. However the number of cells that interacted with the substrate was relatively low resulting in a majority of cells escaping separation. This caused the 'waste' stream to have nearly the same relative concentration of HL60:microspheres as the original stream.

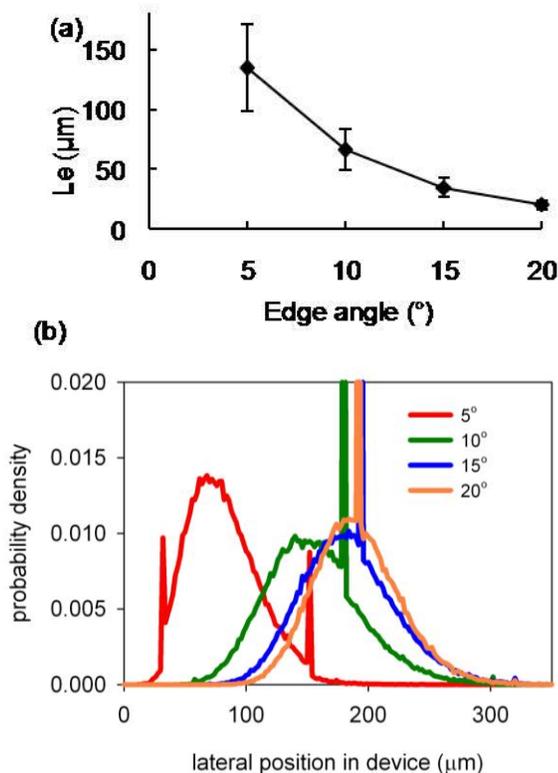


Figure 4. (a) Average length travelled along the edge ( $L_e$ ) for different edge angle at shear stress of  $0.5 \text{ dyn/cm}^2$ . (b) Simulation results showing probability density of net lateral deflection of cells after flowing a total distance of 1 cm through a microfluidic device patterned with receptors at particular angles (shown in colors). Shear stress is  $0.5 \text{ dyn/cm}^2$ .

## 4 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 edge.

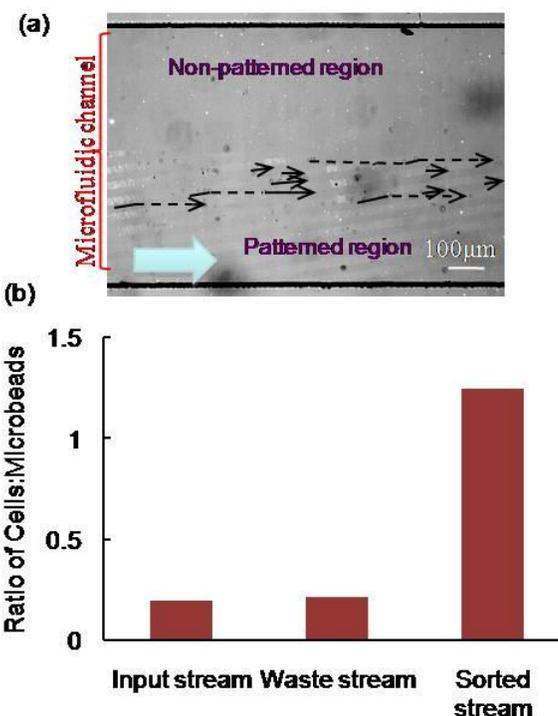


Figure 5. (a) Optical micrograph showing the P-selectin pattern along with the microchannel walls. Cells can be seen rolling along the receptor edges toward the buffer stream. (b) Ratio of concentration of HL60 cells to microspheres in input stream, waste stream and sorted stream.

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