

MICROFLUIDIC DEVICES FOR RAPID LABEL-FREE SEPARATION 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 using selective surface chemistry could direct trajectories of rolling cells along the edges leading to separation of rolling HL60 cells from non-rolling K562 cells in a microfluidic device. The displacement of the cells and hence the resolution of separation was found to be a function of the device length.

KEYWORDS: Cell separation, Leukocyte rolling, Selectin

INTRODUCTION

Separation and analysis of cells based on surface markers plays an important role in biological research and clinical diagnosis. Existing microscale cell sorting techniques are either non-specific to antigen type, require expensive accessory equipment for their operation, or capture cells on adhesive surfaces and necessitate special elution procedures for recovering the cells. 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).

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. In our earlier work we studied the dynamics of the rolling of HL60 cells on P-selectin pattern edges and described the optimal condition under which the deflection of cells on these asymmetric patterns can be maximized [3]. In this work we report design of microfluidic devices for continuous-flow separation of HL60 cells from K562 cells based on transient adhesive interactions between PSGL-1 ligand expressed on HL60 cells with P-selectin receptors patterned in the device. P-selectin was patterned by selective surface chemistry on gold-patterned glass slides. The design allows target cells to settle gravitationally along the length of the channel, after which the cells get displaced laterally into a non-patterned region for quick elution.

EXPERIMENTAL

Gold coated glass slides (EMF corp.) were patterned using a positive photoresist as an etching mask. The substrates were cleaned with piranha (3:1, H₂SO₄ : H₂O₂) and immersed in 1% PEG-trimethoxysilane (Gelest) solution in toluene overnight followed by treatment with 1 mM dithio-bis-succinimidyl propionate (DSP, Pierce Biotech.) in DMF for 2 h, after which they were washed in ethanol. Finally the substrates were incubated with P-selectin (5 μg/ml, R&D Systems) for 3 h and stored in 1% BSA solution until used in the experiments.

The flow cell was molded in PDMS using standard lithography techniques, and consisted of a serpentine channel of rectangular cross section (100 μm × 1 mm) with total length of 20 cm. The device was attached to the substrate after alignment by applying vacuum, creating a reversible bond.

HL60 cells and K562 cells (ATCC) stained with CellTracker Red and Green respectively, were mixed in 1:1 ratio with a total concentration of 1 million cells per mL, and injected into the device assembly alongside a buffer stream. Flux of the flowing cells was recorded at different locations along the channel length using an epifluorescence microscope.

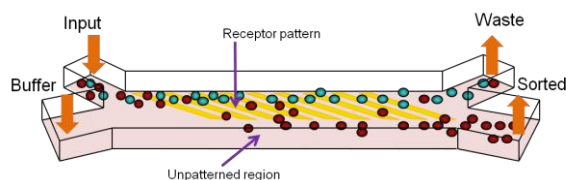


Figure 1: Schematic of the cell separation device. Cells are introduced from one side in a buffer flow. Rolling of cells on patterned receptors causes them to separate laterally from where they can be collected in different channels.

RESULTS AND DISCUSSION

When a stream of HL60 and K562 cell mixture was introduced parallel to a buffer stream in 1:9 ratio at a wall shear stress of 0.5 dyn/cm^2 , we observed that HL60 cells specifically interacted with the patterns, followed the edges of the patterns, and were displaced laterally in a device length-dependent manner (Figure 2). In contrast, the K562 cells showed a passive spreading across the width of the channel, possibly due to cell-cell hydrodynamic interactions. As evident from Figure 2, the efficiency and purity of the separation can be tailored depending on the fraction of the flow isolated at the channel exit. Collecting 25% of the flow from the purified end resulted in a purity of 94% of the separated HL60 population and a recovery efficiency of $\sim 85\%$ as analyzed by flow cytometric measurements. Epifluorescent images of the injected and collected fractions are shown in Figure 3. We operated these devices for more than 3 h and did not find appreciable difference in performance indicating that the surfaces are resistant to bio-fouling.

CONCLUSION

In this paper we demonstrated high efficiency, high purity separation of HL60 cells from K562 cells by rolling on surfaces patterned with P-selectin in a continuous flow. Further development of this technology could lead to separation and detection of different cell types based on their differential rolling behavior on receptor edges. Although P-selectin was used in this study, other molecules that enable transient cell-

surface interactions can potentially be used to target cells based on surface markers. This approach to continuous-flow separation of cells based on surface markers is promising for diagnostic applications and also as a general cell sorting technique.

HL60 (Green) K562 (Red)

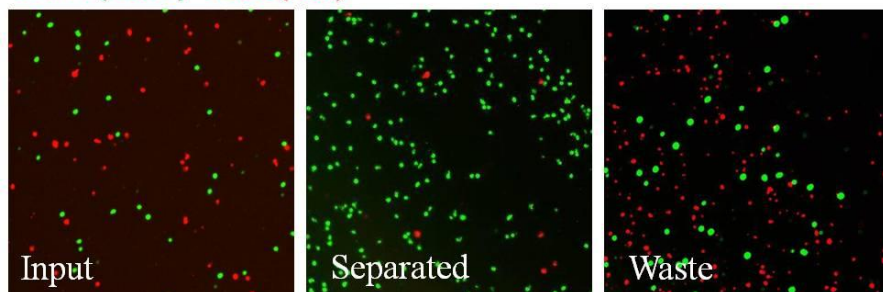


Figure 3: False color images of the input, separated and the waste fraction. HL60 cells are depicted in green while K562 are shown in red. The separated fraction can be seen to be enriched in HL60 cells while there is marked depletion of HL60 cells in the waste population.

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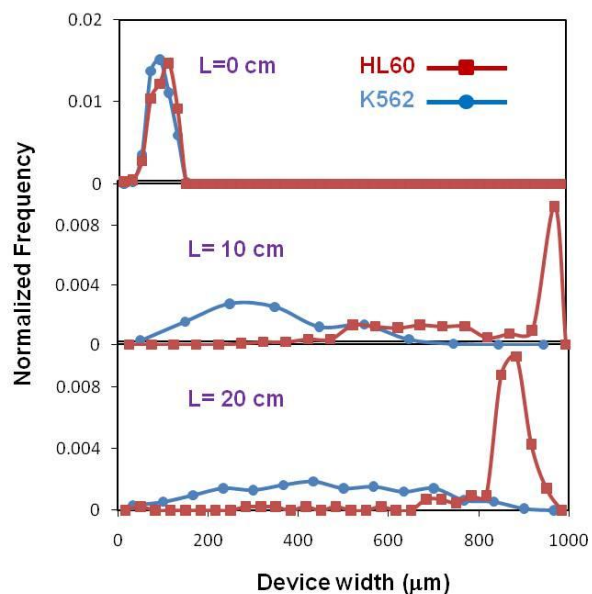


Figure 2: Distribution of the flux of HL60 and K562 cells evolving along the length of device.

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