Steering trajectories of rolling cells by 2D asymmetric receptor patterning

Steering Trajectories of Rolling Cells by 2D Asymmetric Receptor Patterning

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***Abstract*—We demonstrate a simple method to make high-resolution P-selectin patterns based on microcontact printing for controlling cell rolling. SEM characterization revealed well-defined patterns that could direct the trajectories of rolling HL60 cells along the edges. Cell tracking revealed that velocities of rolling cells were higher at the edges as compared to plain P-selectin regions, with a maximum at an edge angle of 20º. The distance traveled by the HL60 cells along the edges decreased with increasing edge angles. These substrates may be used to tune cell adhesion and control the transport of the cells for separation and analysis.**

I. INTRODUCTION

Cell rolling is a physiological phenomenon exhibited by several types of cells including leukocytes, hematopoietic stem cells and cancer cells, involving transient receptor-ligand interactions mediated by glycoproteins known as selectins [1]. We have discovered that when rolling HL60 cells encounter an asymmetric P-selectin edge, an offset between the net force acting on the cell due to fluid flow and forces exerted as the transient adhesive bonds dissociate cause the cell to undergo asymmetric rolling motion and follow the edge [2]. We envision a device for continuous-flow, label-free separation of cells by integrating multiple asymmetric receptor patterned surfaces. This development requires engineered substrates with well-defined patterns for integration into a device, and knowledge of cell rolling on patterned substrates. In this work, we use microcontact printing to pattern P-selectin edges and characterize cell rolling on the patterned surface.

II. EXPERIMENTAL

Microcontact printing (μ-CP) stamps that defined the pattern were fabricated in polydimethylsiloxane (PDMS) by SU-8 molding process. The stamp with multiple straight bands was first inked with a solution of PEG ((1-Mercaptoundec-11-yl)tetra(ethylene glycol), Sigma-Aldrich) molecules, dried, and pressed onto a gold surface to be patterned. The bare gold areas were then filled with P-selectin receptors (Fig. 1). SEM (Jeol 6700) was used to characterize the patterned surfaces. HL60 myeloid cell suspension (~10^5 cells/mL) was flowed over the surface in flow chamber (Glycotech, Inc) at shear rates of 0.5 dyn/cm^2 and edge angles ranging from 5º to 25º. Images of HL60 cells rolling on patterned P-selectin edges were processed using Matlab to obtain rolling velocities, distance traveled along the edge before detachment.

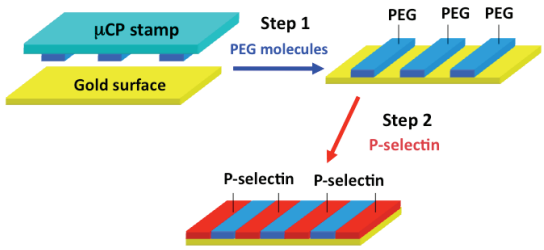

Figure 1. *μ-CP* of P-selectin on a gold substrate.

III. RESULTS AND DISCUSSION

In our earlier work, we patterned multiple edges by microfluidic patterning technique and demonstrated proof-of-concept separation of HL-60 cells from microspheres [4]. Here, we prepared P-selectin patterns at different edge angles by microcontact printing since it has more flexibility in terms of pattern geometry and minimum pattern size as compared with microfluidic patterning. The resulting patterns had well-defined edges that were sharp and straight, as revealed by SEM (Fig. 2). The surfaces were used to characterize rolling behavior of HL60 cells. Fig. 3 shows tracks of HL60 rolling on P-selectin edges at a shear stress of 0.5 dyn/cm^2. Cells were clearly seen to roll on plain P-selectin regions, encounter an edge, and then roll along the edge at an angle to the direction of fluid flow.

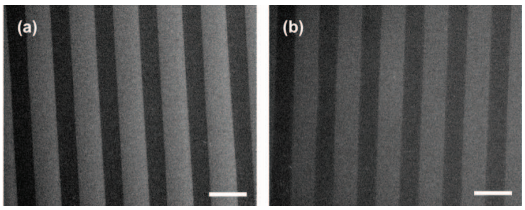

Fig 2. SEM images of surfaces after (a)PEG printing and (b) P-selectin incubation (bright areas correspond to PEG regions). The scale bars are 100 μm.

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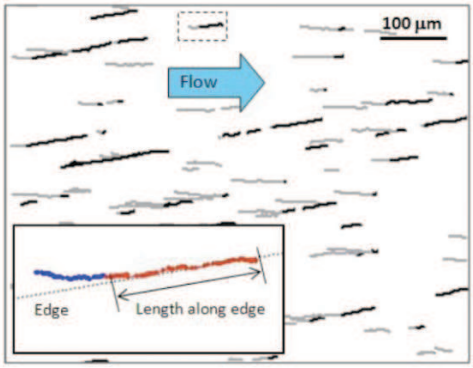

Fig. 3. Automated Matlab analysis of HL60 cells rolling on P-selectin patterns at a shear stress of 0.5 dyn/cm^2 and edge angle of 11°. Black and red lines depict tracks of rolling cells on edge; grey and blue lines depict cells inside patterns.

Data analysis allowed for extraction of the length traveled along the edges (l),and the lateral deflection (d) of HL60 cells rolling on P-selectin edges at different edge angles at a fixed shear stress of 0.5 dyn/cm^2 (Fig. 4). At an edge angle of 6.6º, the cells rolled an average distance of more than 120 μm along the edges before detachment. As the edge angle was increased, the average length on the edges decreased, showing that the ability of the cells to roll along the edges is reduced with increase in edge angle. The lateral deflection that a cell undergoes after rolling a distance l along an edge is given by $d = l \cdot \sin\alpha$, where α is the edge angle. The lateral displacement is important for continuous flow separation of cells by displacing the cells from one stream into another. In our studies, we observed that the deflection exhibited a maximum between the edge angles of 11º and 15.3º (Fig. 4(b)).

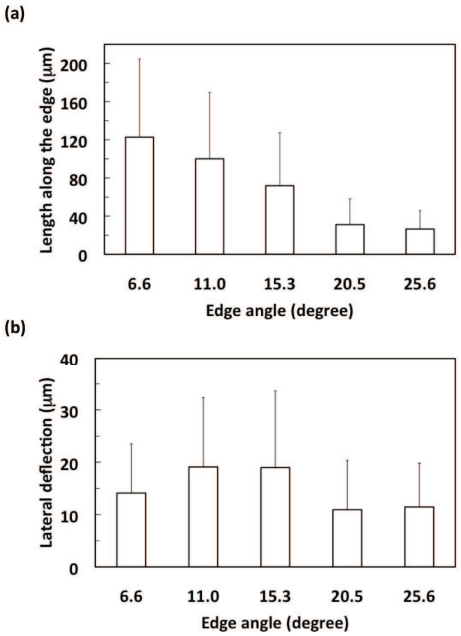

Fig. 4. (a) the length along the edges (l), and (b) the lateral deflection (d) vs. edge angles at the shear stress of 0.5 dyn/cm^2. The error bars in represent the standard deviation.

In addition to the ability to control the direction of rolling, patterned edges can also affect the rolling velocity of the cells. We expect that increasing edge angles will lead to a decreased area of interaction between the cell and the surface, leading to increases in rolling velocity. Cell tracking revealed that velocities of rolling cells were higher at the edges as compared to plain P-selectin regions (3.5μm/s). This effect is clearly visible in a comparison of the rolling velocities of cells at different edge angles at a shear stress of 0.5 dyn/cm^2 (Fig. 5). The rolling velocity increased from 4 μm/s for an edge angle of 6.6º to 7 μm/s at an edge angle of 20.5º. However, we observed that the cell rolling velocity exhibited a maximum at an edge angle of 20.5º, and decreased when the edge angle was increased to 25.6º. A possible explanation for this observation is that the cells with weaker adhesive interactions were unable to roll along the edge, while cells with stronger interactions continued rolling on the edges.

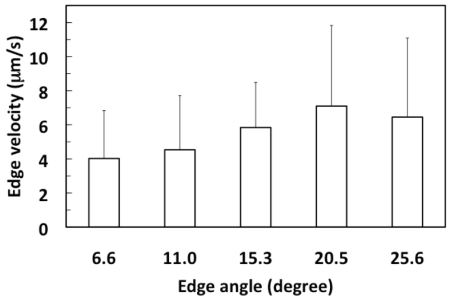

Fig 5. (a) Edge rolling velocity vs. edge angle. Error bars represent one standard deviation.

IV. CONCLUSIONS

In the present work, we demonstrate feasibility of microcontact printing as an easy, versatile method to pattern P-selectin on substrates and characterize cell rolling behavior on the patterns. Such substrates could find applications in separation and sensing of cells for point-of-care diagnostics and therapeutics.

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