EXAMING LATERAL DISPLACEMENT OF CELLS ROLLING ON ASYMMETRIC RECEPTOR PATTERNS

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ABSTRACT

We demonstrate control of HL60 cell rolling trajectories using parallel asymmetric lines of P-selectin receptors patterned using microcontact printing. Cells tracked along edges of the receptor patterns resulting in lateral displacements in the range of 7.5 to 12.5µm per edge, which reached a maximum at an edge inclination angle between 5° and 10°. Detachment of rolling cells after tracking along the edge was consistent with a Poisson process of history-independent interactions. This work supports the feasibility of label-free cell separation and analysis via asymmetric receptor patterns in microfluidic devices.

KEYWORDS: Cell rolling

INTRODUCTION

Separation and isolation of cells from a heterogeneous population is important for diagnostic and therapeutic applications and for biomedical research. We have shown that the trajectories of cells rolling on a substrate can be controlled using asymmetric receptor-patterned edges [1, 2]. This lateral displacement of cells orthogonal to a flow stream presents a new opportunity for label-free separation and analysis of cells. We envision a device for label-free separation or analysis of cells that exploit cell rolling on asymmetric receptor patterns. Characterization of cell rolling trajectories on these engineered substrates is important for design of such devices.

THEORY

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]. HL60 cell surfaces express a specific ligand known as P-selectin glycoprotein ligand-1 (PSGL-1), which binds reversibly to the receptor P-selectin to enable rolling. 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]. Further development of engineered substrates with well-defined multiple patterns for integration into a device requires knowledge of cell rolling on patterned substrates.

EXPERIMENTAL

Microcontact printing 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 thiolated PEG ((1-Mercaptoundec-11-yl)tetra(ethylene glycol), Sigma-Aldrich) molecules, dried, and pressed onto a gold surface to be patterned. After selective deposition of PEG molecules, the bare areas were then incubated within P-selectin solution at the concentration of 15µg/mL. (Figure 1) for 3 hours. SEM (Jeol 6700) was used to characterize the patterned surfaces. HL60 myeloid cell suspension (~10⁵ cells/mL) was flowed over the surface in flow chamber (Glycotech, Inc) at shear rates of 0.5 dyn/cm² and edge angles ranging from 5° to 20°. Images of HL60 cells rolling on patterned P-selectin edges were processed using Matlab. An algorithm was developed to track the cells and identify sections of the tracks that represented cells rolling on an edge. These data were then used to extract the edge tracking lengths, l, lateral displacement d and rolling velocities vₚ and vₑ (Figure 2).

RESULTS AND DISCUSSION

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The P-selectin patterns prepared by microcontact printing had well-defined edges that were sharp and straight, as revealed by SEM (Figure 2). Rolling experiments were performed by incorporating the patterned substrates in a commercial flow chamber to quantify cell rolling of HL60 cells, which are widely used as a model to study leukocyte rolling. 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 (Figure 2). Figure 2 also shows the tracks of HL60 cells along these patterned substrates at different edge inclination angles (α = 5°, 10°, 15° and 20°) at a shear stress of 0.5 dyn/cm². The results show that increasing α significantly decreased l_e (Figure 3(A)). Also, there is an increase in v_e, compared to v_p (Figure 3(B)). The average rolling velocity on the edge v_e increased from α of 5° to that of 20°, though this trend did not reach a degree of statistical significance. In contrast, v_e,y increased significantly with increasing α (Figure 3(C)). Thus, receptor patterns characterized by large edge inclination angles led to greater lateral displacement of cells over a fixed rolling duration. Furthermore, the cumulative probability and the distribution of edge tracking length show that detachment of rolling cells after tracking along the edge was consistent with a Poisson process of history-independent interactions (Figure 4(A)). This correlation suggests that detachment of rolling cells from receptor-functionalized edges is a random process that is affected measurably by cell-surface interactions over the device timescales considered. We also predict the variation of lateral displacement with edge inclination angle based on an exponential fit which agreed well with the experimental data (Figure 4(B)). The curve predicts an optimal edge inclination angle between 5° and 10° that maximizes the lateral displacement for rolling along a single edge. These experimental data indicating the angle dependence of tracking length and its Poisson distribution enable simulation of the rolling trajectories of HL60 cells in a simple device. The prediction of cell tracks can be used to support the feasibility of label-free cell separation via asymmetric receptor patterns in microfluidic devices.

**Figure 2.** Top: Illustration of a typical cell rolling trajectory on the asymmetric patterns: The cell binds within the receptor line, and rolls in the direction of shear flow toward the pattern edge. Bottom: Blue lines depict tracks obtained by image analysis. Inset shows SEM images of the substrate after patterning PEG and P-selectin.

**Figure 3.** Effect of edge inclination angle α on rolling behavior of HL60 cells (τ=0.5 dyn/cm²). Variation of (A) edge tracking length, l_e; (B) rolling velocities v_p and v_e within the P-selectin lines and on the edge, respectively, and (C) lateral displacement, d.
Figure 4. (A) Cumulative distribution function (CDF) of edge tracking lengths $l_e$ (filled triangles) was fitted to a Poisson distribution. Insets show the frequency distribution of the experimentally measured edge tracking lengths, along with that predicted by the Poisson distribution fit to the CDF (solid lines). ($\alpha=10^\circ$, $\tau=0.5\text{dyn/cm}^2$); (B) Variation with the edge inclination angle of the average value of the lateral displacement obtained from the experimental data (bars) and an exponential fit (solid lines) ($\tau=0.5\text{dyn/cm}^2$).

CONCLUSION

In summary, we have designed substrates with multiple P-selectin patterns by a versatile method utilizing microcontact printing. These substrates were incorporated in a flow chamber for studying HL60 cell rolling behavior including quantification of edge tracking lengths and cell rolling velocities. Among the parameters we considered, the pattern edge inclination angle modulated the cell rolling trajectory most strongly. In addition, the nature of cell rolling and detachment along the edge was consistent with Poisson process. The ultimate goal is to develop devices that incorporate asymmetric receptor patterns for point-of-care diagnostic and therapeutic applications where rapid cell sorting with minimal cell processing would be beneficial. In addition to separation, the ability to quantify cell rolling behavior on asymmetric patterns may be useful for analysis of cellular properties and surface ligand expression that influence the adhesive behavior of cells, without necessarily separating the cells. Our work indicates the feasibility of realizing such devices, and also provides quantitative tools for future device design.

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