The Effect of P-selectin Pattern Width on HL-60 Cell Rolling Behavior

by

Minhee Sung

Submitted to the Department of Mechanical Engineering in partial fulfillment of the requirements for the degree of Bachelor of Science in Engineering as Recommended by the Department of Mechanical Engineering/Course 2-A at the MASSACHUSETTS INSTITUTE OF TECHNOLOGY

June 2010

© 2010 Minhee Sung, 2010. All rights reserved.

The author hereby grants to MIT permission to reproduce and to distribute publicly paper and electronic copies of this thesis document in whole or in part in any medium now known or hereafter created.

Signature of Author:

Department of Mechanical Engineering

May 21, 2010

Certified by:

Rohit Karnik
d'Arbeloff Assistant Professor of Mechanical Engineering
Thesis Supervisor

Accepted by:

John H. Lienhard V
Professor of Mechanical Engineering
Chairman, Undergraduate Thesis Committee
The Effect of P-selectin Pattern Width on HL-60 Cell Rolling Behavior
by
Minhee Sung

Submitted to the Department of Mechanical Engineering
on May 21, 2010 in partial fulfillment of the
requirements for the degree of
Bachelor of Science in Engineering as Recommended by the Department of
Mechanical Engineering/Course 2-A

Abstract
The effect of varying the width of P-selectin band patterns on the rolling behavior of HL-60 myeloid cells along edges of the band patterns was studied. The P-selectin and polyethylene glycol (PEG) pattern was produced via microcontact printing using a polydimethylsiloxane (PDMS) stamp. HL-60 cells were flowed across the pattern using a syringe pump at low and high volume flow rates, and the lengths of the cells’ tracks along the edge and velocities along the edge were obtained using a designed Matlab code. It was found that on the 5\textmu m P-selectin band with patterns, the cells were moving horizontally instead of along the edge because the patterns were too thin, while they were rolling along the edge for the wider patterns. The 10\textmu m, 15\textmu m, and 20\textmu m patterns reflected the expected trend of resulting in increased track length along the edge with increasing P-selectin widths. The 15\textmu m band width corresponded to the highest rolling velocities along the edge. The 5\textmu m width gave the next to highest velocity which resulted likely because the 5\textmu m had the smallest P-selectin area to which the cells could interact, except that the cells were not rolling along the edge at this width. In addition, the 10\textmu m band width resulted in the lowest rolling edge velocity, which was unexpected. As expected, the rolling velocities along the edge for the 10\textmu m and 15\textmu m decreased when the flow rate was increased because only the cells with the strongest adhesion interactions remained on the patterns under higher shear stress. But the 5\textmu m and 20\textmu m patterns showed the opposite trend of decreasing with increasing flow rate. The unexpected results suggest that there are still important considerations to be studied when researching cell rolling behavior.

Thesis Supervisor: Rohit Karnik
Title: d'Arbeloff Assistant Professor of Mechanical Engineering
Acknowledgements
The author would like to extend her utmost gratitude to Professor Rohit Karnik, Chia-Hua Lee, and Suman Bose for their constant support and guidance throughout the development of this thesis.
THIS PAGE INTENTIONALLY LEFT BLANK
# TABLE OF CONTENTS

Abstract .................................................................................................................................. 3  
Acknowledgements.................................................................................................................. 5  
1 Introduction .......................................................................................................................... 9  
2 Experimental Methods ......................................................................................................... 11  
  2.1 Materials ........................................................................................................................ 11  
  2.2 Microcontact Printing Stamp Fabrication .................................................................... 11  
  2.3 Pattern Fabrication ........................................................................................................ 12  
  2.4 Cell Culture .................................................................................................................. 13  
  2.5 Cell Rolling Experiments .............................................................................................. 13  
3 Data Analysis ........................................................................................................................ 14  
  3.1 Cell Track Determination .............................................................................................. 14  
  3.2 Cell Track Analysis ....................................................................................................... 15  
4 Results .................................................................................................................................. 17  
5 Discussion .............................................................................................................................. 19  
  5.1 Average Track Length Along the Edge ........................................................................ 19  
  5.2 Average Rolling Velocity Along the Edge ..................................................................... 19  
6 Conclusion ............................................................................................................................. 20  
7 Bibliography .......................................................................................................................... 22  

# LIST OF FIGURES

Figure 1. Neutrophils rolling on endothelial cells via PSGL-1, P-selectin interactions

Figure 2. Images of the 5μm, 10μm, 15μm, and 20μm P-selectin width patterns and respective PEG widths of 30μm, 30μm, 35μm, and 40μm.

Figure 3. Tracks of cells rolling on 5μm, 10μm, 15μm, and 20μm P-selectin widths

Figure 4. Image before and after contrast correction
**Figure 5.** Edges drawn by Matlab code using coordinates of two determined edges on a 15µm P-selectin pattern

**Figure 6.** Average track length along the edge among varying P-selectin widths and flow rates

**Figure 7.** Average velocity along the edge among varying P-selectin widths and flow rates

**LIST OF TABLES**

**Table 1.** Average track lengths at varying P-selectin widths and volume flow rates

**Table 2.** Average velocities along P-selectin edges \( V_{\text{edge}} \)
1 Introduction

Continuous flow cell separation techniques hold much importance in the field of medical diagnostics. However, the current methods of cell type separation using ligand-receptor interactions involve many processing steps such as labeling of the cells and label removal, which is not ideal for point-of-care diagnostics or for cells that are sensitive to such processing\(^1\). In addition, developing countries do not have the luxury of having the expensive equipment and materials needed for such processing. Therefore, an alternative method of using receptor patterned surfaces for cell separation based on transient adhesive receptor-ligand interactions of cells seems to be promising in that such methods would require little processing steps with no need for labeling while retaining the specificity provided by these interactions\(^2\).

Cell rolling is the behavior in which cells form transient ligand-receptor bonds as they flow past surfaces, usually in the blood stream along the walls of blood vessels (see Figure 1). The ligand-receptor bonds dissociate readily and are easily broken by shear stresses caused for instance by the flow of blood. This cell rolling behavior is primarily mediated by glycoproteins called selectins. Leukocytes, neutrophils, metastatic cancer cells, and hematopoietic stem cells make use of this rolling phenomenon to perform important physiological functions, thereby making these cell types receptive to cell separation via ligand-receptor interactions. For instance, leukocytes roll along vascular endothelium to reach sites of inflammation and metastatic cancer cells use rolling to reach secondary sites of tumor formation\(^3\).

![Figure 1. Neutrophils rolling on endothelial cells via PSGL-1, P-selectin interactions\(^5\).](image-url)
It has been shown that through surface patterning of receptors, specifically P-selectin, cell rolling trajectories could indeed be directed via an edge effect in two dimensions\(^2\). The cells used for study were HL-60 myeloid cells because these cells express a significant quantity of P-selectin glycoprotein ligand-1 (PSGL-1), proteins that interact well with P-selectin ligands, making these HL-60 cells similar to leukocytes. When the HL-60 cells encountered an angled edge of P-selectin patterning, a majority of them would follow the edge at the angle to the horizontal fluid flow. Therefore, it seems promising to pursue the application of cell rolling behavior for continuous flow cell separation. However, there remain many questions that need to be answered in order to implement this technique such as what is the optimal angle to promote the edge effect, do different cell types exhibit different rolling behavior, and the question that is addressed in this paper: what is the effect of P-selectin pattern width on the rolling behavior of cells? This study would provide insight on the effect of P-selectin pattern width on rolling when the width is comparable to the diameter of rolling cells.

In this study, HL-60 cells were used as a model for leukocyte rolling. HL-60 cells were flowed at low (75\(\mu\)L/min) and high (300\(\mu\)L/min) flow velocities via a syringe pump across a PEG (polyethylene glycol) and P-selectin angled pattern on gold-coated glass slides. The high-definition pattern was produced by microcontact printing of self-assembling PEG molecules in order to block rolling on selected areas followed by incubation of P-selection by physisorption\(^4\). The pattern contained four different P-selectin widths: 5\(\mu\)m, 10\(\mu\)m, 15\(\mu\)m, and 20\(\mu\)m, with PEG gaps of 30\(\mu\)m, 30\(\mu\)m, 35\(\mu\)m, and 40\(\mu\)m respectively in between the P-selectin lines. The pattern was at an angle of 10° with respect to the direction of fluid flow. Image sequences were captured using a camera mounted to a microscope, after which the images were analyzed using a Matlab code to identify tracks and analyze them for track length and rolling velocity along the edge\(^4\). The results were analyzed to study the effect of P-selectin band widths on cell rolling behavior along an edge, which could give further insight and therefore headway in producing a robust continuous flow cell separation technique.
2 Experimental Methods

2.1 Materials
Prior to use for experiments, the gold-coated glass slides (EMF Corp in Ithaca, NY) were cleaned using piranha solution consisting of a 3:1 ratio of sulfuric acid to 30% hydrogen peroxide in order to rid them of organic residue. The slides were then washed with pure ethanol (EtOH; Pharmo-AAPER), then deionized (DI) water, ethanol again, and finally dried with Nitrogen (N₂) gas. (1-Mercaptoundec-11-yl)tetra(ethylene glycol), a PEG alkanethiol, was diluted in pure ethanol to a 5 mM concentration prior to use in microcontact printing and the recombinant human P-selectin (R&D Systems Inc.) was diluted to 15 μg/mL in 1X Dulbeco’s phosphate buffered saline or DPBS (Mediatech, Inc.). The sulfuric acid, hydrogen peroxide, and PEG alkanethiol derivative were all purchased from Sigma-Aldrich. The PEG alkanethiol, (1-Mercaptoundec-11-yl)tetra(ethylene glycol), will be referred to as “PEG”.

2.2 Microcontact Printing Stamp Fabrication
The microcontact printings stamps were fabricated from polydimethylsiloxane (PDMS) via a SU-8 molding process. The stamp consisted of four sections for the four different P-selectin widths: 5μm, 10μm, 15μm, and 20μm, and respective PEG widths of 30μm, 30μm, 35μm, and 40μm (see Figure 2). The widths of the corresponding PEG lines for each P-selectin width was chosen based on cell size and fabrication capabilities. All the lines were at a 10° angle with respect to the horizontal. The angle was chosen based on previous work that showed angles at such a range showed maximum cell deflection⁴. The stamp was stored in pure EtOH when not in use.
Figure 2. Images of the 5μm, 10μm, 15μm, and 20μm P-selectin width patterns and respective PEG widths of 30μm, 30μm, 35μm, and 40μm. The light regions are the P-selectin and the dark regions are the PEG.

2.3 Pattern Fabrication
The PEG/P-selectin pattern was fabricated onto the gold slide via a two-step method, where the first step consisted of inking the stamp with PEG, and the second step was incubating the PEG pattern with P-selectin. For the first step, 5μM PEG solution (made from 3.8μL PEG diluted in 200μL pure ethanol) was mixed by ultrasound and filtered through a syringe filter. The stamp was inked with the PEG solution and dried for thirty minutes. The piranha-cleaned gold slide was then stamped gently with the PEG-inked stamp for thirty seconds, after which it was rinsed with EtOH, DI water and dried with N₂ gas.
In the second step, the PEG pattern was subsequently incubated with 75μL of 15μg/mL P-selectin for three hours using a sealed perfusion chamber, after which the gold slide was cleaned several times with DPBS. Care was taken to make sure the pattern was wet at all times with DPBS because drying out would ruin the P-selectin pattern. After rinsing, the gold slide was then stored in a Petri dish filled with DPBS.

2.4 Cell Culture
The cells used for experiments were HL-60, human promyelocytic leukemia cells, which were cultured in Dulbecco’s Modified Eagle Medium within Invitrogen 75cm² tissue culture polysterene flasks. A cell concentration of about 10⁵ cells/mL was maintained.

2.5 Cell Rolling Experiments
HL-60 cells (concentrated to about 10⁶ cells per mL) were flowed across the PEG/P-selectin patterned gold slide using a rectangular flow chamber (Glycotech Inc.) of the following dimensions: width = 1.0cm, length = 6 cm, thickness = 0.005 in. The cells were flowed in via a 5mL syringe (diameter = 11.86 mm) and tubing using a computerized syringe pump (Standard Infuse/Withdraw PHD Ultra Syringe Pump; Harvard Apparatus), where volume flow rate was set at either 75μL/min or 300μL/min, where corresponding shear stresses are 0.5 dyn/cm² and 2 dyn/cm², respectively. The gold slide was sealed to the flow chamber with a vacuum. A series of images of the cells flowing past or rolling along the patterned surfaces were acquired via a camera (Andor iXon 885) mounted to a microscope (Nikon TE2000-U) using a 4X objective. The pictures were taken at about 1 frame per second for 300 seconds, giving a total of 300 images. Prior to each recording, cells were flowed in and allowed to settle onto the patterned gold slide for three to five minutes to promote cell to selectin interaction. After ample time for settling, the cells were then flown in at 75μL/min or 300μL/min, ten seconds after which acquisition of the photos was started, so that the pictures were capturing the overall cell rolling behavior, not the immediate effect from suddenly starting an influx of cells. For each P-selectin width, experiments were run at least three times, giving three different image sequences to be analyzed to ensure accuracy of results.
3 Data Analysis

3.1 Cell Track Determination
A Matlab code (Mathworks Inc.) was developed by Suman Bose to analyze the sequence of pictures taken in order to identify the tracks of the cells moving past the patterns. The development of the tracking code is detailed in the Lee et al. paper. The requirements for a track was that the cells identified could not have moved more than 10μm (~diameter of a cell) between consecutive frames and they had to be present in all the frames for the given track (Figure 3). Such requirements eliminated illegitimate tracks of cells that traveled to more than one patterned P-selectin line. Additionally, in order to eliminate any random tracks, any tracks less than 100μm were removed.

![Figure 3. Tracks of cells rolling on 5μm, 10μm, 15μm, and 20μm P-selectin widths.](image-url)
3.2 **Cell Track Analysis**

In order to determine the track length and velocity along the edge, the edge of the patterns needed to be determined. The edge was defined for each image sequence by adjusting the contrast of the first of the image series (Figure 4) so that the pattern became visible and identifying two edges separated by at least ten gaps (Figure 5). The \((x,y)\) coordinates were found along each of the two edges, giving a total of four points. After the edge determination, the code was written such that any tracks that ended within at least 10\(\mu\)m of the four-point determined edge were retained. Additionally, any tracks that were cut off by the field of view or existed before or after the image sequence was taken were removed in order to ensure that the tracks retained were complete.

![Figure 4. Image before and after contrast correction. a) Image before contrast correction b) Image after contrast correction](image)

The retained tracks could be separated into three categories: 1) tracks of cells that rolled on the P-selectin region but not along the edge, 2) tracks of cells that began rolling in the P-selectin region and then started to roll along the edge, and 3) tracks of cells that began with rolling on the edge. The rolling velocity along the edge was computed by dividing distance traveled along the edge by the time elapsed. The details of how the code was written to actually calculate the velocity of rolling cells along the edge \((V_{edge})\) can be found in the Lee et al paper\(^4\). The length of the retained tracks was also determined.
Figure 5. Edges drawn by Matlab code using coordinates of two determined edges on a 15μm P-selectin pattern. Two (x,y) coordinates for each edge and the number of gaps in between the edges were determined and inputted into the Matlab code which drew the remaining edges in between the chosen two.
4 Results

For each P-selectin width and flow velocity, experiments were run at least three to four times. Averages of the track lengths and velocities of rolling cells in along the edge were taken (Tables 1—2). Bar graphs were made of the data (Figures 6—7).

Table 1. Average track lengths at varying P-selectin widths and volume flow rates

<table>
<thead>
<tr>
<th>P-selectin Width</th>
<th>Average Track Length at 75 μL/min flow rate (μm)</th>
<th>Average Track Length at 300 μL/min flow rate (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μm</td>
<td>108.4723 ± 12.9553</td>
<td>145.1574 ± 17.5242</td>
</tr>
<tr>
<td>10 μm</td>
<td>45.7947 ± 11.1708</td>
<td>45.1437 ± 1.6352</td>
</tr>
<tr>
<td>15 μm</td>
<td>52.6010 ± 2.3971</td>
<td>49.4754 ± 6.8190</td>
</tr>
<tr>
<td>20 μm</td>
<td>78.9163 ± 8.1903</td>
<td>91.2778 ± 24.3061</td>
</tr>
</tbody>
</table>

Table 2. Average velocities along P-selectin edges ($V_{edge}$)

<table>
<thead>
<tr>
<th>P-selectin Width</th>
<th>Average $V_{edge}$ at 75 μL/min flow rate (μm/s)</th>
<th>Average $V_{edge}$ at 300 μL/min flow rate (μm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 μm</td>
<td>2.2569 ± 1.7087</td>
<td>2.6165 ± 0.6642</td>
</tr>
<tr>
<td>10 μm</td>
<td>1.1487 ± 0.4609</td>
<td>1.6512 ± 0.5560</td>
</tr>
<tr>
<td>15 μm</td>
<td>3.3822 ± 1.1352</td>
<td>3.4589 ± 0.7846</td>
</tr>
<tr>
<td>20 μm</td>
<td>1.6630 ± 0.2099</td>
<td>2.8031 ± 0.2070</td>
</tr>
</tbody>
</table>
Figure 6. Average track length along the edge among varying P-selectin widths and flow rates.

Figure 7. Average velocity along the edge among varying P-selectin widths and flow rates.
5 Discussion

5.1 Average Track Length Along the Edge
For the 5µm P-selectin width patterns, the cells were primarily moving horizontally rather than along the edge (see Figure 3) likely because the patterns had too little P-selectin area to provide proper adhesion for edge rolling, whereas for the 10µm, 15µm, and 20µm patterns, the cells were rolling primarily along the pattern edges because there was sufficient P-selectin area on which to roll. Therefore, if the 5µm width data is disregarded because the cells were not rolling on the edge, there is an expected trend of increasing average track length with increasing P-selectin width from 10µm to 20µm (Figure 6). This trend was the same for both volume flow rates of 75µl/min and 300µl/min. Interestingly, the edge lengths for the 15µm versus 20µm P-selectin widths are quite different from each other. We would expect the 15µm and 20µm widths to show similar results because both widths are greater than the width of a cell, but instead the 20µm has a far longer average edge length. This may have occurred because in the experiments, the patterns for the 5µm and 20µm were more well-defined than the 10µm and 15µm or there may be an unforeseen cell rolling behavior occurring at these widths.

5.2 Average Rolling Velocity Along the Edge
The bar graphs depicting the average rolling velocities along the edge among varying P-selectin pattern band widths (Figure 7) show that the 15µm width corresponds to the greatest rolling velocity. One would expect the wider the P-selectin patterns, the lower the rolling velocity because there is more selectin area to which the cells can interact with. We do indeed see the next to highest rolling velocity from the 5µm pattern, which can be explained by the fact that these patterns have the smallest area for cells to have ligand-receptor interactions with, except as discussed earlier these cells were not rolling on the edge. Also, the 10µm data corresponds to the lowest rolling velocity along the edge. The 10µm data can be incorrect because the stamped pattern was inconsistent because there were splotches in the pattern being that it was difficult to achieve patterns of such small widths.
The rolling velocities changed when the volume flow rate was increased from 75µL/min to 300µL/min. At 300µL/min, the 20µm instead of the 5µm P-selectin pattern now corresponded to the second highest rolling velocity. The 15µm pattern still showed the highest rolling velocity. It has been shown that higher shear stresses due to higher volume flow rates led to lower rolling velocities because it is likely that at high shear stresses, only cells that have the strongest adhesive interactions remain to roll on the selectin. This was further corroborated with the fact that under a 300µL/min flow rate, most of the cells rolling on the patterns lost ligand-receptor interactions and flew off the patterns due to the high shear stress. Therefore, there were far fewer cells rolling on surfaces under 300µL/min than under 75µL/min. However, our data does not seem to entirely show this trend. The rolling velocities for the 10µm and 15µm did decrease as expected, but those of the 5µm and 20µm increased instead. The reasons for these trends in rolling velocities are unclear and may suggest rolling behaviors not yet considered.

### 6 Conclusion

The effect of P-selectin pattern band widths on the rolling behavior of HL-60 myeloid cells were studied. On the 5µm P-selectin band with patterns, the cells were moving horizontally instead of along the edge because the patterns were too thin, while they were rolling along the edge for the wider patterns. The 10µm, 15µm, and 20µm patterns reflected the expected trend of resulting in increased track length along the edge with increasing P-selectin widths. The 15µm band width corresponded to the highest rolling velocities along the edge. The 5µm width gave the next to highest velocity which resulted likely because the 5µm had the smallest P-selectin area to which the cells could interact, except the cells were not rolling on an edge at this width. The 10µm band width resulted in the lowest rolling edge velocity. As expected, the rolling velocities along the edge for the 10µm and 15µm decreased when the flow rate was increased because only the cells with the strongest adhesion interactions remained on the patterns under higher shear stress. But the 5µm and 20µm patterns showed the opposite trend of decreasing with increasing flow rate.
These unexpected results suggest that there are still important considerations to be studied when researching cell rolling behavior. To better understand the details of cell rolling behavior, future work would include honing in a method to produce robust PDMS stamps especially for the small width patterns and also including in the study the effect of varying shear stresses along with the varying P-selectin widths. By pursuing such research, a novel continuous flow, label-free cell separation device could be engineered, one that could greatly impact the field of medical diagnostics.
7 Bibliography


