Nanomechanical Control of Cell Rolling in Two Dimensions through Surface Patterning of Receptors

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ABSTRACT

We envisioned that label-free control of the transport of cells in two dimensions through receptor–ligand interactions would enable simple separation systems that are easy to implement yet retain the specificity of receptor–ligand interactions. Here we demonstrate nanomechanical control of cell transport in two dimensions via transient receptor–ligand adhesive bonds by patterning of receptors that direct cell rolling through an edge effect. HL-60 cells rolling on P-selectin receptor patterns were deflected at angles of 5–10° with respect to their direction of travel. Absence of this effect in the case of rigid microsphere models of cell rolling suggests that this two-dimensional motion depends on nanomechanical properties of the rolling cell. This work suggests the feasibility of simple continuous-flow microfluidic cell separation systems that minimize processing steps and yet retain the specificity of receptor–ligand interactions.

Techniques for separation of cells rely on the ability to control their transport based on specific characteristics such as size, density, or surface ligands. In particular, control of transport in two dimensions is highly advantageous for the design of continuous-flow separation systems.[1–3] Continuous-flow separation of cells based on specific receptor–ligand interactions is currently limited to external control techniques that harness dielectrophoretic, magnetic, or other forces[4,5] and typically rely on capturing or labeling of the cells. These label-based approaches are very useful for cell separation but involve special apparatus and multiple processing steps for labeling and label removal that are not desirable for sensitive cell samples or for point-of-care diagnostics, especially in third world countries. On the other hand, label-free approaches based on physical characteristics of the cells are often easy to implement but lack the specificity provided by receptor–ligand interactions.[4] Control over the transport of cells based on specific receptor–ligand interactions without labeling and label-removal steps would enable cell separation devices for single use or continuous-flow separation while retaining the specificity of receptor–ligand interactions. In this paper, we wanted to examine whether receptor patterning could be used to achieve nanomechanical control of the transport of cells in two dimensions in a label-free manner through the formation of transient receptor–ligand bonds that result in cell rolling.

The formation of transient receptor–ligand bonds commonly occurs between cells flowing in the blood stream and the vascular endothelium in a physiological process known as cell rolling.[6–8] This phenomenon is mediated primarily by glycoprotein receptors known as selectins, among some other receptors that can also enable cell rolling.[8–11] The adhesive bonds formed by selectins have high dissociation rates and are also responsive to shear stress.[12–14] As the cell rolls under shear force exerted by the flowing blood stream, bonds are formed between the cell and the vascular endo-

thelium on the leading edge of the cell and are broken on the trailing edge of the cell. Leukocytes regulate cell rolling through nanomechanical control via expression of specific adhesion molecules on the surface of specialized 80 nm diameter cell processes known as microvilli that can extend from 350 nm to 1 μm or longer.[15–18] The number of microvilli tethers during cell rolling has been reported to be quite low, on the order of 1−10.[15] Cell rolling has been extensively studied due to its important role in physiological processes such as recruitment of leukocytes to sites of inflammation, homing of hematopoietic progenitor cells after intravenous injection, tumor cell metastasis, and other inflammatory processes.[19–21] Cell rolling has been achieved in vitro and mimicked using microsphere models.[22,23] Researchers have also investigated the possibility of separating cells based on rolling, including a technique for capturing cells in microfluidic devices[24,25] and separation of cells based on differential rolling velocities in a flow chamber.[23] Separation based on cell rolling is applicable to a wide range of cell types such as leukocytes, hematopoietic stem/progenitor cells, and metastatic cancer cells.[19–21,25] Recent work has also shown that coimmobilization of selectins and antibodies that are specific to molecules expressed on the cell surface can affect the rolling behavior of cells, further enhancing the utility of rolling-based separation.[26] Current approaches for separation based on cell rolling exploit the rolling behavior to control the transport of cells in one dimension and rely on differential rolling velocities of different cell types: cells that roll faster elute quickly and are thus separated from slowly rolling cells that are either captured or elute slowly. However, this approach requires all cells to start rolling at the same point in time and requires collection of cell fractions at different points in time, necessitating the use of active mechanisms to separate cells into independent compartments. We envisioned that the ability to control cell rolling in two dimensions would enable a simple system that is very easy to implement and yet retains the specificity due to receptor–ligand interactions (Figure 1b). We therefore explored the possibility of controlling the transport of cells in two dimensions by patterning of selectins on the surface. Herein we demonstrate that edges of P-selectin regions that are at an angle to the direction of fluid flow can be used to direct the motion of rolling cells in two dimensions on the surface (Figure 1a).

We investigated the effect of a single patterned edge of P-selectin on the motion of rolling HL-60 cells, a human myeloid cell line[27] that expresses high levels of P-selectin glycoprotein ligand-1 (PSGL-1) that mediates cell rolling on selectins.[20] The rolling behavior of HL-60 cells is well-characterized in a number of studies, including dependence on shear rate,[20,28] cell rigidity and topology,[29,30] and capture in a microfluidic device.[24] HL-60 cells are robust and easy to maintain and also express levels of PSGL-1 that are comparable to leukocytes, making them suitable candidates for our proof-of-concept study.

Although covalent immobilization of P-selectin enhances surface properties such as functional stability,[31] proof-of-concept studies do not require long-term stability and thus
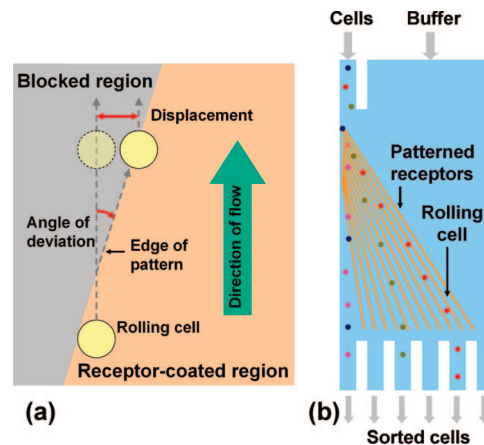

Figure 1. Use of receptor–ligand interactions to direct the motion of cells. (a) Interaction of cells with a receptor-coated substrate may be used to direct the motion of cells along the edge of a receptor pattern. Cells that flow along the direction of fluid flow can be made to follow the receptor edge and thus be diverted from their direction of flow. (b) This two-dimensional control over the transport of cells may be useful for the design of microfluidic devices for continuous flow separation of cells.
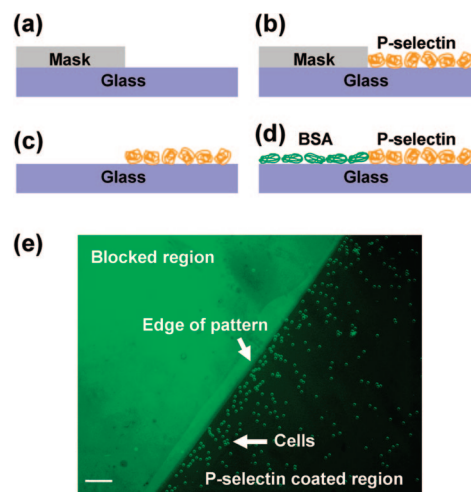
Figure 2. Patterning of P-selectin. A silicone rubber mask was placed on a glass substrate (a), and P-selectin was coated on the exposed area of the substrate by physisorption (b). The silicone mask was then removed from the substrate (c), and BSA was used to block the areas that were not coated with P-selectin (d). Use of fluorescein-labeled BSA enabled visualization of the P-selectin pattern using an epifluorescence microscope (e). HL-60 cells adhered selectively to the P-selectin region, confirming patterning of the substrate with P-selectin. Scale bar: 100 μm.

physisorption of P-selectin on glass substrates is sufficient. We adopted a simple strategy of selective physisorption of P-selectin by using a silicone rubber mask in order to pattern P-selectin on glass substrates (Figure 2) (see Supporting Information). Use of bovine serum albumin conjugated with fluorescein isothiocyanate (BSA-FITC) during the blocking step revealed selective adsorption of BSA in the region occupied by the silicone mask as compared to the P-selectin coated region. The patterns had well-defined edges, showing that the silicone mask did not leak during the physisorption step. Furthermore, when HL-60 cells were flowed over this patterned substrate, the cells selectively interacted with the
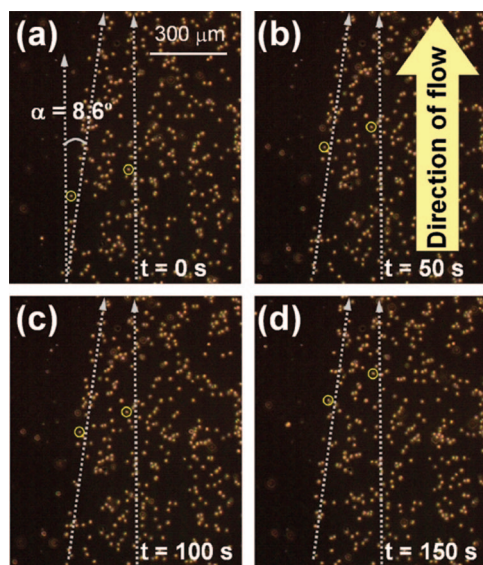
Figure 3. P-selectin edge directs the motion of rolling cells. Rolling HL-60 cells that encountered the edge of a P-selectin pattern making an angle to the fluid flow direction were forced to roll along the edge. The motion of a cell forced to roll along the edge is compared with another cell rolling in the direction of fluid flow, highlighted by circles. The edge succeeded in changing the direction of motion of the rolling cell by 8.6°, resulting in effectively displacing the cell by 0.15 mm from its original position for every 1 mm of length along the direction of flow. Wall shear stress was 1.9 dyn/cm². Video is available in Supporting Information.

region coated with P-selectin, confirming the success of the patterning technique (Figure 2e).

Suspensions of HL-60 cells at densities of $3–5 \times 10^5$ mL^{-1} were flowed over the patterned substrates at a shear stress of 0.32–12.8 dyn/cm² (0.03–1.28 Pa) using a commercially available flow chamber (see Supporting Information). The flow chamber was rectangular with a width of 1 cm, height of either 125 μm (for cells) or 250 μm (for microspheres), and length of 6 cm, with inlet and outlet at either end. Only some of the cells interacted with the surface, and the remaining cells flowed through the chamber without interacting with the surface; here we are interested in only those cells that interacted with the surface. Selective rolling of HL-60 cells was observed on the P-selectin coated region with slower cell rolling velocities than those on the BSA-coated region where cells were not hindered by the formation of adhesive bonds. Typical velocities of the rolling cells in our experiments ranged from 0.3 to 1.2 μm/s for shear stresses ranging from 0.32 to 12.8 dyn/cm², which are either comparable to or smaller than cell rolling velocities reported in other studies.[25,29,31,32] Remarkably, when rolling HL-60 cells encountered the edge of the P-selectin region, they were diverted from their original direction of travel along the direction of the edge, demonstrating that receptor patterning could indeed be used to control the transport of cells through transient receptor–ligand adhesive bonds. This effect was observed only for small angles (<ca. 10−15°) between the edge and the direction of flow, and nearly all cells that encountered the edge were deflected from their original direction of travel and forced to follow the P-selectin edge. No edge effect was observed at larger edge angles; cells that
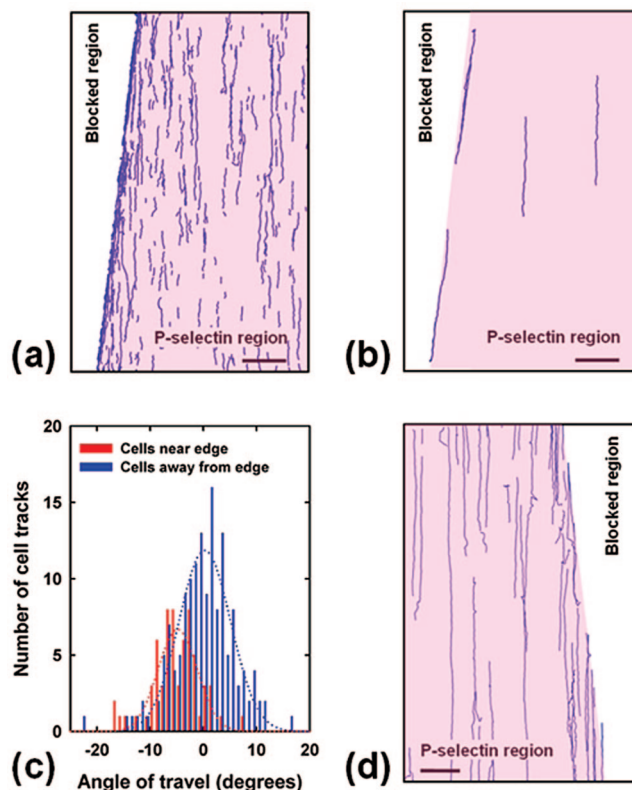
Figure 4. Analysis of cell and microsphere rolling. (a) Matlab tracking of rolling cells generated from a set of 236 images shown in Figure 3 clearly shows the effect of the edge. Inability of cells to cross over the edge resulted in higher density of tracks at the edge. Cell rolling was observed in the P-selectin coated region (pink) but not in the blocked region (white). Scale bar: 300 μm. (b) Longer (>300 μm) tracks of cells rolling on the edge and inside the P-selectin region clearly show that the edge affected the rolling direction. Scale bar: 300 μm. (c) Angular distribution histogram of the direction of travel of cells rolling near the edge (red) with respect to those away from the edge (blue). Wall shear stress was 1.9 dyn/cm² (0.19 Pa, 300 μL/min). (d) Similar experiments done with 9.96 μm diameter sLe^x coated microspheres that roll on P-selectin reveal that the edge did not have a large effect on microspheres as their direction of travel did not change substantially. Wall shear stress was 0.33 dyn/cm² (0.03 Pa, 200 μL/min). Video is available in Supporting Information.

encountered the edge detached from the substrate and continued to flow in the direction of fluid flow. Thus, the direction of travel of the cells could be changed only at smaller edge angles. Figure 3 shows snapshots of cells rolling under a shear stress of 1.9 dyn/cm² (300 μL/min) with two cells highlighted, one cell in the P-selectin coated region that did not encounter the edge and another cell that encountered the edge and was forced to travel along the edge. The cell that encountered the edge was deflected from its direction of fluid flow and traveled at an angle of 8.6° with respect to the other cells that did not encounter the edge, demonstrating that a single P-selectin edge could be used to substantially change the direction of cell rolling and hence control the transport of rolling cells (video available in Supporting Information).

To analyze the rolling behavior of the cells, the sequence of images was processed using Matlab (see Supporting Information). Statically adhered cells were filtered out and
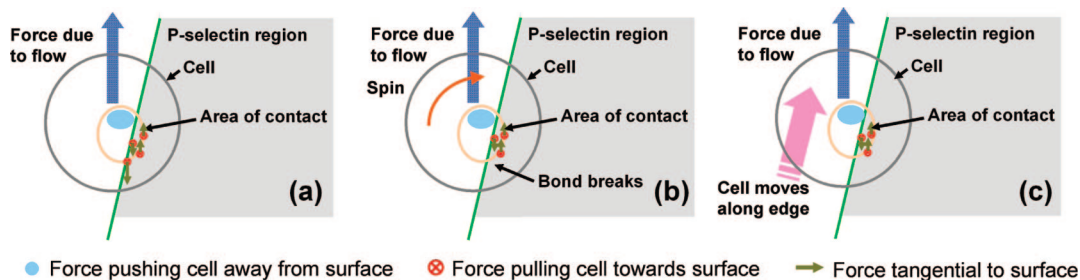
Figure 5. Potential mechanism of cell rolling along a selectin edge. (a) Bonds on the trailing edge experience maximum strain. When these bonds break, it results in an asymmetric rotation of the cell (b) that causes the cell to move along the edge (c). This mechanism is similar to cell rolling on a surface, but in addition to rotation along an axis parallel to the surface, the cell may also spin in plane along an axis perpendicular to the surface as shown in (b). In the case of a rigid microsphere, the area of contact is small and the force due to the flow acts through a point vertically above the area of contact and this asymmetric motion becomes difficult.

tracks of individual cells were plotted, clearly showing the different travel directions of cells rolling on the edge and those rolling inside the P-selectin region (Figure 4a). The image acquisition rate and processing parameters were set so that only those cells that rolled on the surface were tracked. Cells that did not roll moved rapidly as compared to cells that rolled (see Supporting Information), and their large displacements per frame made it impossible to track rolling and free-flowing cells simultaneously. Tracks are not visible in the blocked region as none of the cells rolled in that region. Cells rolling in the P-selectin region that encountered the edge were forced to roll on it instead of crossing over beyond the edge, leading to an accumulation of moving cells being transported at an angle to the fluid flow. This effect is evident in the plotted tracks (Figure 4) but not obvious in the images (Figure 3) because of statically adherent cells that accumulated over a period of time. The effect of the P-selectin edge is very clear if only longer cell tracks are plotted (Figure 4b).

To elucidate the effect of the edge on cell rolling, tracks were divided into two sets: (a) tracks that began within a distance of 30 μm from the edge (cells that encountered the edge), and (b) tracks that began beyond a distance of 90 μm of the edge (cells that were not influenced by the edge). The direction of travel of each track was identified and plotted as a histogram (Figure 4c), with zero angle corresponding to the mean direction of cells rolling in the P-selectin region. Direction of travel of cells that encountered the edge clearly differed from the mean direction of travel of the other cells by 4–10°. This analysis further confirmed the ability of the edge to control the direction of travel of rolling cells. Furthermore, cells near the edge rolled at an average velocity of 1 μm/s, whereas cells away from the edge rolled at an average velocity of 0.5 μm/s. These results demonstrate that the P-selectin edge enabled control over the transport of rolling cells by (a) changing the direction of rolling and (b) increasing the rolling speed.

Similar experiments were performed with Sialyl Lewis(x) (sLe^x) coated microspheres that form transient bonds with P-selectin and are used as models to study cell rolling.[22,23,31] The microspheres rolled selectively on the P-selectin region with average velocity of about 3–4 μm/s at a flow speed of 200 μL/min, corresponding to a shear stress of 0.33 dyn/

cm^2 (0.033 Pa). This velocity is in agreement with our previous work of sLe^x coated microspheres rolling on P-selectin.[31] However, the P-selectin edge did not have a significant effect on the direction of rolling of the microspheres: Almost all microspheres that encountered the edge crossed over beyond the edge and their direction of travel remained unchanged (Figure 4d). Tracks corresponding to these microspheres terminated at the edge instead of following it, as observed in the case of the rolling cells; once the microspheres detached from the edge, they continued flowing in the direction of fluid flow (see Supporting Information) and did not result in tracks due to their much higher speed.

This observation demonstrates that two types of particles that exhibit similar rolling behavior on P-selectin coated surfaces can exhibit dramatically different rolling behavior on patterned P-selectin. This remarkable difference between the rolling behavior of cells and microspheres at the edge that is not evident in one-dimensional rolling provides insight into rolling at the edge and suggests that the edge effect is capable of differentiating rolling particles based on their nanomechanical properties. We hypothesize that when a cell encounters the edge, an offset between the net force acting on the cell due to fluid flow and forces exerted as the adhesive bonds dissociate cause the cell to undergo asymmetric rolling motion and follow the edge (Figure 5). The moment driving the rolling motion in the direction of fluid flow may be expected to be of the order of $F_{drag} \times a$, where a is the radius of the cell or microsphere and F_{drag} is the fluid force acting on the cell. The asymmetric moments that cause the cell to follow the edge may be expected to scale as $F_{drag} \times l_{contact}$, where $l_{contact}$ is the length scale of the contact area within which the cell or microsphere interact with the substrate. This asymmetric moment may be expected to vanish if the area of contact is very small because the net force acting on the cell or microsphere would be aligned with the force due to the adhesive bonds, i.e., $F_{drag} \times l_{contact}$ would not be large enough to sustain this asymmetric motion but $F_{drag} \times a$ would remain relatively unchanged. For the rigid microspheres, the contact length is limited to ~0.4 – 0.6 μm, assuming that either the bonds or linking molecules can extend by 5–10 nm. However, cell rolling depends to a large extent on the mechanical properties of the cell,

including its deformability and the size and extensibility of microvilli, and the contact length can be several micrometers long for rolling HL-60 cells.[15,16,29] Furthermore, rolling cells can extend long tethers due to extension of microvilli to several micrometers that effectively increases the area of interaction between the rolling cell and the substrate.[32,33] Indeed, it is likely to be the reason why sLe[x] coated microspheres selectively rolled on the P-selectin region but did not follow the edge even when it made a small angle with the direction of fluid flow.

Our experiments demonstrate that the transport of cells based on specific receptor–ligand interactions can be controlled in a label-free manner by patterning of receptors that mediate cell rolling. A single edge of P-selectin was capable of substantially changing the trajectory of rolling HL-60 cells with respect to the direction of fluid flow in which the cells would otherwise roll while affecting rolling microspheres to a much lesser extent. This result suggests the feasibility of developing devices based on nanomechanical control of cell rolling for the creation of simple, cost-effective microfluidic systems for separating a population of cells with slight differences in terms of the rolling behavior. Further characterization of rolling on different surfaces and cell types is essential before cell separation devices based on the edge effect can be developed. Practical cell separation devices will need to ensure that all cells interact with the surface, either by decreasing the height of the flow chamber, by gravity, or some other means. Multiple edges will need to be patterned because a single edge may not be sufficient for optimal separation. Because cell rolling is inherently slow, the throughput of such devices will be limited as compared to continuous-flow systems where cell velocities are comparable to the fluid velocity. The operating conditions in these experiments resulted in shear stresses similar to physiological shear stresses experienced by cells in the body. However, higher flow rates may result in shear stresses in large excess of physiological shear stress that may adversely affect the cells. This is a limitation of rolling-based separation, and parallel devices may need to be employed to achieve very high throughput. However, cell separation based on rolling on patterned receptors has the unique advantage in that it can potentially separate cells through specific receptor–ligand interactions in a continuous flow manner without any processing steps. This is a major advantage of this technique, as processing steps such as incubation and label removal are expensive and difficult to perform outside the laboratory. For diagnostic purposes, throughput may not be an issue because small volumes of blood may suffice due to the high density of cells. This technique may therefore be suitable in resource-limited settings for analysis of blood, where sample processing must be minimized. Rolling on patterned receptors may also find use as a research tool for separation of cells and correlating separated fractions with differences in cell behavior. This unique technique may facilitate the incorporation of factors such as cell deformability, morphology, and receptor clustering that influence cell rolling but are typically inaccessible to conventional techniques of cell separation[29,30,34,35] while simultaneously enabling the degree of specificity to be tuned through coimmobilization of antibodies to specific cell markers.[26] With recent research uncovering the links between nanomechanical characteristics of cells and properties such as states of disease[36,37] and homing ability of cancer cells and stem cells,[23] the development of separation techniques based on nanomechanical control of cell rolling through patterning of receptors that depends on both the mechanical properties of cells as well as specific receptor–ligand interactions may prove to be a very useful method for separation and analysis of stem cells, cancer cells, and white blood cells.

Supporting Information Available: Experimental methods. Effect of P-selectin edge on rolling of HL-60 cells. This material is available free of charge via the Internet at http://pubs.acs.org.

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