CONTINUOUS CELL SORTING BY DETERMINISTIC CELL ROLLING
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ABSTRACT
This paper reports a microfluidic cell-sorting device that combines the effect of transient cell receptor-surface ligand interactions and three-dimensional hydrodynamic control by inclined microstructures. The inclined structures not only enhance cell-surface interactions for cell tethering, but also direct the rolling of target cells. This approach enables simple, continuous, rapid, label-free separation of cells and allows for versatile device architectures to manipulate cells based on surface markers.

KEYWORDS: Deterministic cell rolling, Adhesion molecules, Microfluidics, Cell sorting

INTRODUCTION
Fast, efficient isolation of subpopulations of cells from heterogeneous mixtures such as whole blood, is desired in many clinical and research applications. Adhesion-based cell sorting is promising for many applications such as early detection of cancer, isolation of stem cells, and counting of CD4⁺ T-cells. Microfluidics provides advantages, which are specific to adhesion-based separation, such as high surface-to-volume ratio, thin channels for fast settling and adhesion of cells, and higher capture capability. While antibody-functionalized microstructures have been used to enhance cell-surface interactions [1], recovery of target cells is difficult. In contrast, reversible adhesion based on cell rolling enables easy recovery of target cells, but achieving high capture efficiency can be challenging [2].

SORTING PRINCIPLE
To overcome the above challenges, we developed a microfluidic cell-sorting device that enables easy recovery and efficient capture of target cells by utilizing inclined microstructures that alter the flow streamlines and induce repeated collisions between cells and the microstructures (Figure 1) [3]. These collisions result in focusing of non-target cells at the center of the channel by hydrophoresis [3]. In contrast, target cells are captured by the collisions and are deflected into the trench, roll along the trench, and finally detach near the side-ends of the structures (Figure 1).

Figure 1: (a) Capturing of target cells via three-dimensional hydrodynamic control promotes rolling in the lateral direction. (b) Schematic showing trajectories of non-target and target cells passing through the gap between the microstructures coated with specific ligands. Promoting rolling interactions prevents target cells from leaving the trenches. (c) Optical micrographs showing the separation channel formed in PDMS.
EXPERIMENTAL

The device was fabricated in poly(dimethylsiloxane) (PDMS) using soft lithography, with a total channel height of 76 µm and gap height between the top and bottom structures of 20 µm (Figure 1c). The slanting angles of the microstructures relative to the bulk flow were 30° and 45° for the focusing and sorting channel, respectively. The entire channel was incubated with human P-selectin for 3 h and then washed with 2 mg/mL BSA. HL-60 cells that express PSGL-1 and roll on selectins were used as target cells, while K562 cells that do not bind to selectins were non-target cells.

RESULTS AND DISCUSSION

Figure 2a shows the rolling sequence of an HL-60 cell; (1) tethering on the top surface of the microstructure, (2 and 3) rolling in the trench, and (4) detaching from the channel surface. The microstructures inclined to the bulk flow disturb streamlines and induce the repeated collision between cells and microstructures by hydrophoresis [3]. Once cells tether on the microstructure, they are deflected into the trench, continue rolling within the trench, and are finally detached on either of the side-ends of the microstructures. In the trench of the microstructure, rolling cells are exposed to the outward flows toward either of the side walls that direct the motion of the rolling cells. After the complete rolling sequence, HL-60 cells flowed near the side walls. In contrast, K562 cells were focused at the channel center (Figure 2b to 2d).

For evaluation of the rolling process, we defined the sorting efficiency as the ratio of the number of cells going to the target outlet to that of cells going to the non-target outlet. HL-60 is a human myeloid cell line that exhibits rolling on selectins mediated primarily by PSGL-1, while K562 that lacks PSGL-1 does not bind to P-selectin. Thus, HL-60 cells that can roll on the P-selectin coated microstructures can be sorted from K562 cells that can follow the bulk flow. The higher the applied shear rate, the higher the drag force acting on cells, thereby accelerating bond dissociation and decreasing the sorting efficiency (Figure 3). Increasing the P-selectin incubation concentration (from 1 µg/mL to 3 µg/mL) increases the number of the possible bonds formed between cell surface receptors and their ligands, and thus increases the sorting efficiency and potential throughput of the device (Figure 3). After separation of the cell mixture at a flow rate of 6 µL/min, the target and non-target reservoirs exhibited purities of 97.0 ± 2.0 and 90.9 ± 3.1 %, respectively (Figure 3).

Figure 2: (a) Superimposed image of motion sequences of HL60 cell rolling in the channel coated with P-selectin. (b,c) Cells flowing in the channel outlet region after passing the separation channel. (d) Cell flux distributions of K562 and HL60 cells in the outlet region.
**CONCLUSION**

In summary, we demonstrated a new method of affinity cell sorting that uses the selectin-coated inclined microstructures to enhance cell-surface interactions and direct the trajectories of rolling cells. This approach has the distinct advantage of continuous, fast, and efficient label-free cell separation. The simple device architecture can further facilitate massively scalable parallelization to increase the sorting throughput considerably and reach clinically relevant throughput.

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**REFERENCES**


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