Flow-through Cell Separation using Antigen-Antibody Interaction between Cell and Wall Surface

Takahiro Nishimura*, Junichi Miwa**, Shinichi Hashimoto*, Yuji Suzuki* and Nobuhide Kasagi*

We report the development of a continuous label-free micro cell separation method for extraction of rare cells from a limited amount of sample. The separation principle is based on specific adhesion between cells and antibody-functionalized channel wall. Asymmetric pattern of antibody on the wall surface causes cross-flow displacement of the target cells, and they are separated from the other cells. Antibody-immobilized channel wall is fabricated with a versatile technique using functionalized parylene, and the oblique antibody pattern is microfabricated by etching trenches into the channel surface. We found that biotin-coated beads in a streptavidin-immobilized micro channel, which mimic target cells in an antibody-immobilized micro channel, are displaced in the cross-flow direction by 0.8% of the traveling distance. Cross-stream separation with a mixture containing target and non-target beads is also demonstrated.

Keywords: stem cells, cell separation, antigen-antibody interaction, functionalized surface

1. Introduction

Stem cell therapy is a rapidly evolving biomedical technology, in which multipotent stem cells are cultured in vitro and transplanted to regenerate damaged or deficit tissue. Extraction of adult stem cells from our bodies gains importance due to ethical issues with the use of embryonic stem cells. However, these undifferentiated cells are very rare and difficult to identify. Therefore, the development of an efficient and accurate cell separation method of rare cells from cell mixture is necessary.

Cell separation methods often use antibody-conjugated fluorescent molecules or magnetic micro beads that specifically attach to the target cells with antigen-antibody interaction. For example, fluorescence activated cell sorting (FACS)[1] and magnetic cell sorting (MCS)[2], are well-established techniques. However, labeling cell is problematic for regenerative medicine because of unexpected effect of the labels in cultivation and transplantation.

Miwa et al.[3] has proposed label-free separation with antibody-immobilized micro channel, in which the target cells rolling on the wall are decelerated and separated in the direction of main flow. In the present study, we propose a flow-through label-free cell separation method, where the target cells are displaced in the cross-flow direction using asymmetric micro patterns of antibody on the channel wall.

Figure 1 shows the schematic of the present cell separation principle. Oblique patterns of the wall-immobilized antibodies specifically adhere the target cells and cause their movement in the cross-flow direction. In the present study, biotin-coated micro beads are employed as the modeled target cells and the avidin-biotin adhesion is used for the antigen-antibody interaction.

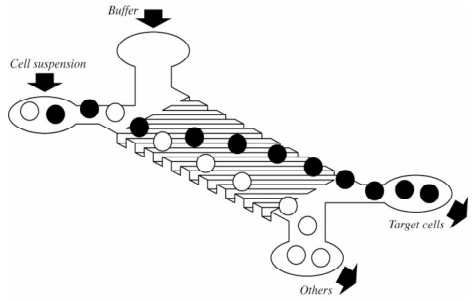

Fig.1 Schematic of the cell separation principle.

2. Microfabrication and surface functionalization

In the present study, it is required that the cells roll along the antibody-immobilized channel wall. Thus, the channel depth is chosen as 40 μm, which is approximately twice the diameter of a typical monocyte. Channel width is 200 μm.

Oblique trenches 1 μm in width and 0.2 μm in depth are etched into the channel surface as the non-adhesive oblique patterns; when cells are located above the trench, the cell surface is displaced from the wall antibody.

Figure 2 shows the fabrication process of the present micro cell separator. Firstly, micro channel structures are etched into a silicon substrate using DRIE and fluidic ports are ultrasonically drilled. Oblique trenches are patterned with EB lithography and etched into a pyrex substrate with ICP. Amino-functionalized surface is formed on the pyrex substrate by coating 0.1 μm-thick diX-AM. The silicon substrate is coated with 2 μm-thick parylene-C. Parylene-parylene thermal bonding between silicon and pyrex surface is accomplished by pressing the substrates at 5 MPa for an hour at 200 ˚C in vacuum. Inlet and outlet ports are made of PDMS blocks, firmly bound to the silicon substrate after oxygen plasma treatment. Figure 3 shows schematic of the present device and channel shape.

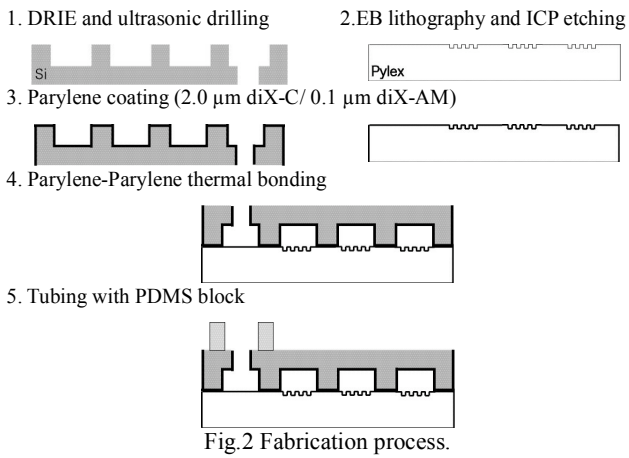

Fig.2 Fabrication process.

*Department of Mechanical Engineering, The University of Tokyo Hongo 7-3-1, Tokyo 113-8656, Japan
**Current address: Department of Microsystems Engineering, University of Freiburg Georges-Koehler-Allee 103, D-79110 Freiburg, Germany

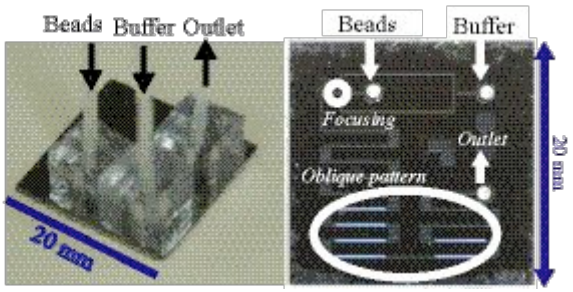

Fig.3 Schematic of the prototype.

Biomolecule immobilization on the channel wall is described in Miwa et al.[3]. The microchannel walls are first biotinylated by conjugating NHS-LC-LC-biotin to the surface amines. NHS-LC-LC-biotin is dissolved into demithylsulfoxide, next into bicine buffered saline (pH 8). The biotin solution is introduced into the micro column and incubated for one hour at 30 ˚C. Streptaviden dissolved into PBS (pH 7.4) are successively incubated (Fig.4). Although streptavidin-functionalized surface is used in the present study, any biotin-conjugated biomolecule can be immobilizated on the surface.

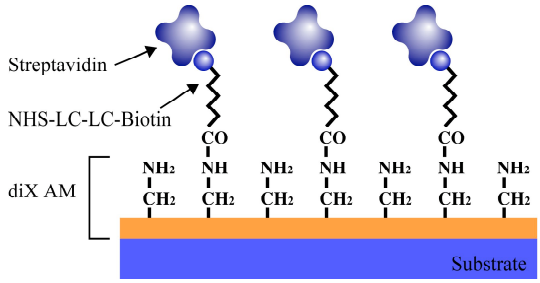

Fig.4 Streptavidin immobilization procedure on diX AM surface.

3. Experimental results

We employ biotin-coated beads, of which diameter is 6-8 μm (Spherotech Inc.), as the modelled target cells, and characterize the beads motion on oblique antibody patterns through microscopic measurement of the beads velocity and their location. Streptavidin-coated beads (7-9 μm in diameter, Spherotec Inc.) are used as the non-target cells. Flow-ratio of the beads mixture and PBS buffer fluid is set 1:1 to concentrate the beads within 100 μm of the center of the channel, where the flow velocity is constant. The oblique angle is 45˚. Figure 5 shows the velocity and cross-stream displacement of the biotin-coated beads, where u_b is the bulk mean velocity, $u_{particle}$ is the velocity of micro beads, and $\Delta y/\Delta x$ is the cross-stream displacement non-dimensionalised with the traveling length.

The mean velocity of the streptavidn-coated beads is larger than u_b. On the other hand, the biotin-coated beads are markedly decelerated, and their mean velocity is about 0.4 u_b. The cross-stream displacement is 0.8 %, and independent of the bulk velocity. Note that the cross-flow displacement of the streptavidin-coated beads is less than 0.2 %, showing the effect of the secondary flow or the flow inside the trench is negligible.

Finally, the cell separation performance is examined using a mixture of biotin/streptavidin-coated beads. Flow-ratio of beads mixture and the buffer fluid is set 1:2.5, so that the beads are introduced within 50 μm of enter of the channel. The bulk mean velocity is set 1.0 mm/s.

Figure 6 shows histograms of the cross-flow positions of the beads. The sample beads are successfully separated into each type of beads after 10,000 oblique patterns, showing the effectiveness of the present device.

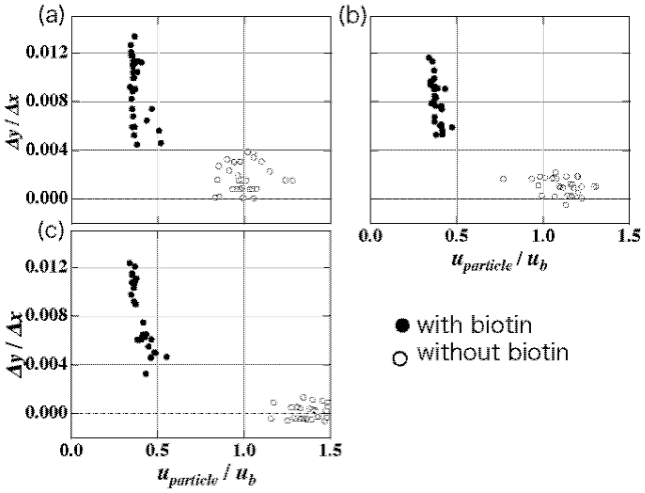

Fig.5 The velocity and cross-flow displacement of the biotin/streptavidin-coated micro beads on oblique streptavidin pattern (a) u_b=0.25 mm/s, (b) u_b=0.50 mm/s and (c) u_b=1.0 mm/s.

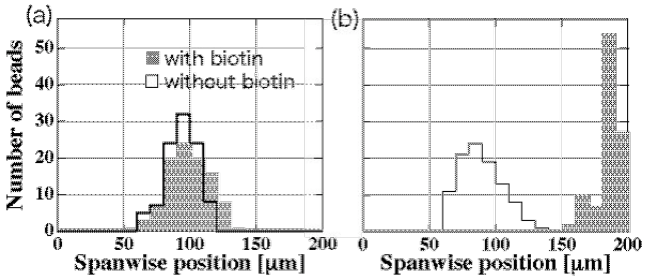

Fig.6 Distribution of biotin-coated beads and streptavidin-coated beads in cross-flow direction. (a) near the inlet and (b) after 10,000 patterns.

4. Conclusion

A label-free specific-adhesion-based cell separator has been developed using surface asymmetric antibody patterns. It is demonstrated that beads having specific adhesion can be significantly displaced in the cross-flow direction by the effect of oblique antibody pattern on the channel wall. Considering the merits of label-free and continuous separation, the present cell separator provides an efficient method for rare cell extraction.

Reference

[1] Y. Fu, C. Spence, A. Scherer, F. H. Arnold and S. R. Quake, “A Microfabricated Fluoresence-Activated Cell Sorter,” Nature Biotech., vol. 17, no. 11, pp. 1109-1111 (1999)

[2] W. H. Tan, Y. Suzuki, N. Kasagi, N. Shikazono, K. Furukawa and T. Ushida,”Lamination Micro Mixer for Micro-immunomagnetic Cell Sorting,” JSME Int. J. Ser. C, vol. 48, no. 4, pp. 425-435 (2005)

[3] J. Miwa, Y. Suzuki and N. Kasagi “Adhesion-Based Cell Sorter With Antibody-Coated Amino-Functionalized-Parylene Surface,” J. Microelectromech. Syst., vol. 17, no. 3, pp. 611-621 (2008)