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Evaluation of Lamination Micro Mixer for Micro Immunomagnetic Cell Sorter

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Abstract

A split-and-recombine lamination mixer for μ-immunomagnetic cell sorting system has been developed for extracting stem cells from peripheral blood. Cell suspension is mixed with antibody-coated magnetic beads, and target cells are labeled with antigen-antibody reaction. In this report, the mixer performance is evaluated quantitatively by using human umbilical vein endothelial cells (HUVEC). The number of magnetic beads attached to the target cell is evaluated in two different arrangements of the mixer units.

Keywords: Micro immunomagnetic cell sorter, Lamination micro mixer, Magnetic beads, Antigen-antibody reaction

1 INTRODUCTION

Stem cells, or an upstream of all the differentiated cells are expected to play a key role in regenerative medicine, which purposes the regeneration of damaged tissues or organs. Stem cells have pluripotency and almost infinite lifetime, and can differentiate into various kinds of cells. Even the congenital failure or damaged tissues that cannot be self-regenerating are expected to be regenerated by culturing the extracted stem cells from human body, and transplanting them into the affected part. Stem cells have such immense possibilities in medical applications, but the extraction is not a straightfoward process because of its rarity [1]. Therefore, the development of simple but robust cell sorting systems to extract stem cells with high efficiency is needed for the spread of regenerative medicine.

Mesenchymal stem cells [2] also have pluripotency and can differentiate into cells of mesodermal origin, such as bone, muscle, or myocardium. Mesenchymal stem cells are contained not only in bone marrow or umbilical cord blood but in peripheral blood, However the number density is very low, say only $1/10^8$ of all the blood cells. Besides, it is difficult to distinguish mesenchymal stem cells from the other mononuclear blood cells, because of little difference in density and diameter.

Antigen-antibody reaction is one of the common techniques to separate stem cells from other cells. The antigen specifically expressed on the stem cells are labeled with antibody on magnetic beads or fluorescent samples, and the labeled cells are separated by applying an external magnetic or electric field.

We are developing a micro immunomagnetic cell sorting system to extract mesenchymal stem cells from peripheral blood with high efficiency [3]-[6] (Fig. 1). The system consists of a micro mixer and a micro magnetic separator. The cell mixture is first mixed with antibody-coated magnetic beads in the mixer. The stem cells are combined and labeled with magnetic beads through the antigen-antibody reaction. The labeled cells are continuously separated from other cells by applying a magnetic field in the separator. Rapid processing can be accomplished by continuous labeling and separation in a single device.

Tan *et al.* [4] proposed a split-and-recombine type of lamination micro mixer to effectively mix fluids containing cells and magnetic beads in a low-Reynolds-number flow (Fig. 2a). The mixer has a series of mixer units with three-dimensional configuration. The mixing is enhanced with a process similar to baker's transformation. The flow horizontally splits into two streams, and are bended four times, before those streams are combined and stacked vertically. With a series of mixer units, the fluids are multifolded and finally fully-mixed. One advantage of the present mixer over conventional split-and-recombine mix-

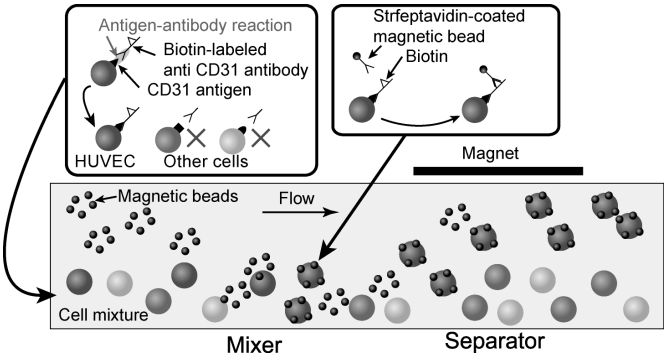

Figure 1: Schematic diagram of μ-IMCS.

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ers is that the multifolded layers of fluids are rotated by 180°, and become upside-down in every mixer to suppress the sedimentation of cells and magnetic beads.

The final goal of the present study is to evaluate in detail the mixer performance under several cell/flow conditions. In this report, the number of magnetic beads attached to the target cell is evaluated and compared in two different arrangements of the lamination mixer units.

2 ARRANGEMENTS OF LAMINATION MICRO MIXER

The split-and-recombine lamination micro mixer with two different arrangements are fabricated and evaluated. Figure 2 shows the unidirectional rotation arrangement, which is identical to that of the mixer proposed by Tan *et al.* [4] (Fig. 2b). The rotational direction of fluid is the same for all the mixer units. Figure 2c shows a mixer configuration with alternate rotation arrangement, in which the same mixer units and these with mirror symmetry configuration are connected alternately

Numerical simulation by Chaktranond [5] revealed that the alternate rotation arrangement archieves better mixing performance than the unidirectional one (Fig. 3). In the unidirectional rotation mixer, an unmixed area remains after the 9th mixer unit, and it corresponds to the Kolmogorov-Arnold-Moser(KAM) tube. The KAM tube can be broken in the alternate rotation mixer, because the periodicity of flow is disturbed. As a result, the unmixed area disappears in the alternate rotation arrangement.

Figure 4 shows a prototype micro mixer device. The device consists of 9 mixer units and an observation chamber. The flow channel cross section is 200 × 200 μm^2. The fluids containing cells and magnetic beads are respectively introduced in two inlets and mixed in the mixer. A buffer fluid is introduced at the upstream of the observation chamer. It passes through the chamber and drains into the outlet. The chamber volume is designed to be 3 $\mu\ell$, with the size of about 4 mm × 4 mm × 200 μm, containing more than 100 cells to be observed under the experimental condition mentioned below. The present three-dimensional configuration is microfabricated with three PDMS layers of 200 μm thickness and a thin glass cover. A 170 μm-thick glass cover is employed to observe clearly the cells sinking onto the glass cover through oil-immersion objective lens. All the PDMS layers and the glass cover are treated with oxygen plasma, and are aligned and bonded together[7].

3 EVALUATION

Human umbilical vein endothelial cells (HUVEC) are chosen as the model cell. HUVEC cells are conjugated with biotin-labeled anti CD31 antibody. Streptavidin-coated Dynabeads MyOne beads 1 μm in diameter are employed as the magnetic beads. The number density of HUVEC is $10^6/\mathrm{m}\ell$, and that of the magnetic beads is $10^8/\mathrm{m}\ell$. In the present study, the number of magnetic beads counted by taking microscopic images of HUVEC. Thus the number density of magnetic beads in this experiment is diluted to 1/10 of that in the experiments by Tan *et al.* [4].

Fluids containing cells and magnetic beads are introduced into the mixer inlets at a flow rate of 5.9 $\mu\ell$/min. The buffer fluid is introduced at a flow rate of 11.8 $\mu\ell$/min.

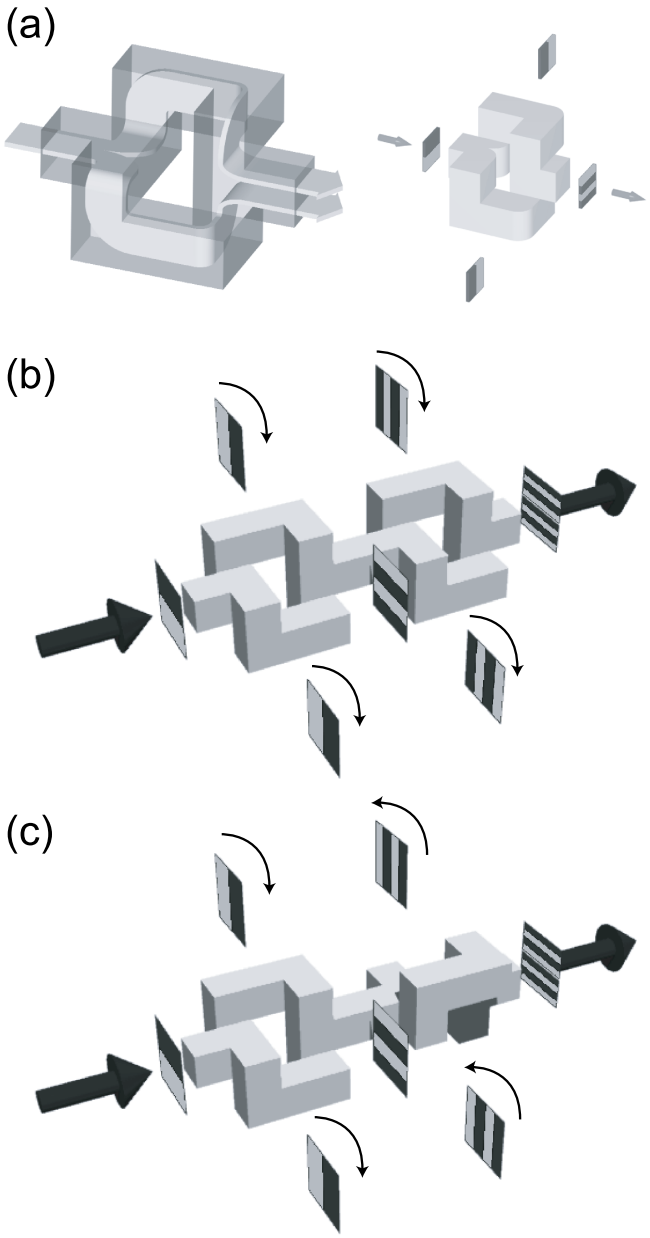

Figure 2: Schematic diagram of the lamination micro mixer. (a) Mixer unit, (b) Unidirectional rotation mixer), (c) Alternate rotation mixer.

After flowing the fluids for 10 minutes, HUVEC remaining in the chamber are observed under an inversion microscope. Cell images are taken at several different focal planes displaced in the out-of-plane direction (Fig. 5a), and the number of magnetic beads attached to each HUVEC cell is counted.

4 RESULTS

Figure 5(b) shows relative frequency distributions of the total number of magnetic beads attached to HUVEC. The number of HUVEC cells observed is about 150. Although variance of the distributions is large, the mean number of magnetic beads attached to HUVEC is 9.32 per cell for the unidirectional rotation arrangement. It is found that, in the alternate rotation mixer, the number of magnetic beads becomes 11.4, which is 22 % larger than the original configuration. Therefore, the mixer with the alternate arrangement should be superior to the single arrangement, and this fact is in accordance with the numerical simulation [2]. Inokuchi *et al.* [6] have developed a micro magnetic separator for cells labeled with magnetic beads. The separator is designed based on the results of Tan *et al.* [4] in such a way that the cells with more than 50 magnetic beads can be extracted. Under the present experimental condition, cells with more than five magnetic beads can be separated in the magnetic separator. Cells with more than five magnetic beads are 85 % of all the observed cells in the alternate rotation mixer, and 72 % in the unidirectional rotation mixer.

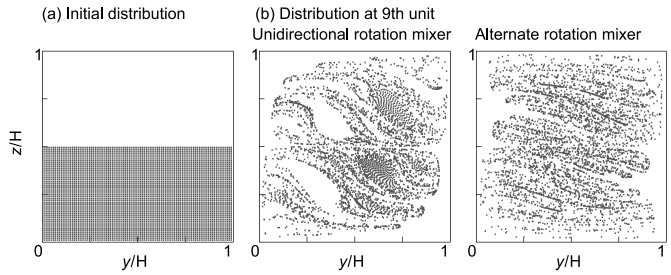

Figure 3: Numerical simulation by Chaktranond[5]. (a) Initial particle distribution at the inlet cross section. (b) Particle distribution at the outlet cross section of the 9th unit of unidirectional rotation mixer and alternate rotation mixer.

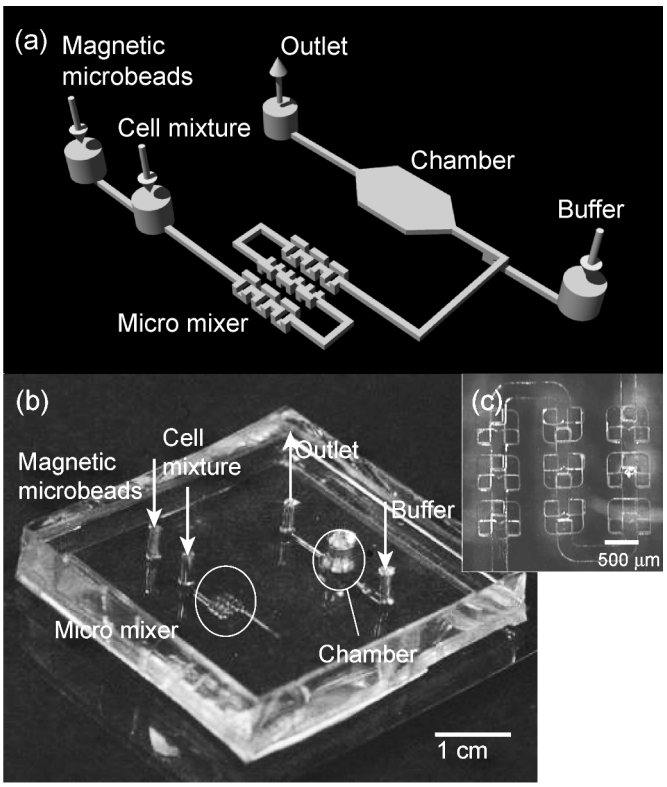

Figure 4: Micro mixer device (a) Schematic. (b) Photograph of prototype device. (c) Close-up view.

4 SUMMARY

In conclusion, a lamination micro mixer for μIMCS has been developed and evaluated through cell attachment ex-

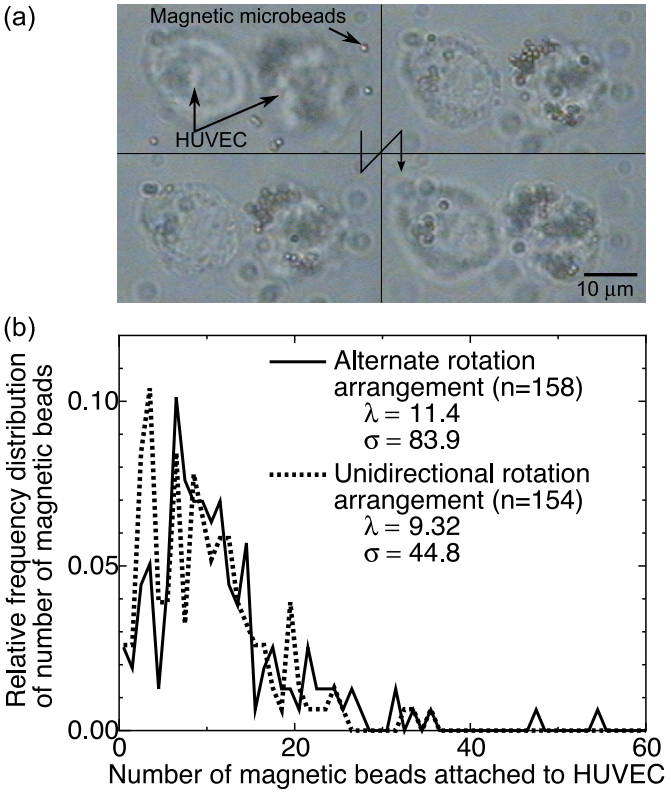

Figure 5: (a) Cell images taken at four different focal planes displaced in the out-of-plane direction. (b) Relative frequency distribution of the number of magnetic beads attached to HUVEC for the alternate rotation mixer and unidirectional rotation mixer.

periments using HUVEC and magnetic beads. The number of magnetic beads attached to HUVEC is evaluated and compared by employing two different arrangements of lamination micro mixer. As a result, it is revealed that the alternate rotation mixer offers better performance to label cells compared with the unidirectional rotation mixer.

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REFERENCES

[1] Wexler, S. A., Donaldson, C, Kendall, P. D., Rice, C., Bradley, B., and Hows, J. M., "Adult bone marrow is a rich source of human mesenchymal 'stem' cells but umbilical cord and mobilized adult blood are not," British Journal of Haematology, **121**, 368-374 (2003).

[2] Jiang, Y., Jahagirdar, B. N., reinhardt, R. L., Schwarts, R. E., Keene, C. D., Ortiz-Gonzalez, X. R., Reyes, M., Lenvik, T., Lund, T., Blackstad, M., Du, J., Aldrich, S., Lisberg, A., Low, W.C., Largaespada, D. A., and Verfaillie, C. M., "Pluripotency of mesenchymal stem cells derived from adult marrow," Nature, **418**, 41-49 (2002).

[3] Suzuki, H., Ho., C.-M., and Kasagi, N., "A Chaotic Mixer for Magnetic Beads-Based Micro Cell Sorter," H. Suzuki, C. M. Ho, and N. Kasagi, "A Chaotic Mixer for Magnetic Beads-Based Micro Cell Sorter," J. Microelectromech. Sys., **13**, No.5, 779-790 (2004).

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

[5] Chaktranond, C., "Performance Assessment of Micromixers for Bio-fluidic Processing with Magnetic Beads," Doctoral thesis, The University of Tokyo, (2006).

[6] Inokuchi, H., Suzuki, Y., Kasagi, N., Shikazono, N., Furukawa, K., and Ushida, T., "Micro Magnetic Separator for Stem Cell Sorting System," Proc. 22nd Sensor Symp., Tokyo, 125-128 (2005).

[7] Xia, Y. and Whitesides, G.M., "Soft Lithography," Annu. Rev. Materials Sci., **28**, 153-184 (1998).