Development of micro immuno-magnetic cell sorting system with lamination mixer and magnetic separator

Hiromichi Inokuchi, Yuji Suzuki and Nobuhide Kasagi

A novel micro immuno-magnetic cell sorting system has been developed for rare cell extraction. The present system consists of a lamination mixer for labeling target cells with magnetic beads, and a separator with an embedded coil, where continuous cell separation is accomplished. We have fabricated a prototype system using soft lithography, and evaluated the separation performance. We successfully achieved the continuous labeling and separation of target cells, although the separation rate remains lower than the designed value. It is conjectured that longer residence time is needed for adhesion of magnetic beads due to antigen-antibody interaction.

Keywords: immuno-magnetic cell sorting, antigen-antibody interaction, mixer, magnetic separator

1. Introduction

In regenerative medicine, clinical use of somatic stem cells is attractive because of their multipotent ability without ethical issues. However, since their number density is very small, cell separation with high purity and high throughput is required.

Final goal of the present study is to develop a micro immuno-magnetic cell sorting system, in which both high-purity and high throughput are possible. In our previous studies, we have developed a lamination micro mixer [1] and a micro magnetic separator [2]. In this report, we have integrated these components and evaluated the system performance.

2. Micro immuno-magnetic cell sorting system

Figure 1 shows the concept of the immuno-magnetic cell sorter. The cell mixture is firstly introduced in the mixer, and the target cells are bound with magnetic beads with antigen–antibody interactions. Then, the target cells with magnetic beads are continuously separated in the separator with the magnetic force.

Figure 2 shows the arrangement of the system. We employ a split-and-recombine lamination mixer developed by Tan *et al*. [1]. In a mixer unit, the fluid is split into two separate passages at the inlet, turned over, and merged at the outlet to form a laminated layer. For n number of mixer units, the number of layers in fluid become 2^n, which makes the thickness of each layer thin to $1/2^n$ of the original thickness. The sedimentation is suppressed by 180º rotation of the stream in each mixer unit.

For the separator, a micro magnetic separator developed by Inokuchi *et al*. [2] is employed. The separator consists of a serpentine separation channel and embedded magnetic coils along the channel. Two layers of fluids are introduced at the inlet of the separation channel. The lower layer is the cell mixture, and the upper layer is the buffer fluid. Target cells labeled with magnetic beads in the mixture migrate to the buffer fluid by the magnetic field. At the outlet of the separation channel, the target and non-target cells are recovered separately by splitting the flow into two streams. They evaluated the separation performance through experiments using plastic particles as model cells, and demonstrated that the number of separated particles increases with increasing electric current.

Department of Mechanical Engineering, the University of Tokyo, Hongo 7-3-1, Bunkyo-ku, Tokyo, 113-8656, Japan

3. Design & Fabrication

Although our micro mixer can completely mix two fluids at the exit, cells and magnetic beads cannot combine readily because mean spacing between cells and beads is much larger than the Brownian diffusion length. Thus, we develop a numerical model to estimate the number of magnetic-beads bindings using time scale of collision between cells and magnetic beads.

Under the shear flow in the present micro channels, the cell velocity is lower than the local flow velocity due to the effect of the velocity gradient. Thus, cells and magnetic beads collide due to their velocity difference. We make the following assumptions for simplicity. 1)The collision between a single cell and a magnetic bead occurs when the magnetic bead passing through the collision cross-section of the cell. 2)All the collided magnetic beads are bound to the cell when the colliding point on the cell surface has a remaining binding site. In the initial condition, cells and magnetic beads are fully mixed with no binding between them. Based on these assumptions, the number of binding magnetic beads is expressed as follows:

$$N_B(t) = \frac{n_B{}^{\circ} N_{B,\max}(1-\exp(-\kappa t))}{n_B{}^{\circ} - N_{B,\max} n_C{}^{\circ} \exp(-\kappa t)} \quad \text{LLL(1)}$$

$$\kappa = \frac{(n_B{}^{\circ} - N_{B,\max} n_C{}^{\circ})\omega \Delta V}{N_{B,\max}} \quad \text{LLL(2)}$$

where $N_B(t)$ is the average number of binding magnetic beads at time t, $N_{B,max}$ is the maximum number of magnetic beads that can bind to a single cell. $n_C{}^{\circ}$, and $n_B{}^{\circ}$ are the initial concentration of cells and unbinding magnetic beads respectively in the solution at $t = 0$. ω is the collision cross-section computed from the diameters of the cell and the magnetic beads. ΔV is the average relative velocity between the cell and the magnetic beads estimated from the cell diameter, the flow rate, and the width and height of the channel.

The cell is 10 μm in diameter, and its number density is 10^6 /mL. Diameter of the magnetic beads is 1 μm, and the maximum number of the binding is 50. Flow rate is 10 μL/min. From this equation, at the magnetic beads concentration of 5 x 10^9 /mL, 30 magnetic beads can be bound to cells for one second, and 50 magnetic beads for 10 seconds. Therefore, we need finite

residence time after the mixer to get enough bindings between cells and magnetic beads.

We fabricated a prototype device using the softlithography [3] (Fig. 3). PDMS layers of the mixer and the separator are fabricated, and then bonded onto a polyimide film with the coil patterns. The channel length between the mixer and the separator is determined in such a way that the residence time is 1 s.

4. Experimental results

The performance of the device is evaluated by measuring the separation rate, which is defined as the ratio of the number of recovered particles at the target-cell outlet divided by the total number of recovered beads at both outlets.

In the present experiment, biotin-coated Sphero TP-60-5 10 μm in diameter, and streptavidin-coated Dynabeads MyOne 1 μm in diameter are employed for the model cell particles and the magnetic beads, respectively. The particle solution is 10^6 /mL in concentration. The particles, magnetic beads, and buffer solution are introduced into the device at the flow rates of 10, 10, and 25 μL/min, respectively.

We evaluated the system performance with two different experiments. Firstly, the particles are labeled with about 50 magnetic beads beforehand. Then, the particles and buffer solution are respectively introduced into the cell and magnetic beads inlet. With this experiment, we evaluated the separation rate of the magnetic separator for pre-labeled particles. Secondly, we introduce the particles and magnetic beads mixtures respectively into the cell and magnetic beads inlets for evaluation of labeling and separation. In this experiment, the concentration of magnetic beads is determined with the model equation in Chap. 3 to obtain 30 magnetic beads per particle.

Figure 4 shows the separation rate versus the electric current through the coil. In the case of the separation using pre-labeled particles, the experimental data are in good agreement with the computational results, in which particle motion with magnetic beads is solved with magnetic field obtained with FEM analysis. When I=1 A, almost all the particles are recovered from the target-cell exit. On the other hand, in the case of the simultaneous labeling and separation, separation rate also increases with increasing current. However, the separation rate remains much lower than the designed value. It is conjectured that the number of magnetic beads is less than the designed value of 30, and longer residence time is needed for adhesion between particles and magnetic beads through antigen-antibody interaction.

5. Conclusions

We have designed and developed a micro immuno-magnetic cell sorting system, and evaluated its separation performance through a series of experiments. It is found that continuous labeling and separation of the model cell particles have been achieved, although the separation rate is less than the designed value.

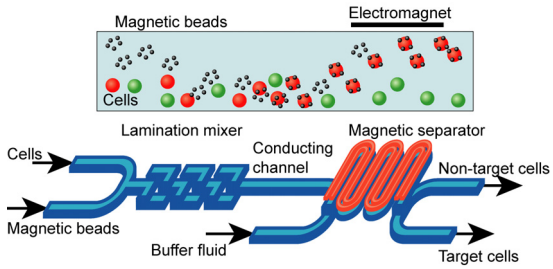

Figure 1: Schematic of micro immuno-magnetic cell sorting system.

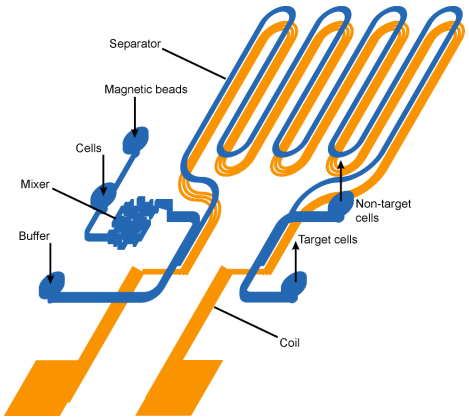

Figure 2: Arrangement of micro immuno-magnetic cell sorting system.

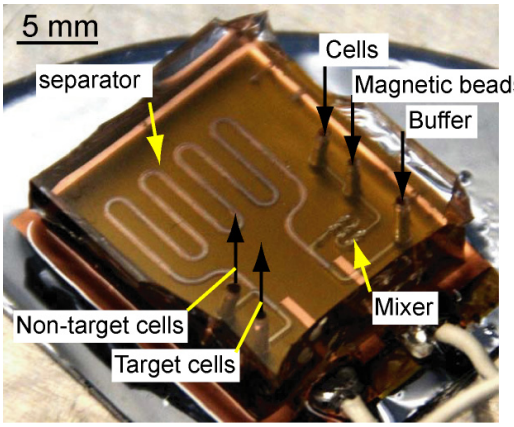

Figure 3: Photo of micro immuno-magnetic cell sorting system.

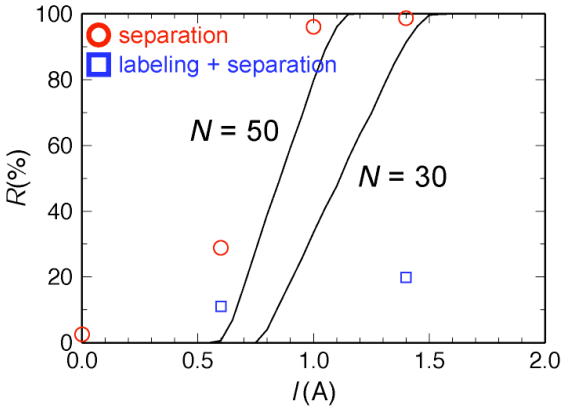

Figure 4: Separation rate versus electric current through the coil.

References

(1) W.-H. Tan, Y. Suzuki, N. Kasagi, N. Shikazono, K. Furukawa and T. Ushida, JSME Int. J., Ser. C, 48. 4, pp.425-435 (2005).

(2) H. Inokuchi, Y. Suzuki, N. Kasagi, N. Shikazono, K. Furukawa and T. Ushida, "Magnetic separator for stem cell sorting system," Proc. of the 22nd Sensor Symposium., Tokyo, pp. 125 – 128. (Octover, 2005).

(3) B. H. Jo, L. M. V. Lerberghe, K. M. Motsegood and D. J. Beebe, "Three-dimensional micro-channel fabrication in poly-dimethylsiloxane(PDMS) elastomer," J. Microelectromech., 9, pp.76-81(2000).