MICRO MAGNETIC CELL SORTING SYSTEM WITH A FUNCTION OF CONTINUOUS LABELING AND SEPARATION

Hiromichi Inokuchi[1], Yuji Suzuki[2] and Nobuhide Kasagi[2]
[1]National Institute for Materials Science (NIMS) and
[2]Department of Mechanical Engineering, University of Tokyo, JAPAN

ABSTRACT

We report development of a novel micro magnetic cell sorting system for the precise extraction of stem cells from a small amount of concentrated sample. The system consists of a lamination mixer for labeling target cells with magnetic beads, and a magnetic separator with an embedded coil, where continuous cell separation is accomplished. We fabricated a prototype device using soft lithography, and evaluated the separation performance. We successfully achieved the continuous labeling and separation of target cells. The results show that the number of binding magnetic bead for each cell ranges widely from several to dozens.

KEYWORDS: Immuno-magnetic cell sorting, Antigen-antibody interaction, Micro mixer, Micro magnetic separator

INTRODUCTION

Tissue engineering is a promising biomedical technology, which enables us to replace damaged tissues by implanting cells or tissues that are cultured *in vitro*. Although somatic stem cells are inferior in differential ability to multipotent stem cells, the practical use is expected to grow rapidly in the near future, considering safe use and no ethical concern. Mesenchymal stem cell (MSC), derived mainly from bone marrow or peripheral blood, has ability to differentiate into various cell types of mesodermal origin. However, MSC is very rare, with a number density of 10^{-9} of all the blood cells, and is difficult to identify with physical properties. A system for precise and high-throughput separation is required.

Currently, recognition of specific cell-surface antigen is available for accurate separation of stem cells. Magnetic cell sorter is one major candidate, in which target stem cells are separated after labeled with antibody-conjugated magnetic beads through antigen-antibody interactions. Labeling process generally requires long time to bind reasonable number of magnetic beads to the separation, although separation process finishes immediately. In this paper, we report the development of a newly micro magnetic cell sorting (MMCS) system, in which labeling and separation are sequentially accomplished. The continuous processing in miniaturized device has the potential to perform cell separation more effectively and rapidly.

DESIGN

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.

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Figure 2(a) shows the arrangement of the system. We employ a split-and recombine lamination mixer developed by Chaktranond *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. We have developed a micro magnetic separator for continuous separation of target cells [2]. 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.

FABRICATION AND EXPERIMENT

We fabricated two prototype devices with different channel length beween the mixer and the separator, using the softlithography [3] (fig. 2(b)). PDMS layers of the mixer and the separator are fabricated, and then bonded onto a polyimide film with the coil patterns. The channel lengths are determined in such a way that the residence time are 10 s and 1 s.

In the present experiment, biotin-coated Sphero TP-60-5 10 μm in diameter, and streptavidin-coated Dynabeads MyOne 1μm in diameter are employed for the cell-model 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 25μL/min, respectively. The concentration of magnetic beads is determeined to obtain 30 and 80 magnetic beads per particles for each device. 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 particles.

RESULTS AND DISCUSSION

Figure 3 shows the separation rate versus the electric current through the coil. 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 (fig. 4), separation rate also increases with increasing current. However, the separation rate remains much lower than the designed value. The results also show that the number of binding magnetic bead for each cell ranges widely from several to dozens. In is conjectured that the number of magnetic beads is less than the designed values.

CONCLUSIONS

We have designed and developed a micro magnetic cell sorting sytem, and evaluated its separation performance through a series of experiments. Results from

separation experiments show the successive achievement of the continuous labeling and separation of target cells.

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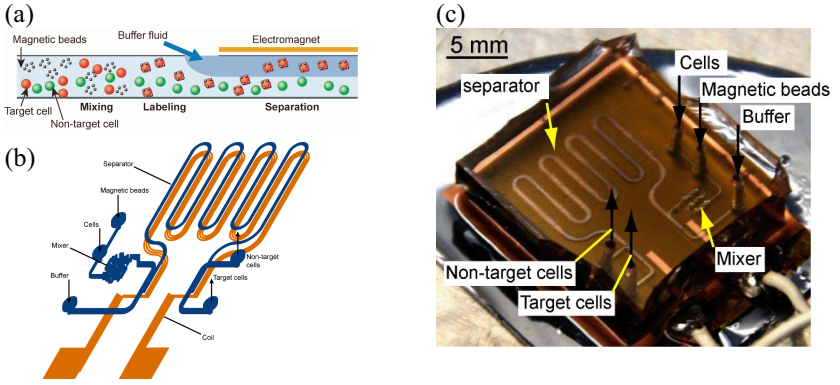

Figure1. (a) Schematic image of the concept of continuous micro magnetic cell sorting, (b) Schematic image of the micro magneti cell sorting device, (c) fabricated device

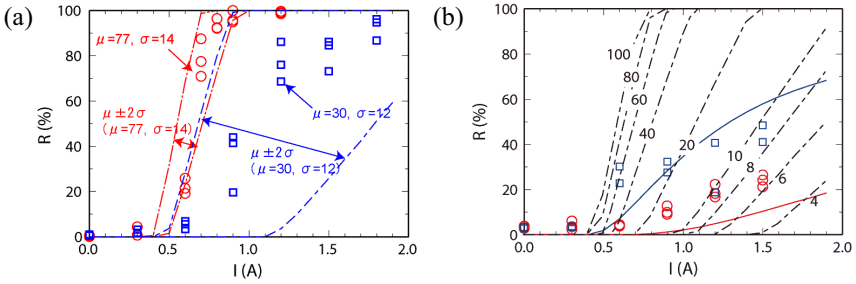

Figure 2. (a)Separation rate versus electric current through the coil for the separation of prelabeled particles. The dashed lines means the upper and lower limits of the separation rate calculated with FEM analysis, (b) Separation rate versus electric current for continuous labeling and separation. The black dashed lines are the computational results for equivalent number, N, of the binding magnetic beads.