

# Micro Magnetic Separator for Stem Cell Sorting System

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Final goal of the present study is to develop micro immunomagnetic cell sorting (uIMCS) system for extracting stem cells from peripheral blood. In this report, micro magnetic separator is designed to extract target cells bound to magnetic beads with the aid of on-chip micro coil. Channel geometry and coil arrangement are determined through a series of numerical simulation of magnetic field and the flow field. Prototype device with embedded coil is microfabricated with the soft lithography technology.

**Keywords:** Regenerative medicine, micro immunomagnetic cell sorter, magnetic beads, PDMS

## 1. Introduction

Regenerative medicine is a promising biomedical technology in the near future, in which pluripotent stem cells extracted from a patient's body are cultured to differentiate into various tissues, and transplanted to damaged tissues. Using this technology, it is expected that serious diseases such as Parkinson's disease, diabetes, etc., can be healed. Embryonic stem cell is currently a major candidate for regeneration of tissues, but its use often encounters ethical concerns. On the other hand, mesenchymal stem cell (MSC) derived from adult bone marrow or blood can also differentiate into various kinds of cells.<sup>(1)</sup> However, MSC is a very rare cell with a probability of  $1/10^9$  in whole blood, and hard to distinguish from other cells by size, weight, charge and so on. Therefore, a safe, inexpensive and highly efficient method for separating stem cells from whole blood or bone marrow is necessary.

The most preferable way to distinguish stem cells from other kinds of cells is by their specific set of surface markers (antigens), which play an important role in immunological reactions in our body. Microfluidic cell sorting devices capable of utilizing immunoreaction for target cell recognition such as fluorescence activated cell sorter (FACS)<sup>(2)</sup> have already been developed, but most of them require bulky external optical systems, or electric fields which may affect the multipotency or plasticity of stem cells.

Our objective is to develop an automated and flow-through cell sorting system using immunomagnetic cell recognition as shown in Fig. 1. Figure 2 shows the

schematic diagram of the immunomagnetic cell sorting (IMCS) system, which consists of a mixer and a separator. Through this system, labeling and separating of target cells are made sequentially and automatically. First, mononuclear cells are extracted from peripheral blood, and introduced into the micro mixer, where target cells are labeled with antibody-coated magnetic beads. Subsequently, in the micro separator, the cell-beads complexes are separated into the buffer fluid by an external magnetic field.

Recently, we have developed a chaotic lamination micro mixer for labeling cells<sup>(3)</sup>, and successfully separate target cells using external permanent magnet.

In this report, we design and develop an on-chip magnetic separator for more efficient and controllable separation of labeled target cells.

## 2. Structure of micro magnetic cell separator

Figure 3(a) shows the schematic diagram of micro magnetic separation channel with embedded magnetic coils. In the separation channel, we introduce two layers of fluid under laminar flow condition. The lower layer is cell mixture, and the upper layer is buffer fluid. Target cells labeled with magnetic beads migrate from the cell mixture to the buffer fluid by the magnetic field generated by the coil.

**2.1 Arrangement of coil for effective cell sorting** We design the arrangement of the magnetic coils, in such a way that the vertical cell velocity becomes as uniform as possible. Since the magnetic force is rapidly decreased with increasing the distance, the channel height should be much smaller than the width. Therefore, the channel height and width are assumed to be  $60 \mu\text{m}$  and  $400 \mu\text{m}$ , respectively. In this study, the coil is parallel to the streamwise direction.

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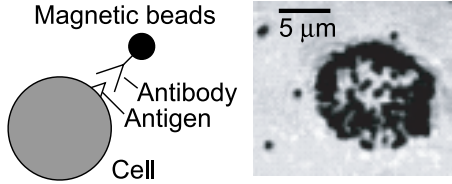


Fig. 1. (a) Schematic diagram of antigen-antibody reaction. (b) HUVEC bound to magnetic beads.

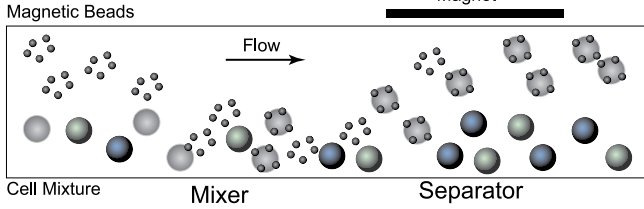


Fig. 2. Schematic diagram of micro immunomagnetic cell sorting system.

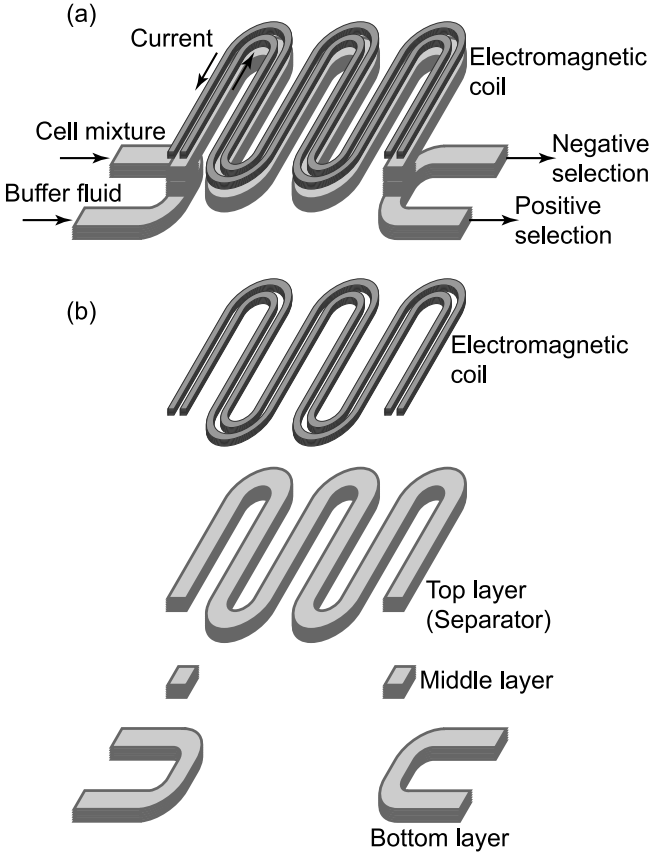


Fig. 3. (a) Micro magnetic cell sorter prototype schematic. (b) Exploded view.

The magnetic field  $\mathbf{H}(\mathbf{r})$  in separation channel is computed by using the Biot-Savart law. Magnetic force acting on a magnetic bead  $\mathbf{F}$  is given by

$$\mathbf{F} = (1 - N_d)\mu_0\mu_r V_m(\mathbf{H} \cdot \nabla)\mathbf{H}, \dots\dots\dots (1)$$

where  $\mu_0$  is the permeability of vacuum, and  $\mu_r$ ,  $V_m$ ,  $N_d$  is relative permeability, the volume, and the demagnetizing factor of the beads, respectively. We assume that the force acting on a cell can be expressed by the summation of the force acting on the magnetic beads attached to the cell. Then the cell velocity  $\mathbf{v}_m$  is given by

$$\mathbf{v}_m = N \frac{\mathbf{F}}{3\pi\mu D_p}, \dots\dots\dots (2)$$

where  $N$  is the number of magnetic beads attached,  $\mu$  is the viscosity, and  $D_p$  is the diameter of the cell.

Figure 5(a) shows the schematic of the channel cross section with the embedded coil. As mentioned above, the cell mixture is introduced to the bottom fluid layer, and the target cells bound to magnetic beads move upward into the buffer fluid by the magnetic force. The cross section of the coil is assumed to be  $35 \mu\text{m}$  by  $150 \mu\text{m}$ . The distance between the coil surface to the channel wall is  $30 \mu\text{m}$ .

Figure 5(b) shows the cell velocity in the vertical direction induced by the magnetic force for an electric current of 1.0 A. The number of magnetic beads  $N$  is assumed to be 50. The cell velocity is in the order of a few  $\mu\text{m}/\text{s}$ , and changes rapidly in the vertical direction. We adopt the three-electrode configuration, since it provides better uniformity in the cell velocity. In this simulation, 95 % of the target cells can move into the upper buffer fluid in 6.7 s.

**2.2 Length of the separation channel** To achieve high separation accuracy with minimum sedimentation loss, we estimate the range of the separation channel length required.

The channel is assumed to have  $L$  in length, and  $H$  in height. The bulk mean velocity is  $U$ . Residence time  $T_r$ , separation time  $T_m$ , and sedimentation time  $T_s$  are given by

$$T_r = \frac{L}{U}, \dots\dots\dots (3)$$

$$T_m = \frac{H}{v_m - v_s}, \dots\dots\dots (4)$$

$$T_s = \frac{H}{v_s}, \dots\dots\dots (5)$$

where  $v_m$  is the induced velocity with the magnetic field given by Eq (2).

The residence time should be longer than the separation time and be shorter than the sedimentation time, i. e.,

$$T_r > T_m, \quad T_r < T_s. \dots\dots\dots (6)$$

Therefore, the length of the separation channel should satisfy

$$\frac{U}{v_m - v_s} < \frac{L}{H} < \frac{U}{v_s}, \dots\dots\dots (7)$$

Sedimentation velocity  $v_s$  can be given by

$$v_s = \frac{(\rho_p - \rho_f)gD_p^2}{18\mu}, \dots\dots\dots (8)$$

where  $\rho_m$ , and  $\rho_f$  are the density of the particle and the fluid, respectively.

In the present study, the cell diameter  $D_p$  and the density of the cell  $\rho_p$  is respectively assumed to be  $20 \mu\text{m}$  and 1005. With these assumptions as well as Eq. (2),  $v_m \sim 3.02 \mu\text{m}/\text{s}$  and  $v_s \sim 1.2 \mu\text{m}/\text{s}$ , inequality (7)

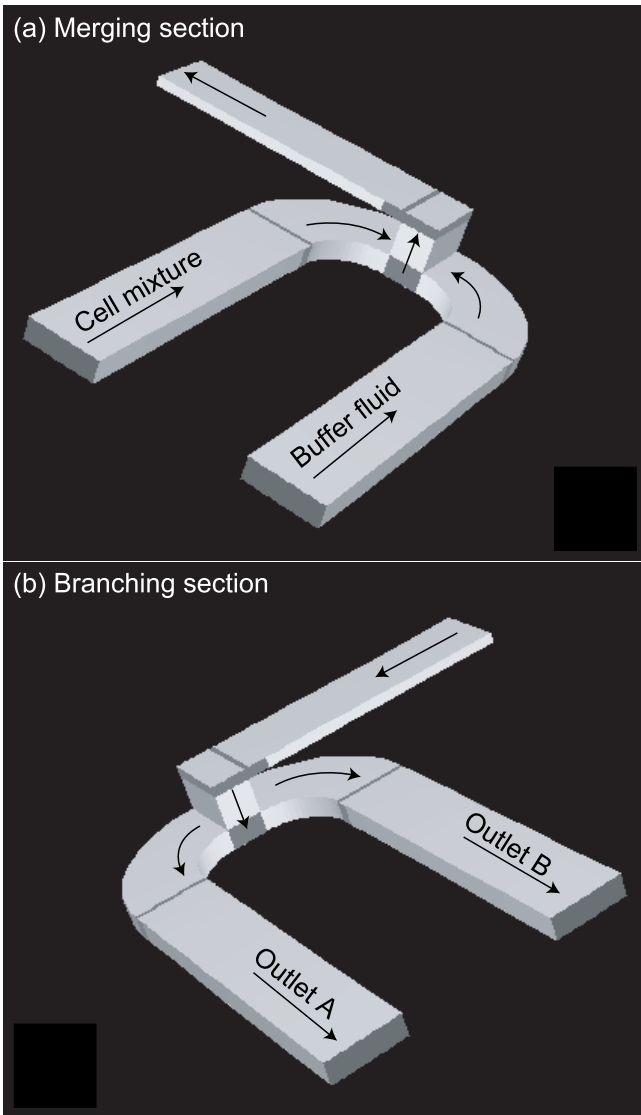


Fig. 4. Computational model. (a) merging section (b) branching section.

leads to

$$1098 < \frac{L}{H} < 1666, \dots \dots \dots (9)$$

when  $U = 2.0$  mm/s. In the present prototype, we choose  $L/H = 1666$ .

### 2.3 Design of merging and branching section

At the merging section of the present device, the cell mixture and the buffer fluid should be stacked layer by layer in the vertical direction. On the other hand, the top and bottom layers should be separated well into the two streams at the branching section. Since the aspect ratio of the present channel is about 1:7, it is not straightforward to design these merging and branching sections. Based on preliminary computations of the flow field, we adopt a U-shaped channel as shown in Fig. 4. At the merging section, the cell mixture and the buffer fluid are introduced into the U-shaped channel at each end, and two streams impinge and flow upward at the symmetry plane of the U-bend. Finally, the stream is introduced into the separation channel through a short

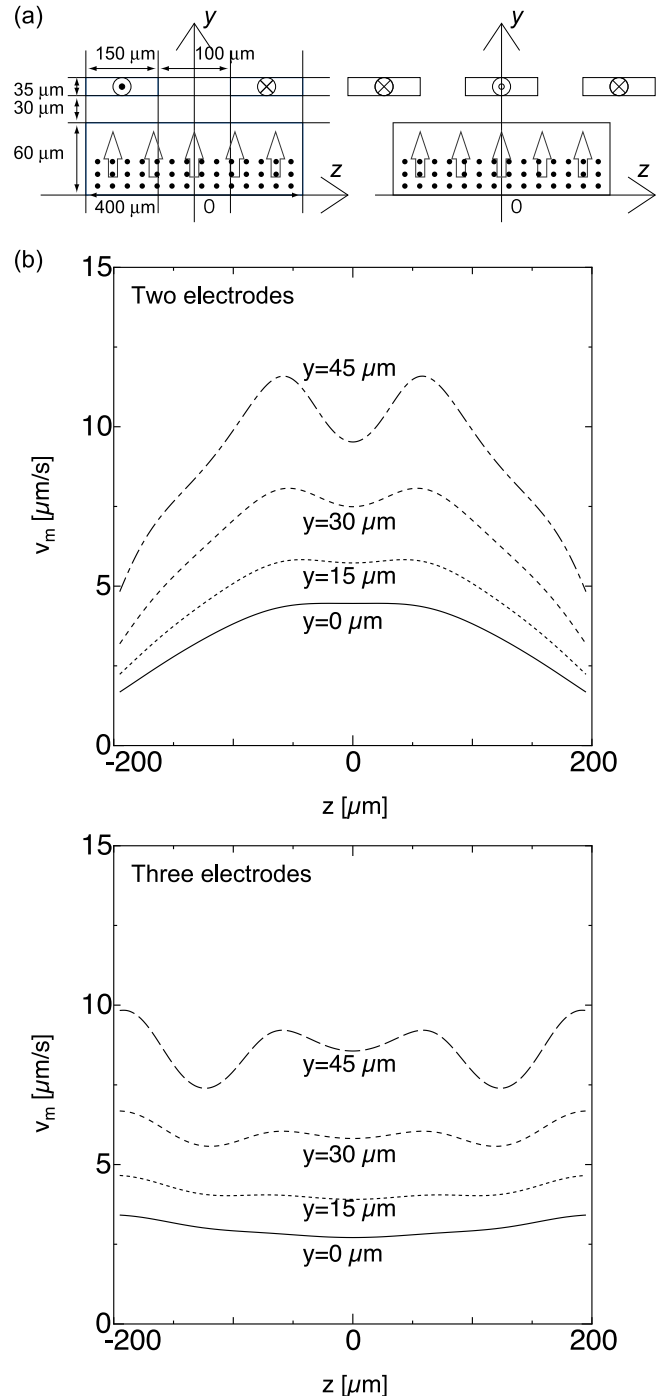


Fig. 5. (a) Arrangement of coils for efficient cell separation. (b) Vertical velocity distribution.

connecting conduit (Fig. 4a). Fig. 4(b) shows the branching section having the same geometry but the opposite flow direction. The channel height of the U-bend is  $120 \mu\text{m}$ , and the length of the connecting conduit is  $200 \mu\text{m}$ . Flow field and cell trajectories are calculated with Fluent 6. The bulk mean velocity at the inlet is  $2.0$  mm/s. Figures 6(a) and 6(b) show the cell distribution at the two exits of the branching section. It is shown that almost perfect separation is obtained.

### 3. Microfabrication

As shown in Figure 3, the current micro magnetic cell

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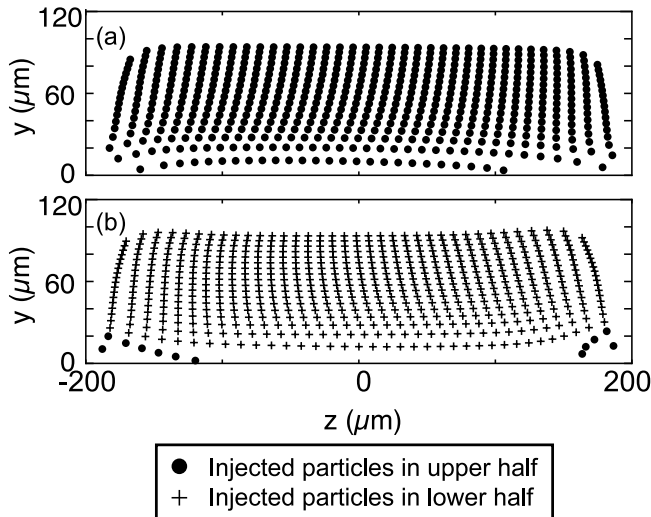


Fig. 6. Cell distribution in the cross-section of  $400 \mu\text{m} \times 120 \mu\text{m}$ . (a) Outlet A (b) Outlet B.

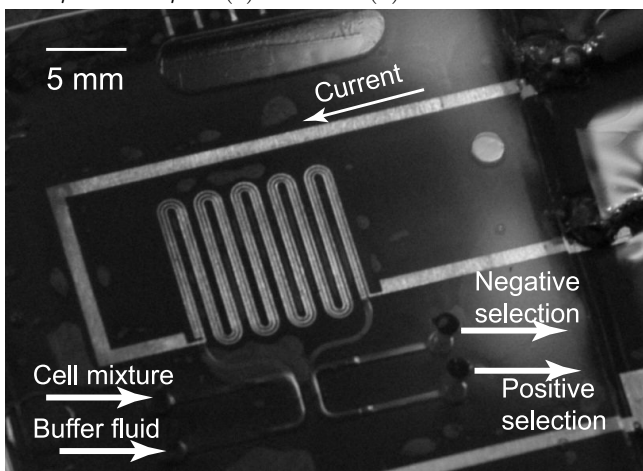


Fig. 7. The prototype device of the micro magnetic cell separator

separator consists of three layers for microfluidic channel and a single layer for the magnetic coil. In this study, poly-dimethylsiloxane (PDMS) is chosen as the material of the micro channel structure.<sup>(4)</sup>

SU-8 mastermolds are made on silicon wafer using standard lithography technique, and each PDMS layer is casted from the mastermolds. The coil is made on  $25 \mu\text{m}$ -thick polyimide film electroplated with  $35 \mu\text{m}$ -thick copper. The polyimide/copper film is glued on silicon wafer with spun-on photoresist. Copper coil is patterned with wet etching. All the PDMS layers are chemically bonded to each other by oxygen plasma treatment of the surface. Figure 7 shows the prototype device.

## 4. Summary

Micro magnetic cell separator for stem cell sorting system is studied. Channel geometry and coil arrangement are designed through a series of numerical simulation of magnetic field and the flow field. Prototype device with embedded coil is microfabricated with the soft lithography technology.

## Acknowledgements