Development of a Magnetic Separator for Biomaterials Labelled by the Magnetic Beads

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Development of a Magnetic Separator for Biomaterials Labelled by the Magnetic Beads

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This paper presents a new high-throughput magnetic separator for biomaterials labelled by magnetic beads. The separator consists of three rectangular coils, two circular coils, and a separation chamber. These instruments were designed by a numerical analysis of magnetic field and a movement of magnetic beads. A separation rate over 90 % has been obtained in this system.

Key Words: Magnetic separation, Magnetic beads, Gradient magnetic field

1. Introduction

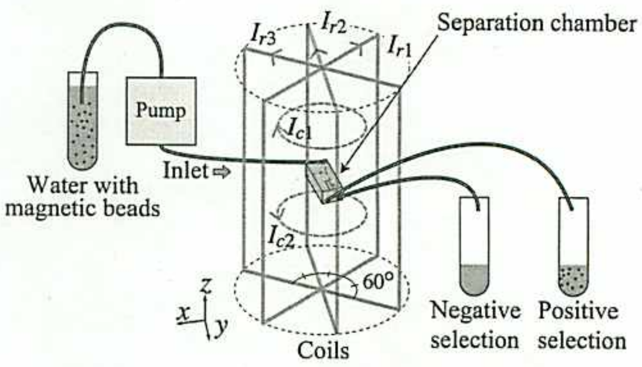
The purification technology to separate cells or biomaterials is required for most cell or pharmaceutical analyses. Conventional methods such as a centrifugation and FACSs (Fluorescence Activated Cell Sorters) are widely used in these fields. A magnetic separation also came to be used as an advancement of magnetic labelling technology. In this method, magnetic beads, which are polystyrene beads containing iron oxide and typically 1-5 μm in diameter, convey targets of separation. Targets are immunochemically fixed to the beads by a highly specific antigen-antibody bridge [1], [2].

The problem is that normal batch magnetic separators using a vessel have a limitation with respect to a volume of separation. At present, some kinds of magnetic separators, which enable continuous separation, have developed to solve the problem and they have worked properly [3]-[5]. However, these solutions have a low flow rate: for example, it is 1 μl/min in [5].

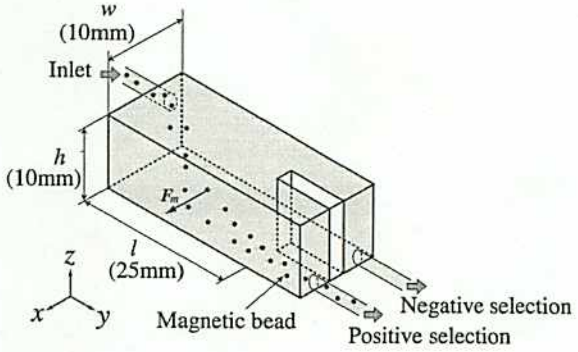
In this paper, we introduce and develop a novel magnetic separator for biomaterials labelled by the magnetic beads, which enables continuous separation with high-throughput compared to the conventional one.

2. Numerical Analysis and Design

A proposed magnetic separator consists of three rectangular coils, two circular coils, and a separation chamber, which is located between circular coils as shown in Fig. 1.



(a) Schematic illustration of a magnetic separator



(b) Enlarged illustration of a separation chamber

Fig. 1. Proposed magnetic separator

Targets labelled by magnetic beads are infused from an inlet of the chamber. When they are in the chamber, they are exposed to gradient magnetic field generated by the coils. Then, they move to x-direction by magnetic force precipitating to negative z-direction and flow out from an outlet "Positive selection". As the result, the targets are separated into bead rich content and others.

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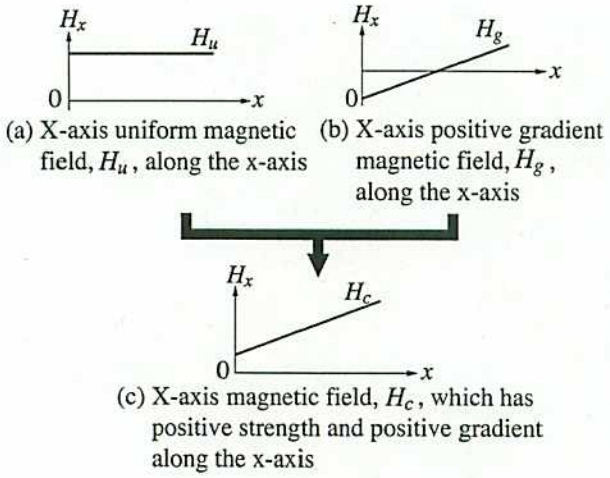
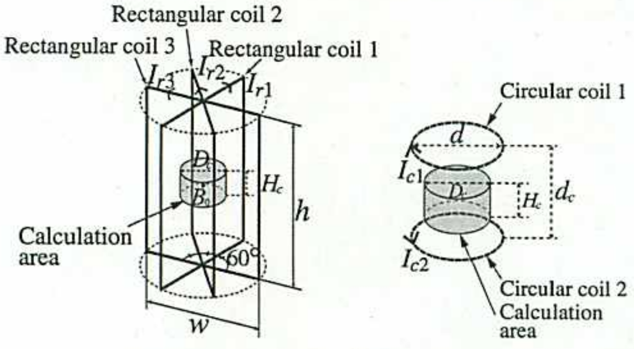


(a) X-axis uniform magnetic field, H_u, along the x-axis (b) X-axis positive gradient magnetic field, H_g, along the x-axis

(c) X-axis magnetic field, H_c, which has positive strength and positive gradient along the x-axis

Fig. 2. Gradient magnetic field for magnetic separation



(a) Rectangular coils (b) Circular coils

Fig. 3. Calculation area for determining coil dimensions

2.1 Coils

In order to generate a magnetic field for dragging magnetic beads toward a direction, two magnetic fields as shown in Fig. 2(a) and (b) are combined, because the strong magnetic force in x-direction is required for the proposed technique.

A uniform magnetic field H_u and a gradient magnetic field H_g are generated by magnetic vector potentials A_u and A_g as follows

$$A_u = (A_x, A_y, A_z) = (0,0,\alpha y) \tag{1}$$

$$A_g = (A_x, A_y, A_z) = (\alpha yz, -\alpha xz, 0) \tag{2}$$

where α is an arbitrary constant [6].

A relationship between a vector potential and a current distribution is given:

$$A = \frac{\mu_0}{4\pi}\int_v \frac{\boldsymbol{i}}{r}dv \tag{3}$$

where r is a distance between dv and A. Therefore, current distributions for uniform and gradient magnetic fields in cylindrical coordinates are respectively given:

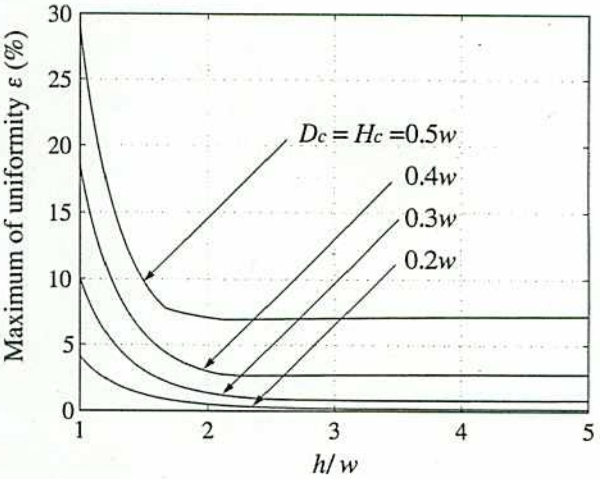


Fig. 4. Maximum of magnetic field uniformity ε at each ratio of a height and a width of rectangular coils (h/w)

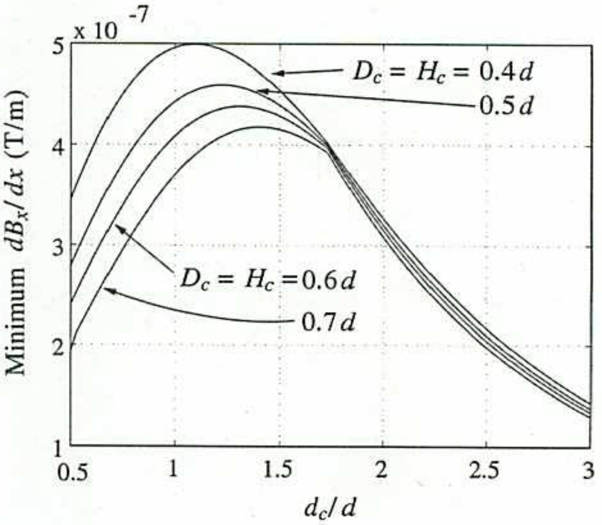


Fig. 5. Minimum of magnetic field gradient dB_x/d_x at each ratio of an interval d_c and a diameter d of circular coils (d_c/d)

Table 1. Coil dimensions and number of turns

Width of rectangular coils	8 cm	Height of rectangular coils	16 cm
Diameter of circular coils	4 cm	Interval between circular coils	4.4 cm
Number of turns	140		

$$\boldsymbol{i}_u = (i_r, i_\theta, i_z) = (0,0,\beta r\sin\theta) \tag{4}$$

$$\boldsymbol{i}_g = (i_r, i_\theta, i_z) = (0,-\beta rz,0) \tag{5}$$

where β is a constant.

A current distribution which satisfies (4) is approximately generated by three rectangular coils with current strength ratio 1:2:1, respectively.

To obtain an optimal ratio of a height and a width of rectangular coils that maximize a uniformity of magnetic field, a calculation area was assumed as shown in Fig. 3(a). Magnetic field uniformity is defined as

$$\varepsilon = \frac{\sqrt{(B_x - B_{0x})^2 + {B_y}^2 + {B_z}^2}}{B_0} \times 100\ (\%) \quad (6)$$

where B_0 is a magnetic field strength at the centre of the area. Fig. 4 shows a maximum of the magnetic field uniformity ε in the area. When a ratio of a height h and a width w (ratio, h/w) is higher than 2, the uniformity is steady state.

A Current distribution which satisfies (5) is approximately generated by two circular coils with the same current in an opposite direction.

To obtain an optimal ratio of an interval and a diameter of circular coils that maximize a gradient of magnetic field, a calculation area was assumed as shown in Fig. 3(b). Fig. 5 shows a minimum of a magnetic field gradient (dB_x/d_x). When a ratio of an interval d_c and a diameter d (ratio, d_c/d) is between 1.1 and 1.4, a minimum of the gradient takes maximum values.

Coil dimensions and numbers of turns are shown in Table 1. These values were determined by assuming that an induced velocity becomes several μm/s at the centre of the circular coils.

2.2 Separation chamber

To accomplish a high separation performance, we estimate dimensions of the separation chamber, which is l in a length, h in a height, and w in a width.

A condition for separation of magnetic beads is given by

$$T_m < T_r < T_s \quad (7)$$

where T_m, T_r, and T_s are separation time, residence time, and sedimentation time of the beads in the chamber, respectively.

At least $w/2$ of movement of the beads from the inlet to positive x-direction make them come out from the outlet "Positive selection". Therefore, T_m is given by

$$T_m = \frac{w}{2v_m} \quad (8)$$

where v_m is the induced velocity of the beads in x-direction. T_r and T_s are given by

$$T_r = \frac{l}{v_f} \quad (9)$$

$$T_s = \frac{h}{v_s} \quad (10)$$

respectively, where v_f and v_s are the velocity of fluid in y-direction and the negative sedimentation velocity in z-direction, respectively. Thus, (7) becomes

$$\frac{v_s l}{h} < v_f < \frac{2v_m l}{w}. \quad (11)$$

Magnetic force exerting on a magnetic bead, $\boldsymbol{F}$ is given by

$$\boldsymbol{F} = (1 - N_d)\mu_0 \mu_r V_m (\boldsymbol{H} \cdot \nabla)\boldsymbol{H} \quad (12)$$

where N_d, μ_0, μ_r, V_m , and H are the demagnetising factor, the permeability of vacuum, the relative permeability, the volume of a bead, and magnetic field around a bead, respectively. N_d is 0.33 for a sphere. Therefore, the induced velocity v_m is given by

$$\boldsymbol{v}_m = N \frac{\boldsymbol{F}}{3\pi\eta D_p} \quad (13)$$

where N, η , and D_p are the number of magnetic beads attached to the target, the viscosity, and the diameter of a target, respectively.

The sedimentation velocity v_s is given by

$$v_s = \frac{(\rho_b - \rho_f) g {D_p}^2}{18\eta} \quad (14)$$

where ρ_b and ρ_f , and g are the volume density of the bead and the fluid, and acceleration due to gravity , respectively.

In the present study, we assume N equal to 1, bead characteristics are 1.2- μ m diameter, 1580-kg/m^3 volume density, and relative permeability of 11.3. The dimensions of the chamber, a length(l), a height(h), and a width(w) are 25 mm, 10 mm, and 10mm, respectively. Therefore, (11) becomes

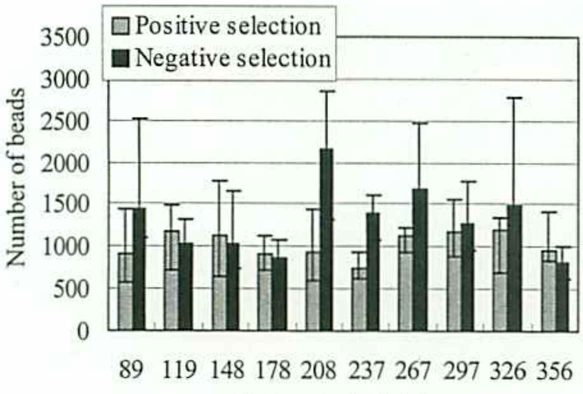
$$1.28 \times 10^{-6} < v_f\ (\mathrm{m/s}) < 1.89 \times 10^{-5} \quad (15)$$

The range of a flow volume Q is given by

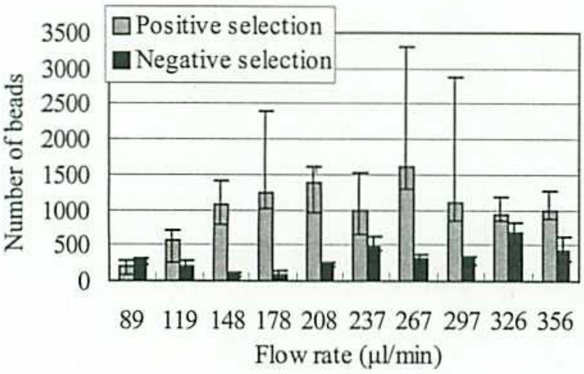
$$1.28 \times 10^{-10} < Q\ (\mathrm{m^3/s}) < 1.89 \times 10^{-9} \quad (16)$$

3. Separation Experiment for the Magnetic Beads

The experimental configuration for performance test of the separator consists of the separator, a tube pump for controlling a bead flow in the chamber, and a power source supplying current to the coils. Applied current strength of rectangular coil 1 and 3 (I_{r1} and I_{r3}) are 1.5 A, rectangular coil 2, circular coil 1, and 2 (I_{r2}, I_{c1}, and I_{c2}) are 3.0 A.



(a) Without magnetic field.



(b) With magnetic field

Fig. 6. Number of magnetic beads obtained from each outlets.

The magnetic beads used for the experiments were SPHERO™ CM-10. And it was 20 times diluted with distilled water.

Fig. 6 shows the number of beads obtained at the outlets at each flow rate. The number of beads was counted by image processing software with captured picture of a microscope, which is a magnification of 200X.

When a current is applied to the coils, most of magnetic beads flew out from the outlet "Positive selection" compared to the case without magnetic field.

Fig. 7 shows a separation rate at each flow rate. The separation rate ξ_s is defined as

$$\xi_s = \frac{N_p}{N_p + N_n} \times 100\,(\%) \qquad (17)$$

where N_p, and N_n are the number of beads obtained from "Positive selection" and "Negative selection".

The maximum separation rate 94.4 % is obtained at 178 μl/min of the flow rate. It may not reach 100 % because some of clustered beads precipitate onto the bottom faster than calculated in (10), and flow out from "Negative selection". For lower flow rate, a decrease of the separation rate may be attributed to retention of the beads in the chamber. For higher flow rate, a decrease of the separation rate may be attributed to a shortage of movement to x-direction by the magnetic force in the chamber.

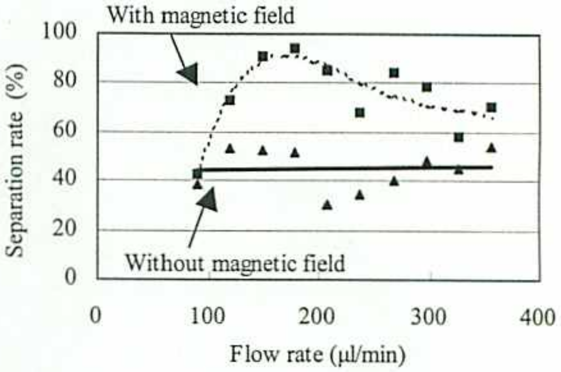


Fig. 7. Separation rate at each of flow rate

4. Conclusion

In this research, the new high-throughput magnetic separator has been proposed. The coils and the separation chamber were properly designed, and have achieved 94.4 % in the separation rate with 178 μl/min of flow rate. The future tasks are to improve the separation chamber in order to obtain more high performance. The chamber with properly sloped bottom may improve the separation rate. And practical experiments using biomaterials is essential.

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