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High Throughput Cell Sorting Device Using Dielectrophoresis and Fluid-Induced Shear Force

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Abstract— **Cell sorting is important technology in many areas such as medical diagnosis in hospitals and cell engineering. Dielectrophoresis (DEP) is one of the promising approaches for cell separation because it does not require any fluorescent dye or antibody. In our previous study, we developed DEP cells sorting device, however the amount of throughput was not sufficient. In this study, we developed high throughput cell sorting device using dielectrophoretic and fluid-induced shear force. As the pilot study, mixed solution containing bovine chondrocytes and polystyrene beads were precisely separated using our novel DEP cell sorting device.**

I. Introduction

Cell sorting is important widely in many applications such as medical diagnosis in hospitals and cell engineering. Commercial sorting method such as fluorescence-activated cell sorters (FACS) involve bulky and high-cost instruments and require fluorescent dye. Magnetic-activated cell sorters (MACS) can be performed by not-expensive instruments, however those require magnetic antibodies.

From these points, dielectrophoresis (DEP) is one of the most promising approaches for manipulating and separating cells [1-7]. DEP is a phenomenon caused under an applied non-uniform electric field inducing dipoles within a polarized cell in a buffer solution. This phenomenon can lead to a nonzero columbic net force on the cell causing its movement. Cells can be manipulated by DEP forces to move toward high or low electric field regions, depending on the relative electric property of the cells in suspending medium [8]. In our previous study, a novel cell sorting device using DEP and fluid-induced shear force was developed. The accuracy of our sorting device was sufficient for cell engineering application, however the amount of throughput was poor because the trapped cells cover the electrodes.

The final goal of this study is to develop high throughput cell sorting system by evaluating the DEP force using a liquid flow control system. As the fundamental study, living cells and polystyrene micro-beads were separated using our novel cell sorting device. The DEP forces caused on the cell or polystyrene microbead were characterized using microfluidic chamber containing electrode-array with fluid-induced shear force. To increase the amount of throughput in DEP separation, dielectrophoretic cell manipulation was controlled by computer system in conjunction with flow rate control of cell-bead solution. Using this control system, the trapped cells covering the electrodes were removed to another port, and cell sorting process could be performed continuously. Finally, the sorting efficacy of our novel DEP cell sorting device was evaluated.

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II. Dielectrophoresis

For a spherical particle in a non-equal electric field, the time-averaged DEP force is generated on the particle as

$$\vec{F}_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m \,\mathrm{Re}\left[f_{CM}(\varpi)\right] \nabla E_{rms}^2 \qquad (1)$$

where r is the radius of micro particle, ε_0 is the permittivity of the vacuum, ε_m is the relative permittivity of the medium, $\mathrm{Re}[f_{CM}]$ is the real part of the Clausius-Mossoti (CM) factor, E_{rms} is the root mean square value of the electric field [8].

The CM factor is related to the magnitude and direction of the DEP force. If the CM factor is plus, the DEP force caused on particle is directed toward the region of high electric field intensity (p-DEP). Conversely, if the CM factor is minus, the DEP force is directed toward the low field intensity. The CM factor is shown as

$$f_{CM}(\varpi) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* + 2\varepsilon_m^*} \qquad (2)$$

where ε^*_p and ε^*_m are the complex permittivity of the micro-particle and the suspended medium. Each complex permittivity is defined as

$$\varepsilon^* = \varepsilon_0 \varepsilon - \frac{j\sigma}{\varpi} \qquad (3)$$

where $j = \sqrt{-1}$, ε is the relative permittivity of the particle or medium, σ is the electric conductivity and ω is the angular frequency of the applied AC electric field. This equation shows the dependency of CM factor on not only the electric property of the particle and medium but also on the frequency of the applied AC electric field. Therefore, DEP forces have possibility to separate cells based on their own electric properties that reflect cell functionality.

III. Materials and Methods

A. Cell sorting device using dielectrophoretic and fluid-induced shear forces

To fabricate a transparent parallel-line-electrode array on the glass slide, ITO (Indium-Tin-Oxide)-coated glass (Geomatec, Kanagawa, Japan) was used for conductive substrate. The thickness of ITO-layer was 1500 A^o and resistance was 5 Ω/sq. The pattern of the parallel-line electrodes was made using laser-etching techniques. The

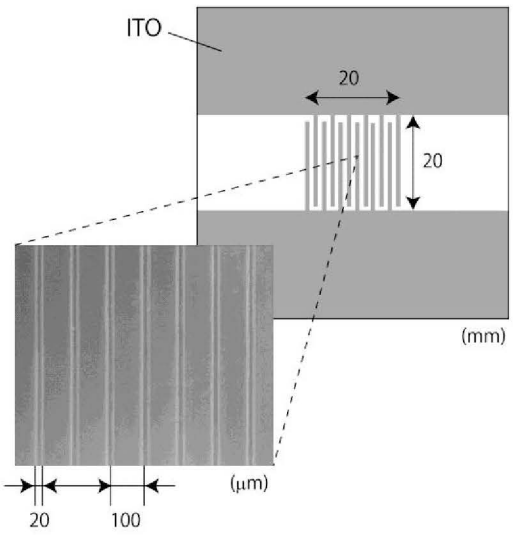

Figure 1. Pallarel-line electrode array constructed on ITO-coated glass.

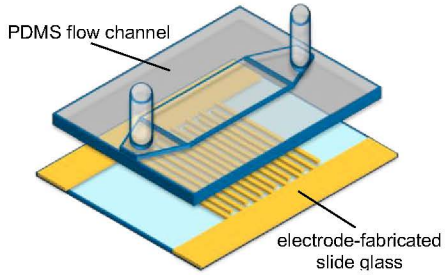

Figure 2. Schematic of DEP chamber.

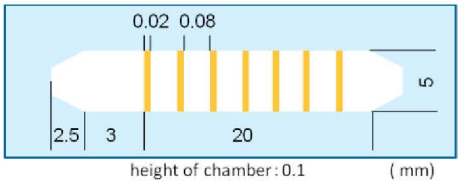

Figure 3. Geometry of PDMS flow channel.

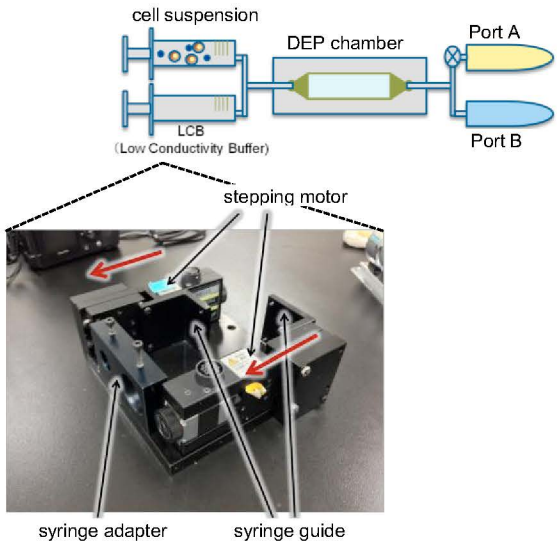

Figure 4. Schematic of DEP cell sorting system and custom-made twin syringe pump.

parallel-line microelectrodes were designed to generate a highly non-equal electric field (Fig. 1). Each electrode line has a width of 20 μm and was spaced 100 μm apart.

The flow channel was made from Polydimethylsiloxane (PDMS) polymer to establish rectangular volume (Fig.2). The DEP chamber was formed by contacting the PDMS flow channel on the electrode-fabricated glass. The resulting geometry of DEP chamber was 5.5-mm long from the inlet to electrodes, 5-mm wide, and 100-μm high (Fig.2). The length of the flow channel was determined to ensure whole cells/microbeads were dropped on the parallel-line electrode array. In the DEP chamber, living cells were trapped on each electrode by positive-DEP force (Fig. 3).

The flow rate of cell-suspended solution and bulk LC buffer were controlled by a custom-made twin syringe pump (Fig. 4). The process of DEP sorting is described as follows. At first step, the cell-bead mixed solution was induced into the chamber at 0.024 ml/min to trap the chondrocytes on the electrodes by DEP and the microbeads passed through the chamber to port B (Fig. 5a, b). Next, AC voltage was switched off to remove the trapped cells from electrodes at the flow rate of 2.4 ml/min and collect them into port A (Fig. 5c). To repeat this process continuously, the amount of throughput of DEP cell sorting cloud be increased. In this study, the process was repeated 2times to evaluate the efficacy of DEP cell sorting.

B. Cell culture and polystyrene micro-beads

In this study, bovine chondrocytes and polystyrene microspheres were used as the objects for DEP cell sorting experiments.

Chondrocytes were harvested from bovine articular cartilage [9-10]. Briefly, cartilage explants were excised from bovine articular joints. The explants were then shaken gently in 0.15% collagenase type II solution to dissolve cartilage matrix. The cells were isolated from the digest by centrifugation. Finally, isolated chondrocytes were cultured in 75 cm^2 flasks in Dulbecco's Modified Eagle Medium/Ham's F12 (DMEM/F12; Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, antimycotics-antibiotics and incubated in 5% CO_2, 100% humidity at 37°C. The cells were passaged 3-5 times before the DEP experiments. Prior to the experiments, the cells were suspended in low-conductivity buffer (LC buffer; 10 mM HEPES, 0.1 mM $CaCl_2$, and 59 mM D-glucose in sucrose solution) [11].

Polystyrene microspheres, 2.5% solids (w/v) aqueous solution, concentration 4.55 x 10^7 particles/ml, were used as cell simulant (Polyscience, Warrington, PA). Stock microsphere solutions were diluted by the LC buffer to make the concentration of spheres equal to that of chondrocyte suspension before the experiments.

C. Cell-bead sorting experiment by DEP & fluid-induced shear forces

Before the experiments, the DEP chamber was degassed and sterilized. To sterilize the chamber, the flow channel was filled with 70% ethanol for 5 min and washed twice with LC buffer. Next, a 5 ml-disposable syringe containing cell-bead suspended solution and 30 ml-disposable syringe containing

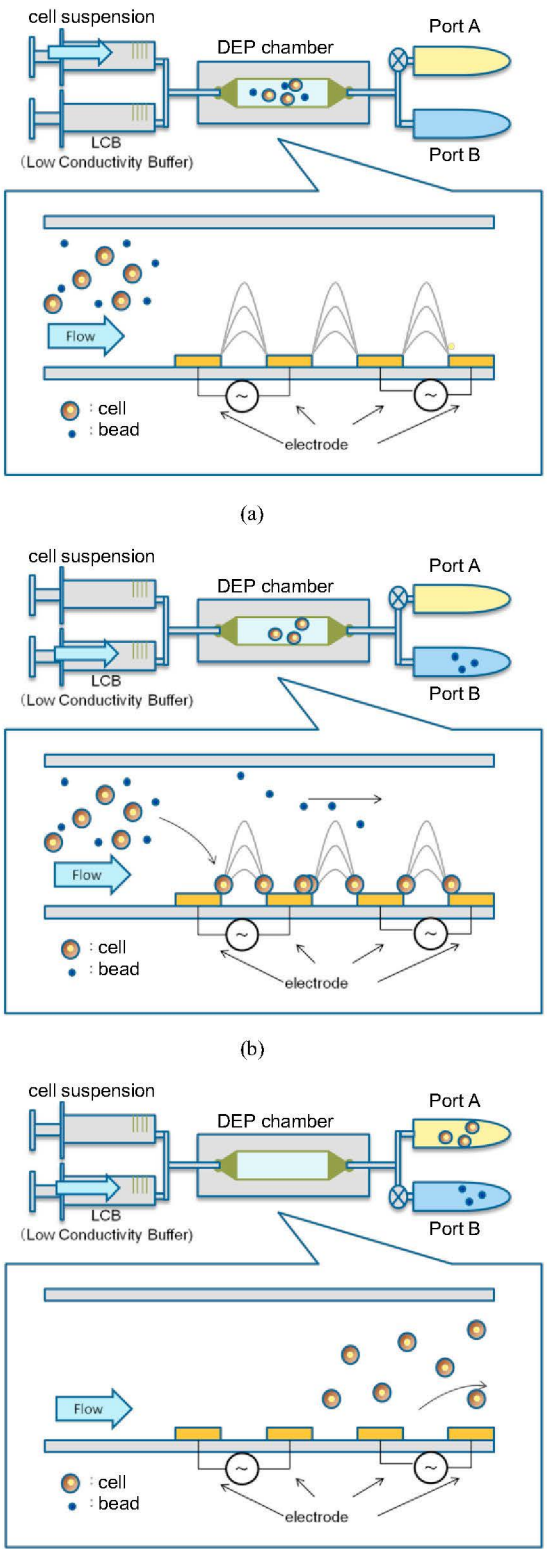

Figure 5. Schematic of DEP cell sorting process to separate cell-bead mixed solution. (a) Step 1: cell-bead suspension was induced into the chamber, (b) Step2: cells were trapped on the electordes by positive DEP force, (c) Step 3: Trapped cells were removed to clear the surface of electrode-array.

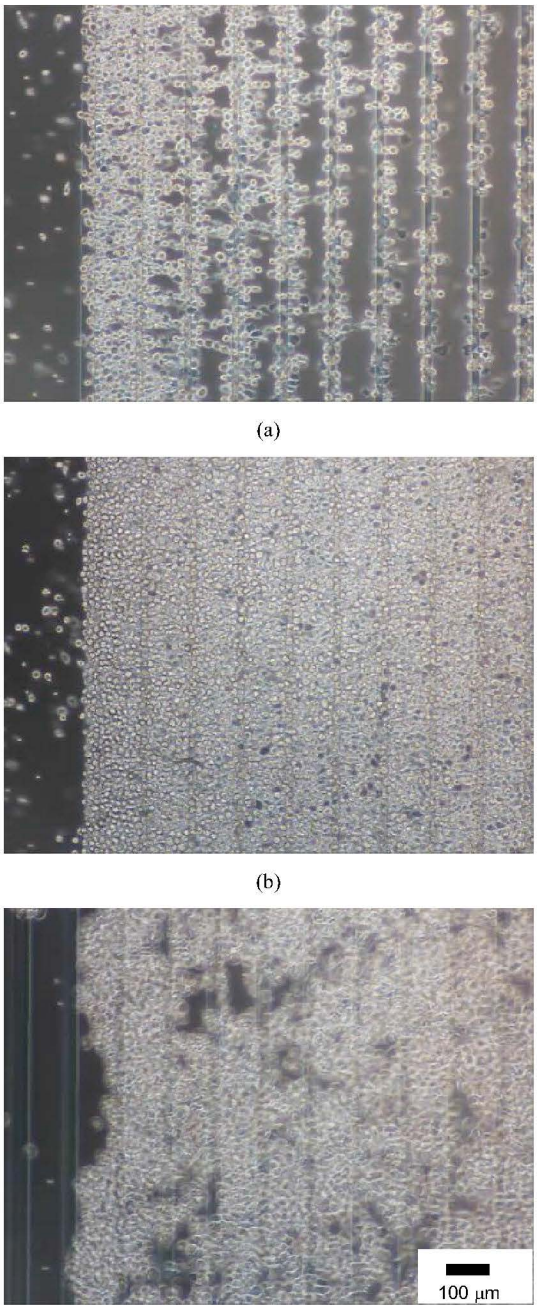

Figure 6. Photomicrograph of DEP cell sorting process at step1 (inducing cell-bead solution) (a), step2 (trapping cells on the electrodes) (b), and step3 (gathering trapped cells) (c).

bulk LC buffer were connected to the inlet of DEP chamber and the flow rate was controlled by the twin-syringe pump according to the DEP cell sorting process.

An AC electric voltage (20 $V_{p\text{-}p}$, 500 kHz) was applied between the parallel-line electrodes, using a function generator (WF1974, NF, Kanagawa, Japan) and amplifier (BA4850, NF, Kanagawa, Japan), with monitoring the voltage by an oscilloscope (TDS1001B, Tektronix, Beaverton, OR) connected in parallel. The movements of the cells and micro-beads within the DEP chamber were observed, using a

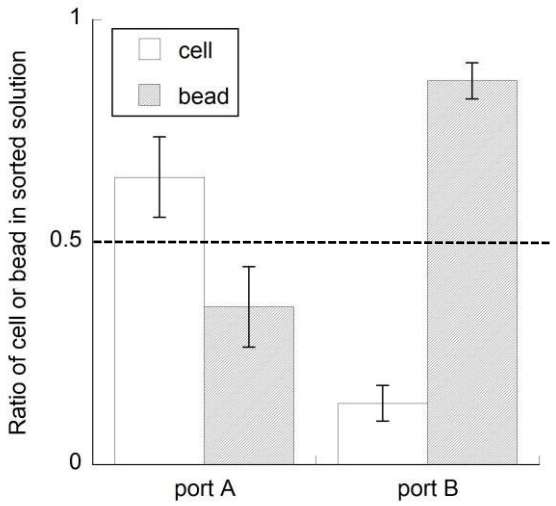

Figure 7. Ratio of cells and micro-beads in port A and port B. Dashed-line indicates inial ratio of cell and bead solution.

phase-contrast microscope (Nikon Eclipse TE300, Nikon, Tokyo, Japan) with a digital video camera. The efficacy of cell sorting was evaluated to count the numbers of chondrocytes and beads in the port A and B.

IV. Results and Discussions

Fig. 6 shows the photomicrograph at each step of DEP cell sorting. At first step, the cell-bead suspension was introduced into the chamber (Fig. 6a), then the chondrocytes were trapped on the electrodes against fluid-induced shear force, whereas the beads passed through the electrodes (Fig. 6b). Next, the DEP force was turned off and the chondrocytes were gathered into port B (Fig6 .c).

The ratio of cells increased to 65% whereas that of beads decreased to 35% in port A. On the other hand, the ratio of cells decreased to 24% whereas that of beads which are simulant for target cells increased to 76% (Fig. 7). Considering that the sorting efficacy of commercial sorting method, MACS, is about 80%, the results of this study suggested that our novel DEP cell sorting device could work at practical level.

It should be noted that our method was only applied for the cell-bead mixed solution in this study, not applied to cell-cell solution. To apply our method for the cell-cell mixed solution, we plan to characterize dielectrophoretic properties of various kinds of living cells. It could be speculated that the dielectrophoretic properties of cells are different depending on their membrane's electric properties. Moreover, the design of electrode-array and fluid channel will be improved to increase the efficacy of DEP cell sorting.

V. Conclusion

In this study, we developed a microfluidic DEP chamber on which parallel-line electrode array was established to impose dielectrophoretic force and fluid-induced shear force on the living cells and polystyrene beads. This device enabled the DEP cell sorting process to be perfomed continuously, and cell purity rose around 80%. From these results, our novel device using DEP and flow-induced shear force was established and enabled high throughput cell sorting.

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