

A continuous cell separation chip using hydrodynamic dielectrophoresis (DEP) process

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Abstract

We present a high-throughput continuous cell separation chip using hydrodynamic dielectrophoresis (DEP) process. The continuous cell separation chip uses three planar electrodes in a separation channel, where the positive DEP cells are moved away from the central streamline while the negative DEP cells remain in the central streamline. In the experimental study, we use the mixture of viable (live) and nonviable (dead) yeast cells in order to obtain the continuous cell separation conditions. For the conditions of the electric fields frequency of 5 MHz and the medium conductivity of $5 \mu\text{S}/\text{cm}$, the fabricated chip performs a continuous separation of the yeast cell mixture at the varying flow-rate in the range of $0.1\text{--}1 \mu\text{l}/\text{min}$; thereby, resulting in the purity ranges of $95.9\text{--}97.3$ and $64.5\text{--}74.3\%$, respectively, for the viable and nonviable yeast cells. The present chip demonstrates the constant cell separation performance for varying mixture flow-rates.

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1. Introduction

The integrated biological analysis systems have received an increased attention in the areas of point-of-care (POC) diagnostics, food pathogen screening, environmental monitoring and biomedical research for drug discovery by providing automated and potentially portable solutions to a wide range of fluid-base solutions [1]. One of the key issues in developing these systems is simple and high-throughput cell separation.

Previously, filtration [2] and fluorescence activated cell sorter (FACS) [3] are used for cell separation in micro regime. Filtration has a critical drawback, clogging at the pore, which makes it impossible to separate repetitively and in F/MACS, a label process is inevitable. Dielectrophoresis (DEP) has been extensively studied for cell separation because it does not require a labeling process and DEP devices can be fabricated easily by same technology required for the remainder of mi-

crosystems. Moreover, cell separation using DEP has many applications since it can separate cells on the basis of physical phenomena occurring inside the cells such as a change in cytoplasmic conductivity or the presence of an extra membrane [4].

In the previous DEP devices [5,6], cell mixture is injected into the separation chamber and the electric fields are applied for DEP separation. After separation, the buffer solution washes out the negative DEP cells and the weakly positive DEP cells while the electric fields are applied. Then, the electric fields are turned off in order to collect the positive DEP cells remained in the separation chamber. Markx and Pethig [7] tried to automate the above-mentioned troublesome process using valves for the simultaneous separation of positive and negative DEP cells. This process uses the discontinuous cell mixture flow controlled by the external valves and it requires the larger electrode area for a high throughput cell separation.

In this work, we propose a high-throughput continuous cell separation chip using hydrodynamic DEP process. The present device, separating the cell from continuous cell mix-

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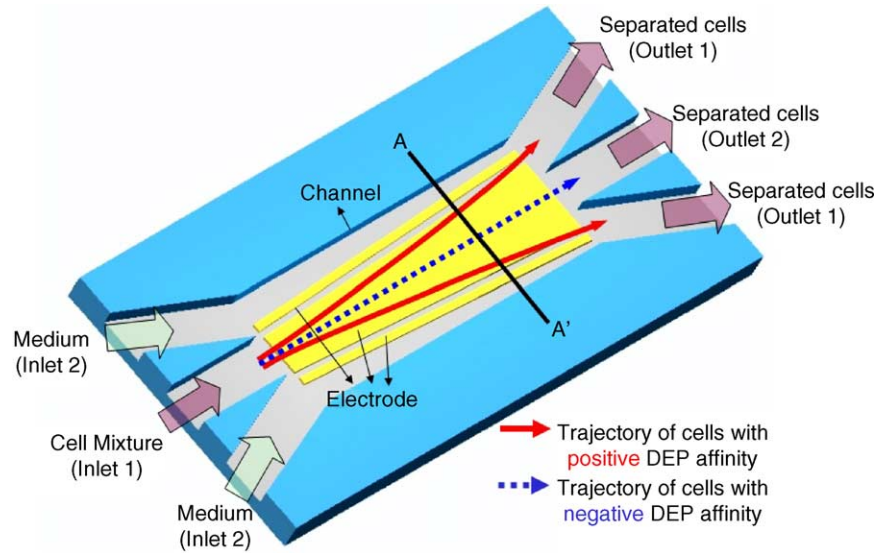


Fig. 1. Conceptual view of hydrodynamic dielectrophoresis (DEP) process.

ture flow, eliminates valves and achieves high-throughput cell separation simply by increasing the mixture flow-rate for a fixed electrode area.

2. Working principle and design

Fig. 1 illustrates the basic principle of the hydrodynamic dielectrophoresis process. The present cell separation chip is composed of the microchannel and planar electrodes parallel to a microchannel at the bottom of the microchannel. The cell mixture flow (injected from inlet 1) is guided along the central streamline by the sheath flow from inlet 2. The AC signals applied to electrodes generate the DEP force to move the cells in the mixture flow in the normal direction to the streamline direction, where the DEP forces and directions are dependent on cell properties. Due to the different DEP force directions, the cells with different DEP responses move continuously to

the different location across the channel as they flow, thus continuously separated into the different outlets.

DEP is the movement of cells in the non-uniform electric fields [1,4–10]. When cells are subjected to nonuniform electric fields, charges are induced at the interfaces resulting in electrical polarization along the direction of electric fields. If the electric fields are uniform, then the electrostatic forces acting on opposite ends of the dipole are equal and there is no net movement, unless cells carry a net charge and the electric field frequency is equal to zero. However, if the fields are spatially non-uniform, then the forces on either side of cells will be different, and the net DEP force can induce translational movement of cells [4].

If electrical polarizability of cells exceeds that of the suspending medium, DEP force is the same direction as the gradient of electric fields (Fig. 2(a)). In this case, cells move to the strong electric field region (positive dielectrophoresis, pDEP). On the contrary, when the electrical polarizability of

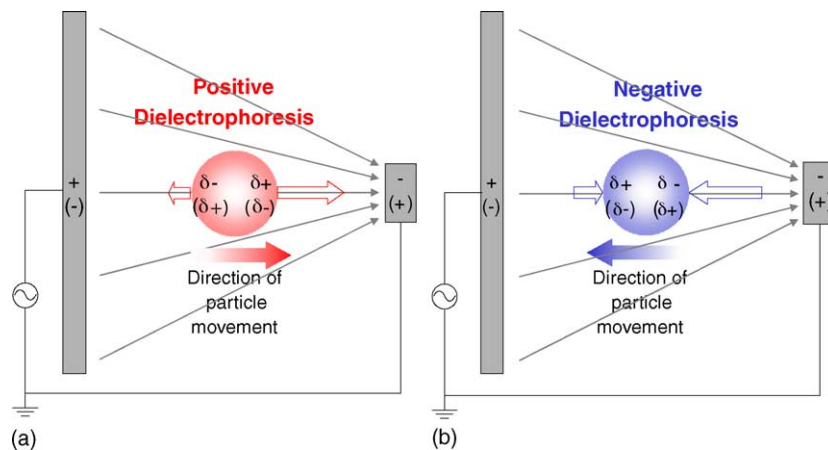


Fig. 2. Direction of cell movement depending on DEP response: (a) positive DEP cells and (b) negative DEP cells.

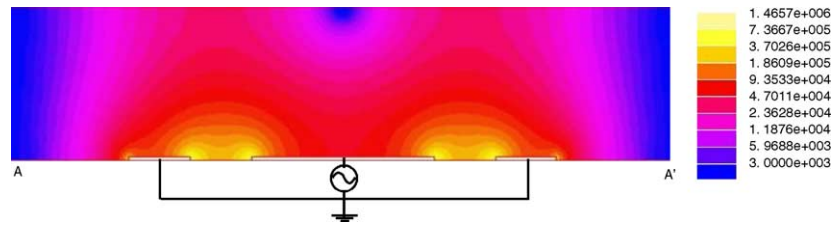


Fig. 3. Numerical simulation of the electric field distribution over the electrodes at the cross-section, A–A' of Fig. 1.

cells is less than that of the medium, the direction of DEP force is reverse to the gradient of electric fields (Fig. 2(b)) and cells move to the weak electric field region (negative dielectrophoresis, nDEP). The polarizability of cells depends strongly on their composition, morphology, phenotype and the electric field frequency [1]; therefore, the cells of different types or physiological states including viability can be discriminated by the DEP.

The time-averaged DEP force is given by [4,8],

$$F_{DEP} = 2\pi\epsilon_m r^3 \text{Re}[f_{CM}] \cdot \nabla |E_{rms}|^2 \quad (1)$$

where r is a radius of cell; ϵ_m , the permittivity of the medium; f_{CM} , the Clausius–Mossoti factor; E_{rms} is the root mean square value of an electric field. $\text{Re}[f_{CM}]$ means a real part of the f_{CM} , which can be represented as follows [4,8]:

$$f_{CM} = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad (2)$$

where ϵ^* is the complex permittivity ($\epsilon^* = \epsilon - j\sigma/\omega$); σ , the conductivity; ω is the electric field frequency. Subscripts p and m mean cells and the medium, respectively. $\text{Re}[f_{CM}] > 0$ means that cells show pDEP response while $\text{Re}[f_{CM}] < 0$ means nDEP response.

The electric potential applied to the three electrodes in the microchannel, generates symmetric non-uniform electric fields across the microchannel. Fig. 3 shows the simulation results of the electric fields distribution across the separation

channel (A–A' in Fig. 1), where the two strong electric field regions are apart from the center of the middle electrodes and the weak region is on the center. Therefore, the pDEP cells move away from the central streamline, while the nDEP cells are focused along the central streamline. From the simulation results as shown in Fig. 3, the magnitudes of DEP forces are estimated in the range between several pN (at channel center) and several tens of pN (near the electrode edges), thus generating the forces sufficient to move the cells as mentioned in other publications [8,9].

3. Fabrication process

The fabrication process for the continuous separation chip is shown in Fig. 4. We use 4-in. Pyrex® glass wafers as a substrate. We deposit sputtered 200 Å/1000 Å-thick Cr/Au layer on the wafers for electrodes. Then, we coat 1.2 μm-thick PR (PhotoResist: AZ1512) and define the electrodes. Next, we use 4-in. silicon wafers and coat 50 μm-thick SU-82025 layer and define for microchannel. Then, we pour PolyDiMethylSiloxane (PDMS) prepolymer mixture (PDMS:curing agent = 10:1). After 2 h curing at 85 °C, we peel off PDMS microchannel. Finally we bond glass substrate and PDMS microchannel after air plasma treatment for 10 s at the vacuum pressure of 200 mTorr and 30 W RF power. Fig. 5 shows the fabricated device and enlarged view of the device.

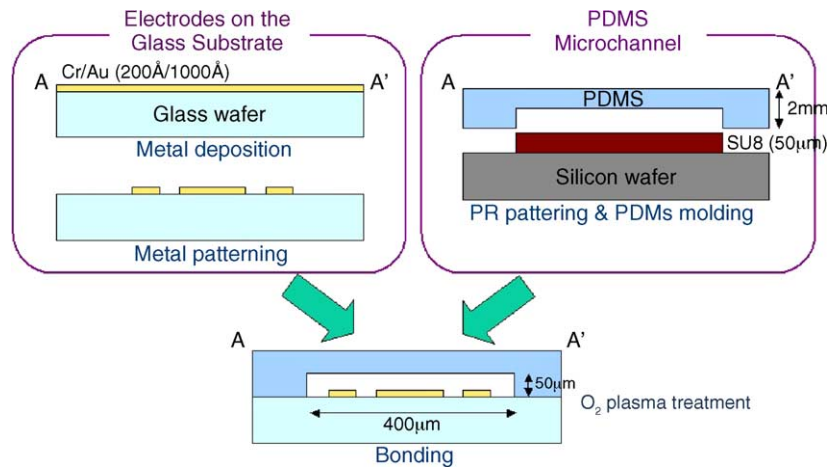


Fig. 4. Fabrication process of present devices illustrating the cross-section, A–A' of Fig. 1.

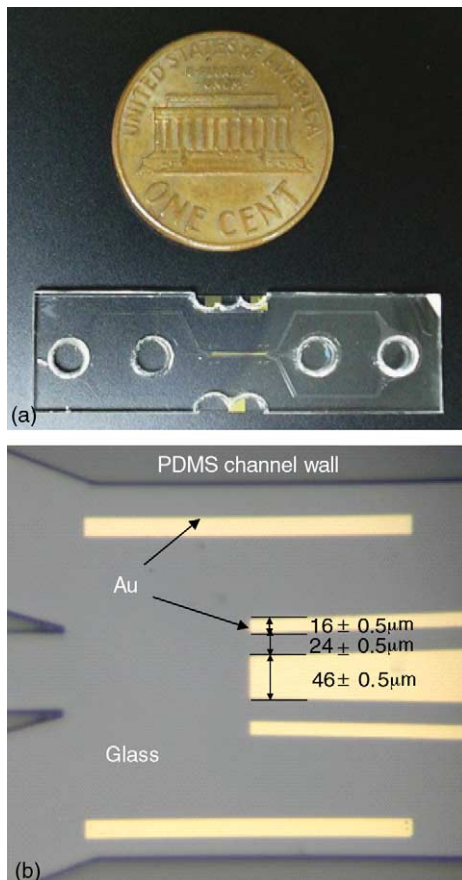


Fig. 5. Fabricated separation chip: (a) overall view compared with a penny and (b) enlarged view of microchannel.

4. Experimental results

4.1. Sample preparation

We use the mixture of viable (live) and nonviable (dead) yeast (*Saccharomyces cerevisiae*) cells as sample cells to be separated. Yeast cells are grown at 30 °C for 24 h in culture medium, washed and then re-suspended four times in deionized water. Conductivity of the medium is adjusted by adding a small amount of NaCl and conductivity is measured by HI8733 (HANNA instruments). Nonviable yeast cells are prepared by heat treatment (90 °C for 20 min) and viability of yeast cells is visualized using a methylene blue stain [11]. In this stain, their color changes into blue.

4.2. Experimental set-up

The schematic view of overall experimental apparatus for the continuous separation is shown in Fig. 6(a). We use syringe pump (KDS 200) with two different syringes for cell mixture and buffer flow-rate control. We attach the separation chip to the die and interconnect the electrical signal to the separation chip by electrical probes and observe

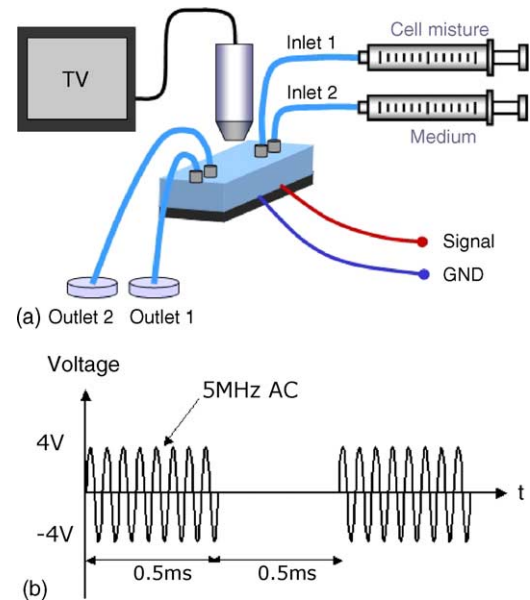


Fig. 6. Experimental set-up for continuous cell separation: (a) experimental apparatus and (b) switched output signal, V_{out} .

the cell separation by CCD camera connected to the microscope. In order to prevent cell trapping at the electrode edges by the pDEP, we use a switched AC electrical signal with duty ratio of 50% and switching frequency of 1 kHz (Fig. 6(b)). This means that there is no DEP force during the half of a period. Therefore, cells in pDEP can flow in the microchannel while they across the microchannel by pulsed DEP force.

4.3. DEP response test

In order to obtain the optimal separation condition, we measure DEP response of viable and nonviable yeast cells using test devices in terms of the medium conductivity and the electric fields frequency before the continuous separation. For example, we fix medium conductivity of 5 $\mu\text{S}/\text{cm}$ and change the electric field frequency as shown in Fig. 7. While viable yeast cells show positive response at both 10 kHz (Fig. 7(a)) and 5 MHz (Fig. 7(b)), nonviable yeast cells show positive response at 10 kHz (Fig. 7(c)) and negative response at 5 MHz (Fig. 7(d)). The results of DEP response at three different medium conductivities (5, 30 and 78 $\mu\text{S}/\text{cm}$) are compared to the estimated response by two-shell model [8] in Table 1. The measured DEP responses have a good accordance with the estimated ones. We choose the medium conductivity of 5 $\mu\text{S}/\text{cm}$ and electric fields frequency of 5 MHz as the separation condition because viable and nonviable yeast cells show different DEP response at this conditions and low medium conductivity is good to avoid heat problem which can damage cells. Under these conditions, viable and nonviable yeast cell mixture can be separated each other as shown in Fig. 8.

Table 2

Experimental conditions for continuous separation

Cell mixture	
Composition	Viable yeast: 62%; nonviable yeast: 38%
Concentration	8×10^7 cells/ml
Flow-rate	0.1, 0.5 and 1 $\mu\text{l}/\text{min}$
Medium conductivity	5 $\mu\text{S}/\text{cm}$
Electric field frequency	5 MHz
Electric voltage	8 $V_{\text{p-p}}$ (sinusoid)
Electrode gap	20 μm

Table 3

Experimental results of the continuous separation

Flow-rate of the cell mixture ($\mu\text{l}/\text{min}$) (throughput in cells/s)	Purity ^a of the separated cells	
	Outlet 1: viable yeast (pDEP) (%)	Outlet 2: nonviable yeast (nDEP) (%)
0.1 (1.3×10^2)	97.3 ± 1.2	72.7 ± 3.4
0.5 (6.5×10^2)	97.0 ± 1.6	74.3 ± 5.9
1 (1.3×10^3)	95.9 ± 1.3	64.5 ± 3.3

^a Purity is defined by the number of target cells over the number of total cells in each outlet.

4.4. Continuous separation

Experimental conditions and results of continuous separation are summarized in Tables 2 and 3, respectively, and Fig. 9 shows the video image of the continuous separation. We obtain the separated viable and nonviable yeast cells at the two different outlets. Fig. 10 shows the microscopic images of yeast cell mixture before separation, and separated viable and nonviable yeast cells. The purity of the separated viable and nonviable yeast cells has been measured in the range of 95.9–97.3 and 64.5–74.3%, respectively, at the mixture flow-rates of 0.1–1 $\mu\text{l}/\text{min}$. We observe that the purity of separated cells remains even though the flow-rate changes. However, the purity of the separated nonviable yeast cells is a little bit lower than the viable yeast cells. We think that nonviable yeast cells block the movement of viable yeast cells across

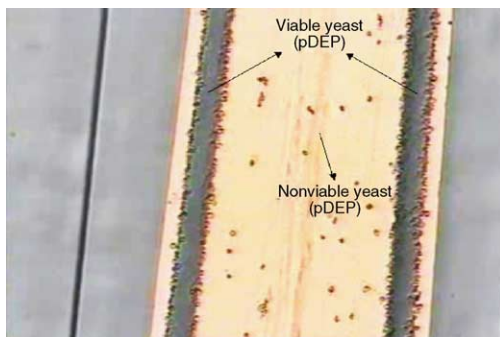


Fig. 9. Video image of continuous separation using viable and nonviable yeast cells in present device, where the electric field frequency of 5 MHz for the sinusoidal potential of 8 $V_{\text{p-p}}$ across the electrode array of 20 μm -gaps immersed in the medium conductivity of 5 $\mu\text{S}/\text{cm}$. The mixture flow-rate is 0.1 $\mu\text{l}/\text{min}$.

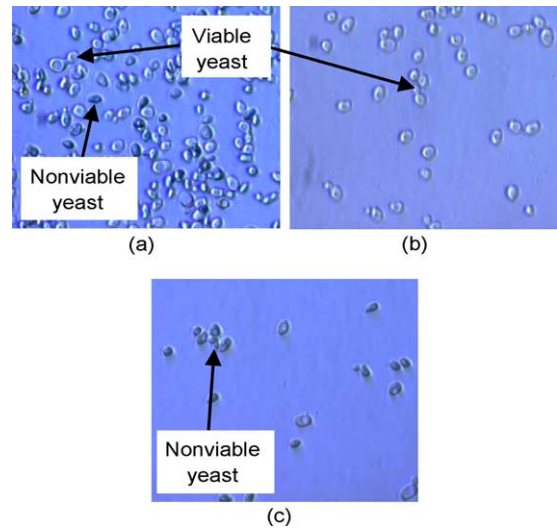


Fig. 10. Microscopic images of yeast cells: (a) yeast cells mixture before separation; (b) separated viable yeast cells at outlet 1 and (c) separated nonviable yeast cells at outlet 2.

the microchannel; therefore, some viable yeast cells are still confined to the central streamline; therefore, the purity of nonviable yeast cells decreases.

5. Conclusions

In this work, we present a high-throughput continuous cell separation chip using hydrodynamic dielectrophoresis process. In the experimental study, we obtained the cell separation conditions where viable and nonviable yeast cells showed different DEP responses, and we verified that present continuous cell separation chip can separate yeast cell mixture continuously. The purity of the separated viable and nonviable yeast cells has been measured in the range of 95.9–97.3 and 64.5–74.3%, respectively, at the mixture flow-rates of 0.1–1 $\mu\text{l}/\text{min}$. We also observed that the purities of separated viable and nonviable yeast cells remain for varying flow-rate conditions. The present chip, capable of separating various kinds of the bio-object mixtures having different DEP responses, is promising for applications to high-throughput integrated biological analysis systems.

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Biographies

Il Doh received a BS degree summa cum laude at Ajou University in 2002 and a MS degree from the Department of Mechanical Engineering at the Korea Advanced Institute of Science and Technology (KAIST) in 2003. His research interests are focused on biofluidic microsystems for point-of-care applications.

Young-Ho Cho received the BS degree summa cum laude from Yeungnam University, Daegu, Korea, in 1980; the MS degree from the Korea Advanced Institute of Science and Technology (KAIST), Seoul, Korea, in 1982; and the PhD degree from the University of California at Berkeley for his electrostatic actuator and microflexure suspension research completed in December 1990. From 1982 to 1986, he was a research scientist of CAD/CAM Research Laboratory, Korea Institute of Science and Technology (KIST), Seoul, Korea. During 1987–1991, he worked as a graduate student researcher (1987–1990) and a post-doctoral researcher (1991) of the Berkeley Sensor and Actuator Center (BSAC) at the University of California at Berkeley. In August 1991, Dr. Cho moved to KAIST, where he is currently an associate professor in the Departments of BioSystems and Mechanical Engineering as well as the Director of Digital Nanolocomotion Center. Dr. Cho's research interests are focused on photonic and biofluidic microsystems with micro/nano actuators and detectors. In Korea, he has pioneered MEMS research and has been active on the development of electromechanical inertial sensors and optomechanical and thermofluidic actuators for automotive, electronics, information and biomedical applications. Dr. Cho is a member of IEEE and ASME.