Rapid Formation of arrayed cells on the electrode with microwells by scanning electrode based on positive dielectrophoresis

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Abstract

We have developed simple and rapid formation of cell-based array on microwell array electrodes by an attractive force of positive dielectrophoresis (p-DEP) even after removing an upper disk electrode stick that was used as a counter electrode to the microwell array electrodes. The attractive force of p-DEP generated by the scanning of the disk electrode allows to forming the cell-based array on the whole microwell arrays. We demonstrated the exploration of the target cells spiked with a low ratio after removing the disk electrode.

Keywords: Dielectrophoresis, Cell-based array, Scanning electrode.
Cell-based arrays have been developed to analyze and screen cellular functions in high throughput. To availability of the simultaneous estimation of the large numbers of individual cells, cell-based arrays are considered as useful tools for biological applications such as toxicology, drug screening, and gene function analysis\(^1\). The surface functionalization techniques\(^2\), embedding in hydorogel\(^3\) and microwell arrays are frequently applied to fabricate the cell-based arrays on the solid substrate. The use of the microwell arrays is advantageous for the exchange of a medium and the concentration of cellular secretions\(^4\text{-}\text{7}\). However, the introduction of cells to each microwell relied on the arrangement based on cell’s own weight, hence relatively long time for forming cell-based arrays.

Microfluidic flows\(^8\), centrifugal separations\(^9\), magnetic forces\(^10\) and electrical forces were used as an external force to form cell-based arrays. A dielectrophoresis (DEP) is the motion of a particle by an interaction of a polarization effect induced by a spatially inhomogeneous electric field\(^11\). Arrays of microwells with the electrodes on the bottom of microwells were combined with fluidic systems to form the cell-based arrays with high-density by positive-DEP (p-DEP)\(^12\). The discrimination of target cells with specific antigens expressed on the cell membranes were conducted by the cell-based arrays with a view of applying to the cell-based biosensors and drug discovery\(^13\). In addition, microwell arrays have also been used to form arrays of cell pairs to develop systems for producing hybrid cells effectively\(^14,15\).

The techniques for recovering the target cells from the arrayed cells is urgently desired to develop the comprehensive systems for analyzing the single cells with high throughput manner. The microdispenser was applied to retrieve target cells with the response to chemical stimuli selectively from the cell-based arrays after the upper substrate was removed. However, the removal of upper substrate with electrodes led to the dispersion of cells by a large drag force, resulting in the loss of cells. In this study, we have used a disk electrode with millimeter order as a counter electrode for the microwell array electrode to form an
inhomogeneous electric field in the space between the disk electrode and the microwell array electrode. By using the present system, we achieved to form the cell-based array in the microwell arrays with a wide space above the microwells occupied with single cells after removing the disk electrode.

The microwell array electrode was fabricated on an indium-tin oxide (ITO) substrate by conventional photolithography which was reported previously\textsuperscript{15}. Figure 1A shows the cross-sectional view of the schematic configuration of the system for fabricating the cell-based arrays. A square shape 10,000 (100×100) microwells (approximately 16 µm) were formed between the micropoles fabricated by negative photoresist (SU-8 3025, MicroChem Corp., Newton, MO), which had a circular shape (30 µm diameter). The top view of the optical image of the device was shown in Figure 3A. PDMS chamber (7 mm diameter and 7 mm height) was attached on the substrate with the microwell array. A gold disk electrode stick (electrode diameter 1.6 mm and total diameter 3.0 mm, BAS Inc., Tokyo, Japan) that was attached on a XYZ manipulator (Suruga Seiki, Tokyo, Japan) is arranged 30 µm above a surface of the photoresist.

The suspension (50 µL) of mouse myeloma cells (average diameter 12 µm and concentration $6.0 \times 10^6$ mL$^{-1}$) in 200 mM sucrose solution was dropped in the chamber before arranging the disk electrode. Trapping of cells to the microwell arrays were conducted by applying AC voltage of 20 V peak-to-peak (20 V$_{pp}$) in the p-DEP frequency region (5.0 MHz) to the disk electrode from the function generator (7075, Hioki E.E. Co., Ueda, Japan), while the microwell array electrode was connected to the ground (Fig. 1B). The disk electrode was horizontally scanned over the whole region of the microwell array at 100 µm s$^{-1}$ (Fig. 1B and 1C) and then removed from the cell suspension by transferring the disk electrode to the upper direction at 50 µm s$^{-1}$ (Fig. 1D). The excess cells in the outside of microwells were removed by repeating the following three stepes three times; 1) the addition of 100 µL culture medium, 2)
the gentle pipetting and 3) the removal of 100 µL suspension medium by a pipet. The behavior of the cells were observed with an optical microscope (IX72, Olympus Co.).

Cell-based arrays were fabricated by trapping myeloma cells in the microwells by using the systems based on p-DEP (Fig. S1).

The disk electrode was horizontally scanned over the microwell array to introduce cells in the whole 10,000 microwells. Figure 2A and 2B show the fluorescence images of cells above the microwell array before and several seconds after applying AC voltage, respectively. The center of the disk electrode was positioned approximately 1.3 mm left from the center of left-hand axis. The brightness of the area with the falcate shape in the left side of Fig. 2A is darker than that in the other area due to the presence of the edge of the disk electrode. Thus, we can observed the fluorescence emitted from the cells dispersed between the disk electrode and microwell array in the area with the falcate shape. The well-ordered array of fluorescence signals was clearly observed in the area with the falcate shape by p-DEP after applying AC voltage, while the cells above well array in the other area were still dispersed randomly (Fig. 2B).

The disk electrode applied AC voltage was then scanned to right direction at 100 µm s$^{-1}$ (Movie 1 in Supplementary). When the disk electrode moved to the right direction, cells were directed in the microwells at the right side of the array occupied with cells, resulting in the formation of the cell-based array along the trajectory of the disk electrode. The microwells with the strong electric field was shifted along the movement of the disk electrode due to the shift of microwells with the closest distance between the disk electrode and the well array electrodes. Although the space above the microwell array after the disk electrode was passed was covered again by the dispersed cells. Figure 2C shows the fluorescence image 25 s after scanning the disk electrode. The cell-based array can be found in the center of the image because of the travel of the disk electrode to 2.5 mm right from the original position in Fig. 2B. The occupation
efficiency was found to be approximately 80-90% that is corresponded to the results obtained without the scanning of the disk electrode (Fig. S1). The cell-based array can be formed in the whole 10,000 microwells within 240 s by scanning the disk electrodes twice along the horizontal axis with 1.6 mm shift to the vertical direction. Most cells trapped in microwells were still stayed at the original position even after the excess cells were removed by repeating three steps.

Finally, we have studied the exploration of the target cells that were spiked with a low ratio. The cells stained in green by Calcein-AM as target cells were spiked in the suspension of cells stained in red by Cyto Red at a rate of 2.0%. The cells containing 2.0% of target cells were arranged by using the microwell array device. The optical image of trapping cells after removing the excess cells was shown in Figure 3A. The cells trapped in microwells still stayed in their without the decrease of the cell occupation efficiency even after excess cells were removed. Unfortunately, there are some cells located on the undesired position such as the top of micropoles. The density of the undesired cells was investigated and found to be 272 mm⁻¹ averagely. This may be due to the nonspecific adsorption of cells to the surface of the photoresist used for fabricating the micropoles.

Figure 3B and 3C show fluorescence images for a major portion of the cells stained in red and target rear cells stained in green, respectively. The well-ordered red signals were observed from the cells trapped in the microwells (Fig. 3B), while three green signals were observed in Fig. 3C, because the rate of content for green cells addressed as target is only 2.0%. In Fig. 3B, no red signal was observed from the position arrowed by yellow arrow where the green signal was observed at the position in Fig. 3C. Thus, we can judge that the cell arrowed by black arrow in the optical image (Fig. 3A) is the target stained in green. There is no position with both red and green signals. In addition, the wells with both red and green signals were found at the upper right in Fig. 3B and 3C. The observation of both red and green signals from
the single microwells is responsible for the trapping each cell stained in red and stained in green in these microwells. The ratio of the cells with green signal to the cells in the microwell array containing cells stayed on undesired position is 2.0±0.3% averagely (trial order number is three) and coincided with that of the spiked green cells.

In conclusion, the well-ordered cell-based array can be formed by using the disk electrode as the upper counter electrode to the lower microwell array electrode. The cells between the disk electrode and the microwell array were quickly trapped in microwells by p-DEP. The scanning of the disk electrode horizontally permits to form the cell-based array along the trajectory of the movement of the disk electrode. Thus, we can introduce the cells in wells in the whole 10,000 microwell arrays within 240 s. The cell-based array can be obtained after removing the disk electrode and excess cells. We also demonstrated the exploration of the target cells spiked with a low ratio from the fabricated cell-based array by detecting the fluorescence signal. Although we have to improve the method for the surface treatment to avoid nonspecific adsorption of cells, the present system could be useful candidate to produce the cell-based array. We will perform the optimization of the size and scan speed of the upper electrode and the distance between the upper electrode and microwell array electrode to improve the efficiency and the time required for forming cell arrays and increase the number of microwells for discriminating rare cells with high-throughput manner. The pickup system of target cells by using the microdisk electrode based on p-DEP will also be applied to recover the target cells arrayed in microwells.

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Supporting Information

Supporting Information includes Movie 1 for forming the cell-based array by p-DEP when the disk electrode was scanned horizontally. This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/.

References


Figure Captions

Fig. 1 (A) Cross-sectional view of the system for fabricating the cell-based arrays. (B and C) Horizontal scan of the disk electrode over the whole region of the microwell array. (D) Removal of the disk electrode from the cell suspension by transferring to the upper direction.

Fig. 2 Fluorescence images of cells above the microwell array (A) before and (B) several seconds after applying AC voltage. (C) Fluorescence image 25 s after scanning the disk electrode.

Fig. 3 (A) Optical image of trapping cells after removing the excess cells. Fluorescence image of cells stained in (B) red and (C) green.
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Graphical Index

disk electrode

scan

removal of disk electrode and excess cells

microwell array electrode

200 μm

100 μm