Rapid Formation of Arrayed Cells on an Electrode with Microwells by a Scanning Electrode Based on Positive Dielectrophoresis

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We have developed a simple and rapid formation of a 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 the formation of a cell-based array on all microwell arrays. We demonstrated an exploration of target cells spiked with a low ratio after removing the disk electrode.

Keywords Dielectrophoresis, cell-based array, scanning electrode

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Cell-based arrays have been developed to analyze and screen cellular functions in high throughput. To make available a simultaneous estimation of large numbers of individual cells, cell-based arrays are considered to be useful tools for biological applications, such as toxicology, drug screening, and gene function analysis.1 Surface functionalization techniques,2 embedding in hydorgel3 and microwell arrays are frequently applied to fabricate cell-based arrays on a solid substrate. The use of microwell arrays is advantageous for the exchange of a medium and the concentration of cellular secretions.4–7 However, the introduction of cells to each microwell has relied on the arrangement based on the cell’s own weight; hence, a relatively long time is needed for forming cell-based arrays.

Microfluidic flows,8 centrifugal separations,9 magnetic forces10 and electrical forces were used as the external force to form cell-based arrays. A dielectrophoresis (DEP) is the motion of a particle by the interaction of a polarization effect induced by a spatially inhomogeneous electric field.11 Arrays of microwells with electrodes on the bottom of the microwells were combined with fluidic systems to form 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 was conducted by cell-based arrays with a view of applying to cell-based biosensors and drug discovery.13 In addition, microwell arrays have also been used to form arrays of cell pairs so as to develop systems for producing hybrid cells effectively.14,15

Techniques for recovering the target cells from the arrayed cells are urgently desired to develop comprehensive systems for analyzing single cells in a high-throughput manner. A microdispenser was applied to retrieve target cells with the response to chemical stimuli selectively from cell-based arrays after the upper substrate was removed. However, removing the 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 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 a cell-based array in 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.15 Figure 1(A) shows a cross-sectional view of the schematic configuration of the system for fabricating the cell-based arrays. Square-shaped 10000 (100 × 100) microwells (approximately 16 μm) were formed between the micropoles fabricated by a negative photoresist (SU-8 3025, MicroChem Corp., Newton, MO), which had a circular shape (30 μm diameter). A top view of the optical image of the device is shown in Fig. 3(A). A PDMS chamber (7 mm diameter and 7 mm height) was attached onto the substrate with a microwell array. A gold-disk electrode stick (electrode diameter of 1.6 mm and total diameter of 3.0 mm, BAS Inc., Tokyo, Japan), which was attached to a XYZ manipulator (Suruga Seiki, Tokyo, Japan), was arranged 30 μm above the surface of the photoresist.

The suspension (50 μL) of mouse myeloma cells (average diameter 12 μm and concentration 6.0 × 10^6 mL−1) in 200 mM sucrose solution was dropped in the chamber before arranging the disk electrode. The trapping of cells to the microwell arrays was conducted by applying an AC voltage of 20 V peak-to-peak (20 Vp0) in the p-DEP frequency region (5.0 MHz) to the disk electrode from a function generator (7075, Hioki E.E. Co., Ueda, Japan), while the microwell array electrode was connected to the ground (Fig. 1(B)). The disk electrode was horizontally scanned over the whole region of the microwell array at 100 μm s−1 (Figs. 1(B) and 1(C)) and was then removed from...
the cell suspension by transferring the disk electrode to the upper direction at 50 μm s⁻¹ (Fig. 1(D)). Excess cells outside of microwells were removed by repeating the following three steps three times: 1) the addition of a 100-μL culture medium, 2) gentle pipetting and 3) removing the 100 μL suspension medium by a pipet. The behavior of the cells was 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, Supporting Information). The disk electrode was horizontally scanned over the microwell array so as to introduce cells in all 10000 microwells. Figures 2(A) and 2(B) show fluorescence images of cells above the microwell array before and several seconds after applying an AC voltage, respectively. The center of the disk electrode was positioned approximately 1.3 mm left from the center of the left-hand axis. The brightness of the area with the falcate shape on the left side of Fig. 2(A) is darker than that in the other area due to the presence of the edge of the disk electrode. Thus, we could observe fluorescence emitted from the cells dispersed between the disk electrode and the 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 an AC voltage, while the cells above well array in the other area were still dispersed randomly (Fig. 2(B)).

The disk electrode applied AC voltage was then scanned to the right direction at 100 μm s⁻¹ (Movie 1 in Supporting Information). 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 a cell-based array along the trajectory of the disk electrode. The microwells with a strong electric field were shifted along with 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. However, the space above the microwell array after the disk electrode was passed was covered again by the dispersed cells. Figure 2(C) shows the fluorescence image 25 s after scanning the disk electrode. The cell-based array can be found at the center of the image because of the travel of the disk electrode to 2.5 mm right from the original position in Fig. 2(B). The occupation efficiency was found to be approximately 80 – 90%, which is corresponds to the results obtained without scanning the disk electrode (Fig. S1). A cell-based array can be formed in all 10000 microwells within 240 s by scanning the disk electrodes twice along the horizontal axis with a 1.6-mm shift to the vertical direction. Most cells trapped in the microwells still remained at the original position, even after excess cells were removed by repeating the three steps.
Finally, we 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 the target cells were arranged by using a microwell array device. An optical image of trapping cells after removing the excess cells is shown in Fig. 3(A). Cells trapped in microwells still stayed in their position without a decrease of the cell occupation efficiency, even after excess cells were removed. Unfortunately, there were some cells located at some undesired position, such as the top of the micropoles. The density of the undesired cells was investigated and found to be 272 mm$^{-1}$ on the average. This may be due to a nonspecific adsorption of cells to the surface of the photoresist used for fabricating the micropoles.

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

In conclusion, the well-ordered cell-based array could 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 one to form a cell-based array along the trajectory of the movement of the disk electrode. Thus, we can introduce cells in wells for all 10000 microwells within 240 s. The cell-based array can be obtained after removing the disk electrode and excess cells. We also demonstrated the exploration of 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 so as to avoid a nonspecific adsorption of cells, the present system could be a useful candidate to produce a cell-based array. We will perform optimization of the size and scan speed of the upper electrode and the distance between the upper electrode and the microwell array electrode to improve the efficiency and time required for forming cell arrays and to increase the number of microwells for discriminating rare cells in a high-throughput manner. The pickup system of target cells by using the microdisk electrode based on p-DEP$^{18}$ will also be applied to recover 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/.

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