Chapter 5
Discrimination of Cells with Specific Antigens Expressed on a Membrane Based on the Dielectrophoresis

Tomoyuki Yasukawa and Fumio Mizutani

Abstract Formation of line pattern with cells based on dielectrophoresis (DEP) was applied to simple and rapid distinction of cells with specific surface antigens from a cell population. Dispersed cells were accumulated to gap areas of the interdigitated band array (IDA) electrode modified with antibody within 5 s by negative DEP (n-DEP) and captured by immunoreactions. Unbounded cells without the specific antigen on the membrane were removed to form another pattern by switching the applied voltage of the band electrode. The time required for the assay was substantially short, 60 s for forcing and 60 s for the separation of unbounded cells. Furthermore, the present method does not require pretreatment such as target labeling or washing of unbound cells.

Keywords Cell patterning · Surface antigen · Label free · Phenotyping

5.1 Introduction

Particles placed in a spatially inhomogeneous electric field experience dielectrophoresis (DEP) force by the interaction of a polarization effect induced in the particles. DEP is attractive for the manipulation of micro- and nano-objects including biological living cells and bacteria [1] and has been used in the wide range of applications, such as a separation and sorting [2–4], trapping [5–7] and patterning. Cell arrays can be fabricated using positive DEP (p-DEP), which is a force directed particles towards the regions of electric field maxima [8–10]. Generally, p-DEP patterning is utilized to attract in array formats with the pair of electrodes at every elements modified with cell adhesive layers to produce cell patterns. Negative DEP (n-DEP) force which is a force to repel the particles from the high electric field regions has also been used to pattern cells [11–13]. The strategy for n-DEP cell patterning is the construction of the localized position enclosed with strong electric fields and allows

T. Yasukawa (✉) · F. Mizutani
Graduate School of Material Science, University of Hyogo, 3-2-1, Kouto, Kamigori, Ako 678-1297, Japan
e-mail: yasu@sci.u-hyogo.ac.jp

T. Arai et al. (eds.), *Hyper Bio Assembler for 3D Cellular Systems,*
DOI 10.1007/978-4-431-55297-0_5

to cell trapping by the repulsion force balanced from every direction. We have also previously contributed to the fabrication of periodic and alternate cell lines incorporating two cell types of cells using n-DEP [14]. Furthermore, by using both p-DEP and n-DEP, individual cell types with different dielectrophoretic properties were separately patterned at the different positions [15, 16]. We can easily and reversibly fabricate two different patterns with particles by controlling the direction of the DEP force with applied frequency. However, one feature was only used in most case of cell patterning. We recently developed rapid and simple sandwich-typed immunosensing systems using accumulation and re-dispersion of microparticles based on n-DEP [17]. The use of n-DEP manipulation of microparticles brought about the separation-free sensing of unreacted target molecules and microparticles within 3 min [18–20].

In this chapter, we introduce the useful application of the DEP manipulation for the rapid and simple discrimination of cells which were expressed a specific surface antigen [21, 22]. Surface antigen patterns expressed on living cells depend on a linage, differentiation and maturation. Phenotyping, which can be identified the presence and proportion of pathogenic cell populations, are useful for early medical diagnosis and prognosis prediction. A common approach is to use fluorescence labeling via antigen-binding to relieve specific antigens at cell surfaces; however, the methods are often qualitative and low throughput, and involve several complex steps for a modification and washing. The cell binding assay to the immobilized antibody was accelerated by n-DEP cell accumulation and the discrimination of the unbound cells was performed with spatial separation by switching the formation of electric field. We demonstrate here that Human promyelocytic leukemia (HL60) cells with surface antigen, CD33, can be trapped on the gap region modified with anti-CD33 antibody.

5.2 Particle Patterning at Different Positions in a Single Device

We demonstrated the formation of aligned structures with microparticles at the different positions in the single device [23]. Interdigitated band array (IDA, 2 mm long, 12 μm wide, and gap 50 μm) electrode was fabricated by indium tin oxide (ITO) layer which is a transparent conductive material. Another ITO electrode was mounted on the ITO-IDA electrode via a 30 μm thick polyester film to fabricate the DEP device with fluidic channel. Top and cross section of DEP device were shown in Fig. 5.1a, b, respectively. The application of the AC voltage with the frequency region for n-DEP formed a non-uniform electric field in the device.

A suspension of the polystyrene particles (6-μm diameter) was injected into the device. The patterns with particles were formed by applying AC voltage in the frequency region for n-DEP (1.0 MHz) to the band A and band B. Figure 5.2a, b show images of particle patterns obtained by use of the glass and ITO as upper substrates, respectively. The application of AC voltage (20 V_{pp}) with an identical frequency and

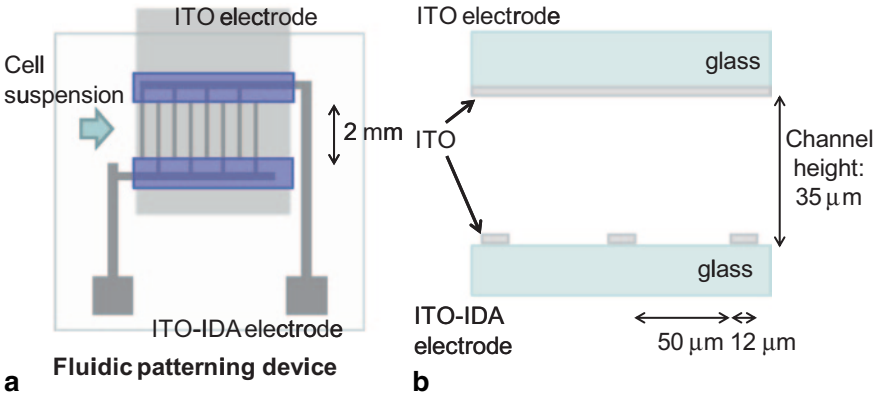


Fig. 5.1 **a** Top and **b** cross sectional views of fabricated DEP device

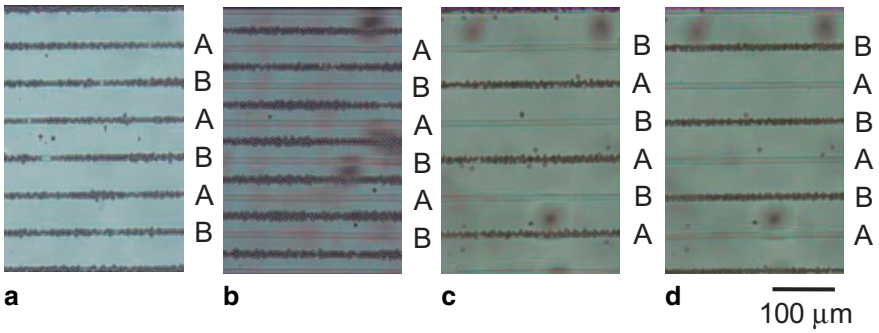


Fig. 5.2 Images of particle patterns obtained by use of **a** glass and **b** ITO as upper substrates. The AC voltage (20 V_{pp}) with an identical frequency and opposite phase to the band A and B was applied and upper ITO was connected to the ground. Images of patterns obtained by applying AC voltage with identical phase to **c** band A and upper ITO, and **d** band B and upper ITO. (Modified from [23])

opposite phase to the band A and B forced particles to form the line pattern along both bands within 1 s. The particles were guided to the glass surface to form periodical lines of positive projection above the band electrodes of the IDA template (Fig. 5.2a). On contrast, when we used the conductive ITO electrode connected to the ground as an upper substrate, the particles were aligned on the ITO surface above the gap of IDA electrodes (Fig. 5.2b).

The use of the conductive electrode as an upper substrate allowed to the formation of various patterns using the same design of device. Figure 5.2c shows an image for the particle formation. In this case, the AC voltage was applied to the upper ITO and band A with the same intensity (20 V_{pp}), frequency (1.0 MHz) and phase, while the AC voltage with opposite phase was applied to band B. Dispersed particles were guided to the areas above band A to form the alternating line pattern

with the half of the positive projection. The electric field formed between the upper ITO and band A is relatively low compared to that between the upper ITO and band B. Thus, no particles were accumulated between the upper ITO and band B. On the other hand, the AC voltage was applied to the upper ITO and band B with the same intensity, frequency and phase to form another line pattern. The particles also rapidly accumulated at the areas above band B due to the repulsive force of n-DEP by the distribution of the strong electric fields generated between the upper ITO and band A and between each band (Fig. 5.2d). Thus, the line patterns with same design can be easily fabricated at the different position in the single DEP device. We applied the techniques to accumulate and capture cells with specific surface antigen via immunoreactions.

5.3 Formation of Two Different Patterns with Cells

DEP line patterning with HL60 cells was studied using a DEP patterning device consisted of the upper ITO and lower ITO-IDA electrode. HL60 cells (4.0×10^7 cells/mL) suspended in the DEP medium, which consisted of 250 mM sucrose and 250 mM HEPES buffer (pH 7.4), adjusted the conductivity to 400 mS/m with PBS were introduced into the device to manipulate the cells with the n-DEP. The AC voltage (15 V_{pp}, 100 kHz) was applied between upper ITO and the lower ITO-IDA electrode to accumulate the cells in the gap region between bands by the repulsive force of n-DEP, because the strong and weak electric fields were formed on the band electrodes and at the gap region. Figure 5.3a, b show the optical microscopic images before and 5 s after the voltage was turned on, respectively. The cells dispersed randomly in the channel (Fig. 5.3a) were rapidly accumulated in the gap region within 5 s (Fig. 5.3b). When the voltage applied to the band B was switched to zero, the cells moved on the band B with the repulsive force generated between the upper ITO and band A, and band A and B, resulting in the formation of another line pattern within 5 s (Fig. 5.3c). Moreover, the formation of the first line pattern in Fig. 5.3b reproduced as the voltage for the band B was again switched on. The results clearly indicated that the different patterns with cells could be easily created by applying the voltages with the different intensity to bands. Figure 5.3d, e show the cross-sectional plots of the electric field formed in the patterning device calculated from the digital simulation when a voltage is applied to both IDA bands and band A, respectively. The distribution of the electric field strength for the DEP patterning device was calculated by the finite element method (FEM) solver (COMSOL Multiphysics, Stockholm, Sweden). The regions with high electric field were found in the areas between the upper ITO and lower both bands (Fig. 5.3d). Thus, the suspended cells moved in the gap region by n-DEP. In contrast, when the voltage is applied to the band B, the regions with high electric field were found between the upper ITO and band A (Fig. 5.3e). Therefore, low electric field regions were formed on the band B. These results indicated that the different applications of the voltage for

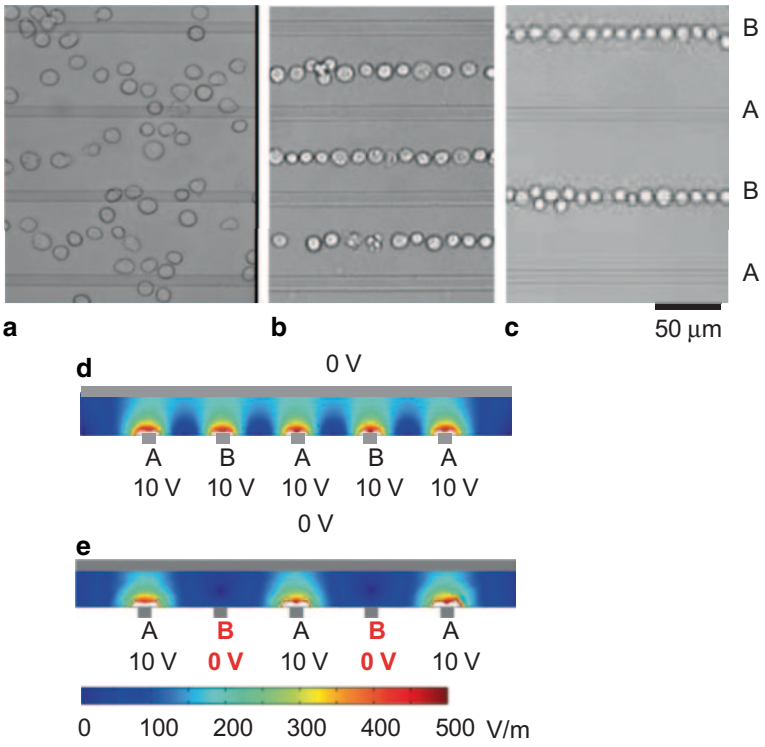
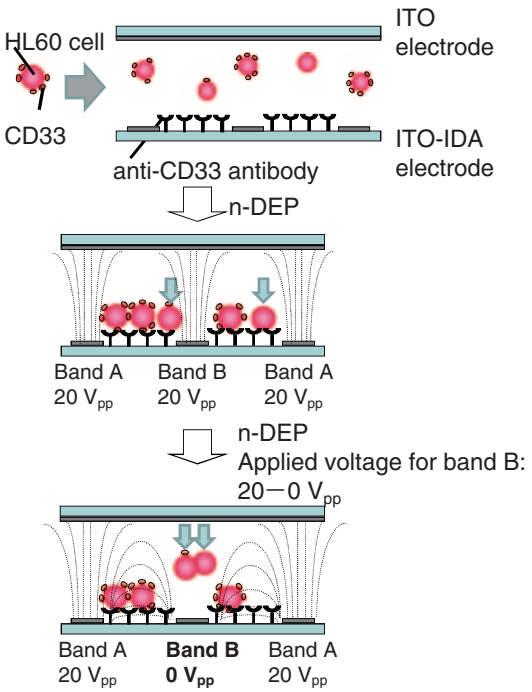


Fig. 5.3 Optical images of cells manipulated by n-DEP **a** before and **b** and **c** 5 s after the application of the ac voltage (intensity, 15 Vpp; frequency, 100 kHz). **b** The voltage was applied between the upper ITO and lower band A and B. **c** The voltage was applied to band A, while the upper ITO and band B was connected to the ground. **d** and **e** Cross-sectional views of the numerically calculated electric field formed in the patterning device. Upper and lower gray bars show the ITO and band electrodes, respectively. Applied voltages for bands A and B and the upper ITO were (D) 10, 10, and 0 V and (E) at 10, 0, and 0 V, respectively. (Modified from [22])

band B forced the cells to move to areas in the gap region or on the band B, which were in good agreement with the experimental results (Fig. 5.3a–c).

5.4 Capture of HL60 Cells with CD33 Antigen by n-DEP

We have investigated the number of cells accumulated in the gap region using the cell binding efficiency. Figure 5.4 depicts the cross sectional view of the DEP device and the present procedure for detecting the surface antigen. Cells were forced to move toward the gap regions between the bands and captured with the antibodies via immunoreactions. Uncaptured cells were removed and separated from the gap region to the band B by switching the applied voltage for band B to zero. As a result, the ratio of captured cell density can be easily calculated from the images



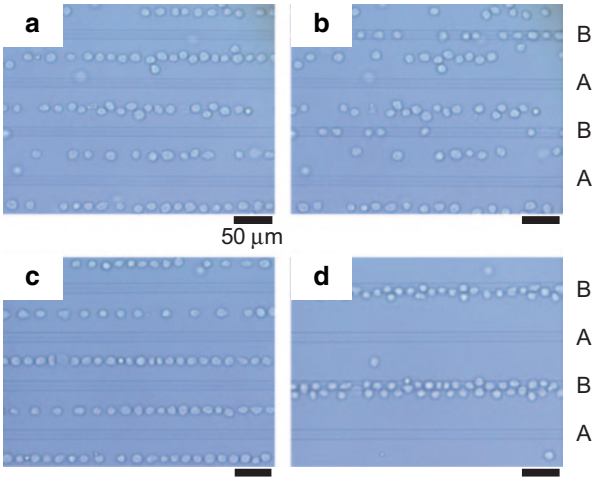


Fig. 5.4 Cross-sectional view of the DEP device and the method for discriminating cells with specific surface antigens using n-DEP [22]

Fig. 5.5 Optical images of cells patterned by n-DEP. **a** Cells accumulated in the gap region modified with the anti-CD 33 antibody by applying AC voltage of same intensity (20 V_{pp}) and frequency (100 kHz) as bands A and B. **b** Cell pattern captured in the gap region modified with the anti-CD 33 antibody after separating unbound cells by switching off the band B voltage. **c** Cells accumulated in the gap region modified with the anti-mouse IgG antibody. **d** Cells removed from the gap region modified with the anti-mouse IgG antibody after the band B voltage was turned off. Duration of voltage application for accumulation and removal with n-DEP: 60 and 60 s. (Modified from [22])

of the line structure of cells in the gap region. Figure 5.5 shows the optical images of cells accumulated in the gap region (Fig. 5.5a) and captured cells after separating the unbound cells (Fig. 5.5b). Cells were accumulated in the gap region with n-DEP for 60 s and then the voltage for the band B was switched off to remove the cells from the gap region. Again, the uniformly dispersed cells initially start to move toward the gap region due to the strong repulsive force for n-DEP to form clear line patterns (Fig. 5.5a). After the voltage of band B was switched to zero, some cells accumulated in the gap region modified with anti-CD33 antibody were remained on the gap region even after the voltage was switched off, while the other moved on the band B (Fig. 5.5b). In contrast, almost all cells moved on band B after switching off the voltage to the band B when cells were accumulated in that with anti-mouse IgG antibody (Fig. 5.5d). The results suggested that the cells with CD33 surface antigens were reacted with the antibodies immobilized in the gap region and irreversibly captured at that position. The number of cells in the gap region quickly decreased in 30 s after switching off the voltage, and then reached at steady-state value. The cell binding efficiency is estimated from the steady-state value and found to be $68.3 \pm 3.2\%$. The slight undesired binding originated from the non-specific adsorption was observed on the bands modified with anti-mouse IgG antibody ($4.2 \pm 1.4\%$). The time as short as 30 s was required for removing unbound cells. Therefore, the cells with CD33 cell surface antigen can be rapidly identified from the cell suspension by the spatial separation based on the immunoreactions and DEP manipulation.

5.5 Specific Cell Binding in a Mixture Containing Nonspecific Cells

Non-specific cells used as a CD33 negative cell was prepared by treating HL-60 cells with anti-CD33 antibody. Cells were also labeled with fluorescent molecules (CFDA SE) to distinguish the antibody-treated non-specific cells. The suspensions of each specific and non-specific cell were mixed at different ratios to study the relationship between the content of CD33 positive cells in suspensions. The mixtures were introduced into the device and accumulated in the gap region by n-DEP for 60 s. The binding efficiency of the cells was calculated after switching off the voltage for band B for 60 s. The efficiency was defined as a ratio of average density of cells in the gap region immediately before and 60 s after AC voltage for band B was switched off to eliminate the unbound cells.

Mixtures of HL-60 cells specific and nonspecific to anti-CD33 were used to determine the number of cells captured in the gap area modified with anti-CD33. Figure 5.6a shows the image obtained by combining optical and fluorescent images, which were obtained 60 s after the applied voltage to band B was switched to zero. The initial concentration ratio of specific cells was set to 50%. Almost all nonspecific cells with a fluorescent signal moved to band B, whereas the specific cells were captured in the gap between the band electrodes, even in the presence of

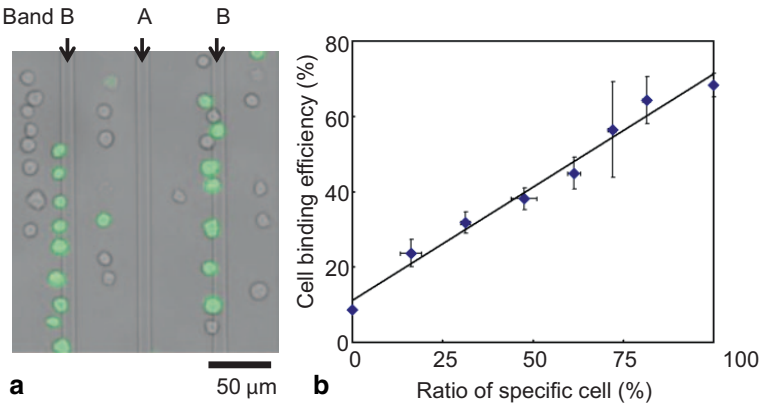


Fig. 5.6 Cells captured in the gap region from mixed suspensions of specific and nonspecific HL-60 cells. **a** Photographs were obtained by combining the optical and fluorescent images. Initial ratios in the original suspensions were set to 50% of specific cells. **b** Ratio of cells captured in the gap. [22]

treated nonspecific cells (Fig. 5.6a). Figure 5.6b shows the ratio of cells captured in the gap between the band electrodes. The ratio of captured cells increased linearly with the increasing ratio of specific cells in the prepared mixture suspension. These results indicate that the presence of cells without the target antigen did not obstruct specific cell binding for detecting cells with surface antigens. The binding efficiency obtained in the present study improved compared to that obtained in our previous study using a combination of p- and n-DEP.

5.6 Conclusion

We propose a novel procedure for rapid and simple cell binding assay based on the manipulation of cells by DEP. On applying an AC voltage, cells were forced to accumulate in the gap region yielding the line formations. On the other hand, cells were repelled from the gap region and formed another line pattern on the band B by switching off the voltage for band B. Therefore, we can easily prepare the different pattern with cells by controlling the intensity of the applied voltage. The formation of second line pattern with cells was markedly inhibited by immunoreactions between the surface antigen and specific antibody immobilized in the gap region. HL60 cells with surface antigen were irreversibly captured in the gap region modified with specific anti-CD33 antibody, as a result, captured cells did not remove even after the voltage for the band B was switched off. Importantly, the capture of cells via immunoreactions was assisted by rapid accumulation with n-DEP. The total time as short as 90 s was required for detecting the antigen expressed on the cell surface. Moreover, no any pre-treatment, such as cell staining with fluorescent molecules was requited for the proposed method.

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