RAPID AND SIMPLE DISCRIMINATION OF CELLS WITH SPECIFIC SURFACE ANTIGEN WITH DIELECTROPHORESIS

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ABSTRACT

In this paper, we describe a development of the rapid and simple discrimination system of cells with specific surface antigen by immobilization of cells accumulated by positive dielectrophoresis (p-DEP) via effective surface immunoreactions and removal of unbound cells by negative DEP (n-DEP). The time required for the determination of the surface antigen is decreased to 60 s compared to that required by a cell binding assay using microtiter plates (30 min). Furthermore, the present method for a novel cell binding assay does not require pretreatment such as target labeling or washing of unbound cells.

KEYWORDS

Dielectrophoresis, Surface antigen, immunoreaction, leukemia cells, separation.

INTRODUCTION

Immunophenotyping, a method in which the presence and proportion of pathogenic cell populations can be identified, is useful for early medical diagnosis and prognosis [1]. A common approach is to use fluorescent labeling to reveal specific surface antigens on cells; however, these methods are often qualitative, have low throughput, and involve several complex steps of modification and washing. Recently, an antibody microarray has been employed to detect surface antigens by direct binding of cells to the antibody spot arrays [2]. However, a relatively long incubation is still required for antibody arrays because of cell integration at spots.

Dielectrophoresis (DEP) is attractive for the manipulation of micro- and nano-objects including biological living cells and bacteria in a microfluidic device because of its non-contact nature. We recently developed a rapid and simple immunosensing system using n-DEP-based accumulation and redispersion of microparticles [3-6]. The particles were irreversibly captured on the substrate to form sandwich-type immuno-complexes in the presence of specific analytes because the particles modified with the antibody rapidly accumulated and pressed against the substrate modified with the antibody by n-DEP. Unreacted particles were automatically removed from the substrate by deregulation of DEP. The use of n-DEP manipulation of microparticles allowed separation-free sensing of unreacted target molecules within 3 min.

We applied rapid immunosensing systems based on the particle manipulation with DEP to the surface antigen CD33 expressed on HL-60 cell. The use of the p-DEP can reduce the transport time and remove the cell diffusion for the capture of target cells by antibodies on electrodes. We positively applied n-DEP to remove non-specific cells uniformly from electrodes. The suspension of cells was introduced into the device comprising an upper indium tin oxide (ITO) electrode and a lower band electrode modified with an antibody (Fig. 1). When an AC voltage in the p-DEP frequency region is applied, the cells are directed toward the band electrode, thereby accelerating the capture of cells via immunoreactions. Uncaptured cells were removed to the gap region by n-DEP. Thus, the ratio of captured cell could be easily calculated by the cell pattern.

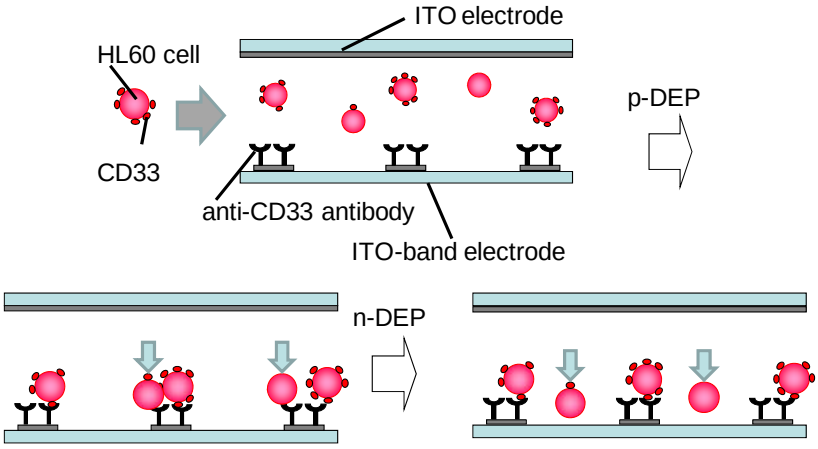

Figure 1. Cross-sectional view of the DEP device and the principle of the present method for detection of surface antigens using a combination of p- and n-DEP.

EXPERIMENT

A patterning device was constructed with an ITO electrode and an ITO band electrode. The band electrode was fabricated by conventional photolithography. Each element was 2 mm long, 12 μm wide, and placed at a distance of

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50 μm from adjacent bands. We modified the band electrodes with mouse anti-CD33 monoclonal antibody (anti-CD33) or goat anti-mouse IgG polyclonal antibody (anti-mouse IgG). The ITO electrode was mounted on the band electrode via a 30-μm thick polyester film used as a spacer.

HL60 cells (CD33+ cells, 4×10^7 cells mL^{-1}) were suspended in DEP medium consisting of 250 mM sucrose and adjusted the conductivity to 80 mS m^{-1} with 250 mM HEPES buffer (pH 7.4) for manipulation of cells by DEP. The AC voltage in the p-DEP frequency region (typically 15 V_{pp} and 10 MHz) was applied between the ITO electrode and the band electrode to accumulate cells on the bands. Then, n-DEP (5–15 V_{pp} and 100 kHz) was used to remove the cells from the bands to the gap region.

We prepared non-specific cells treated with anti-CD33 and used them as CD33-negative cells. Furthermore, the cells were labeled with fluorescent molecules (CFDA SE) to distinguish antibody-treated non-specific cells. Suspensions of specific and non-specific cells were mixed at different ratios to study the relationship between the number of CD33-positive cells in the suspension and the number of cells captured on the band electrode. The cell mixture accumulated on the band electrode by p-DEP for 60 s. The binding efficiency of the cells was calculated after application of the AC voltage for n-DEP for 180 s. The efficiency was defined as the ratio of the average cell density on the band electrode immediately before and 180 s after the AC voltage for n-DEP was applied to remove unbound cells.

RESULTS AND DISCUSSION

Dielectrophoretic patterning with HL60 cells was studied using a patterning device consisting of an ITO electrode and a band electrode without antibody immobilization. The cells dispersed randomly in the channel (Fig. 2A) rapidly accumulated on the band electrode within 5 s (Fig. 2B) of the application (10 MHz). After the frequency was switched to 100 kHz, most of the cells moved toward the gap region within 10 s because of the repulsive force caused by n-DEP (Fig. 2B–2D). Moreover, the formation of line patterns reproduced as the frequency was switched between p- and n-DEP. The results clearly indicated that the different cell patterns could be easily created by applying voltages of different frequencies.

Figure 2E shows the cross-sectional area of the electric field formed in the patterning device calculated from digital simulation in which a voltage is applied to the ITO electrode and the band electrode. Regions with high electric field were found in areas at the edge of the bands (areas labeled as P in Fig. 2E). Thus, the suspended cells moved to area P when the AC voltage in the p-DEP frequency region was applied. However, regions with low electric field were found in the gap region between the band electrodes (areas labeled as N in Fig. 2E). These results indicated that the application of the AC voltage in the p- or n-DEP frequency region to the electrode forced the cells to move to areas P or N, which was in good agreement with the experimental results (Fig. 2A–2D).

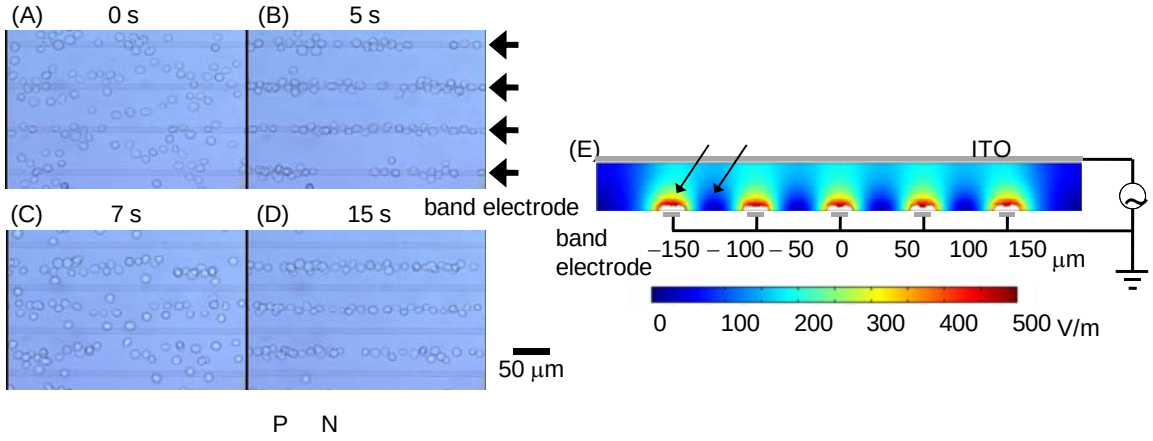

Figure 2. Series of optical images showing cell manipulations by p- and n-DEP (A) before and (B) 5 s after the application of the AC voltage (intensity: 15 V_{pp}, frequency: 10 MHz) for p-DEP manipulation and (C) 2 s and (D) 10 s after the frequency was switched to 100 kHz for n-DEP manipulation. (E) Cross-sectional view of the numerically calculated electric field.

We investigated the number of cells accumulated on the band electrode using cell binding efficiency. Figure 3 shows the optical images of cells accumulated on the band electrode (Fig. 3A) and the captured cells after the separation of unbound cells by n-DEP (Fig. 3B and C). Uniformly dispersed cells initially started to move toward the band electrode to form line patterns by the p-DEP force (Fig. 3A). The number of cells attracted to the bands increased with the duration of voltage application and saturated within 5 s.

Removal of cells was markedly inhibited by the use of band electrode modified with anti-CD33, which corresponded to the CD33 surface antigen expressed on HL60 cells. When the cells accumulated on the band electrode modified with anti-mouse IgG, almost all the cells moved to the gap region after the frequency was switched to n-DEP region frequency after 60 s (Fig. 3C). In contrast, some cells accumulated on the band electrode

modified with anti-CD33 remained even after the frequency was switched for n-DEP (Fig. 3B). These results suggested that the cells with CD33 surface antigens react with the antibodies to irreversibly capture at that position. The number of cells on the band electrode rapidly decreased within 30 s after the frequency was switched and then reached a steady-state value. The cell binding efficiency was estimated from the steady-state value and found to be 46.9 ± 3.4%. Slight undesired binding originating from the non-specific adsorption was observed on the bands modified with anti-mouse IgG (8.7 ± 1.5%). Time as short as 30 s was required for removing unbound cells. Therefore, the cells with CD33 cell surface antigens can be rapidly identified from the cell suspension by spatial separation based on immunoreaction and manipulation by DEP.

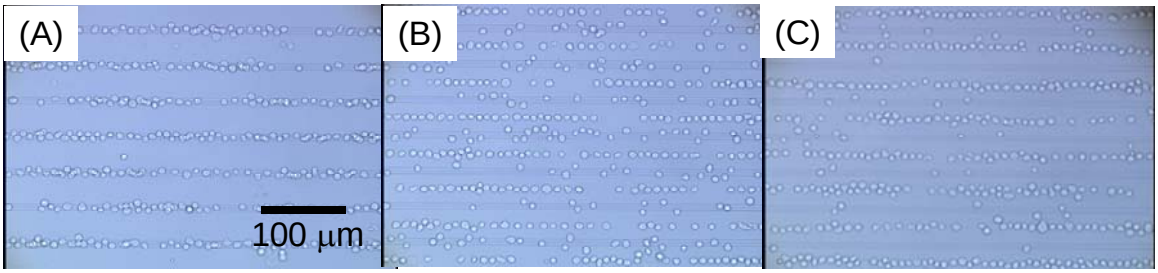

Figure 3. Images of cells patterned by p-DEP and n-DEP. (A) Cells accumulated on the band electrode by p-DEP. (B) Cell pattern captured on the band electrode modified with anti-CD33 after separating unbound cells by n-DEP. (C) Cell pattern captured on the band electrode modified with anti-mouse IgG after separating unbound cells by n-DEP. Conductivity of the medium: 80 mS/m; applied voltage and frequency for p-DEP: 15 V_{pp} and 10 MHz; applied voltage and frequency for n-DEP: 5 V_{pp} and 100 kHz; duration of voltage application p-DEP and n-DEP: 60 and 180 s.

Mixtures of HL60 cells specific and non-specific to anti-CD33 were used to determine the number of cells captured to the band electrode modified with anti-CD33. Figure 4A and B show photographs obtained by combining optical and fluorescent images, which were obtained 180 s after the switching of the direction. Almost no non-specific cells with a fluorescent signal were captured on the band electrode, whereas the specific cells were captured on the band electrode, even in the presence of treated cells (Fig. 5B). Figure 5C shows the ratio of cells captured on the band electrode. The ratio of the captured cells linearly increased with the increasing ratio of specific cells in the prepared mixture suspension. The results indicated that the presence of the cells without the target antigen does not obstruct specific cell binding from detecting cells with surface antigens. Furthermore, the linear relationship could be useful to determine the present ratio of cells with surface antigen in the suspension.

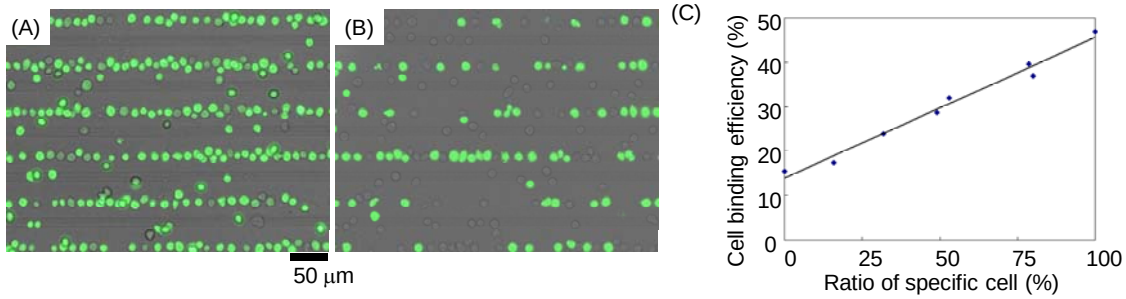

Figure 4. Cell binding to the band electrode modified with anti-CD33 from mixed suspensions of specific and non-specific HL60 cells. Treated cells were stained with a fluorescent molecule (CFDA SE). Photographs were obtained by combining the optical and fluorescent images. Initial ratios in the original suspensions were set at (A) 0% and (B) 50% of the specific cells. (C) Ratio of the cells captured on the band electrode.

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