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Rapid formation of cell-particle complexes via dielectrophoretic manipulation for the detection of surface antigens

Takuma Horii [a], Masashi Yamamoto [a], Tomoyuki Yasukawa [a,b,*], Fumio Mizutani [a,**]

[a] Graduate School of Material Science, University of Hyogo,3-2-1 Kouto, Kamigori, Ako, Hyogo 678-1297, Japan
[b] JST-CREST, 5, Sanbancho, Chiyoda, Tokyo 102-0075, Japan

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ABSTRACT

A rapid and simple method for the fabrication of the island patterns with particles and cells was applied to detect the presence of specific antigens on the cell surface. An upper interdigitated microband array (IDA) electrode was mounted on a lower substrate with the same design to fabricate a microfluidic-channel device for dielectrophoretic manipulation. The electrode grid structure was fabricated by rotating the upper template IDA by 90° relative to the lower IDA. A suspension of anti-CD33 modified particles and HL-60 cells was introduced into the channel. An AC electrical signal (typically 20 V peak-to-peak, 100 kHz) was then applied to the bands of the upper and lower IDAs, resulting in the formation of island patterns at the intersections with low electric fields. Immunoreactions between the antibodies immobilized on the accumulated particles and the CD33 present on the surface of the cells led to the formation of complexes comprising corresponding antigen-antibody pairs. Non-specific pairs accumulated at the intersection, which did not form complexes, were then dispersed after removal of the applied field. The time required for the detection of the formation/dispersion of the complexes is as short as 6 min in the present procedure. Furthermore, this novel cell binding assay does not require pretreatment such as target labeling or washing of the unbound cells.

1. Introduction

Immunophenotyping is used to discriminate clusters of differentiation (CD) antigens expressed on various leukocytes for hematological malignancy diagnosis and prognosis (Belov et al., 2001). Labeling with fluorescent molecules via antibody recognition has enabled the characterization of cells with specific membrane proteins. Flow cytometry has also been used for high-throughput counting and isolation of specific cells (Laerum and Farsund, 1981), but requires high sample volumes, high cell numbers, and highly skilled researchers (Meda et al., 2000; Verstovsek et al., 2002). Moreover, a relatively long incubation (over 30 min) is required for labeling of target antigens with fluorescent molecules. Recently, microfluidic technologies have been applied to the isolation of specific cells in a cell population (Toner and Irimia, 2005; El-Ali et al., 2006). However, an increase in the potential for the labeled antibodies to interact with the target antigens expressed on the cell surface is required for effective detection and separation of target cells based on phenotyping.

Dielectrophoresis (DEP) is used for the manipulation of micro- and nano-objects, including biological living cells and bacteria, in a microfluidic device because of its non-contact nature (Morgan and Green, 2003). Particles placed in a spatially inhomogeneous electric field undergo DEP due to polarization induced in the particles. The direction and strength of the DEP force depend on the cell size, the electrical properties of the cells and the fluid medium, and the magnitude and the frequency of the applied electric field. Thus, the differences in these parameters have been utilized to distinguish different cell types (Voldman, 2006). However, the difference in the dielectric polarizabilities of cells with and without surface antigens is not sufficient for distinguishing their cells via spatial separation. The concentration and separation of highly similar cells depending on their vitality have attractively been achieved by combining the manipulation of particles and cells by DEP force and direction of fluid flow by hydrodynamic force (Li et al., 2013).

Recently, biological recognition was combined with DEP manipulation to accelerate capture reactions and improve capture efficiency. For pathogen detection, bacterial cells in a suspension were concentrated and allowed to come into contact and be captured by antibodies immobilized on the chip surface due to the attractive force of positive DEP (p-DEP) (Yang et al., 2006; Koo et al., 2009;

* Corresponding author at: Graduate School of Material Science, University of Hyogo, 3-2-1 Kouto, Kamigori, Ako, Hyogo 678-1297, Japan. Tel.: +81 791 58 0171; fax: +81 791 58 0493.
** Corresponding author.
E-mail addresses: yasu@sci.u-hyogo.ac.jp (T. Yasukawa), mizutani@sci.u-hyogo.ac.jp (F. Mizutani).

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Yang, 2009). Neutrophils and eosinophils were also selectively captured from a mixed leukocyte suspension on specific regions with antibodies using the repulsive force of negative DEP (n-DEP) (Hashimoto et al., 2009). We recently developed a rapid and simple sandwich-type immunosensing system for two relevant tumor markers using DEP manipulation. The different types of microparticles captured with different marker proteins were spatially separated in the single device to detect the fluorescence signals corresponded to relative markers (Ramón-Azcón et al., 2011). We have also employed cell manipulation techniques based on DEP to detect cells with specific surface antigens within a cell population (Hatanaka et al., 2011; Yasukawa et al., 2012). In this method, different patterns were produced with particles by controlling the strength and frequency of the applied electric field using a device with multiple electrodes (Suzuki et al., 2008; Yasukawa et al., 2013).

Herein, we describe a method for the fabrication of island organizations with cells and particles for the rapid and simple detection of a targeted antigen on the cell surface. Previously, we fabricated island patterns with particles based on n-DEP using a three-dimensional device comprising upper and lower interdigitated array (IDA) electrodes and applied this method to simple and rapid immunosensing (Yamamoto et al., 2012). In the present study, this technique was applied to the fabrication of immuno-complexes of HL-60 cells with CD33 antigens and particles modified with anti-CD33 antibodies accumulated at intersections using n-DEP. The compulsive accumulation of the antibodies on the particles and the antigens expressed on the cell membrane allowed close contact and enhanced the rate of formation of the complexes. Because the cells and particles re-dispersed into the bulk solution in the absence of the specific antigen or antibody in the sample solution after the AC voltage was switched off, the presence of the target surface antigen on cells could be readily detected based on their aggregation behavior.

2. Theory

Particles in a suspended solution are polarized by an alternating electric field. DEP refers to the migration of a particle due to the interaction between the induced polarization and the spatially inhomogeneous electric field (Pohl, 1978; Jones, 1995). The quantitative description of the time-averaged DEP force, $\langle \overline{F}_{\mathrm{DEP}} \rangle$ (N), is given by

$$\langle \overline{F}_{\mathrm{DEP}} \rangle = 2\pi\varepsilon_s a^3 \mathrm{Re}[\underline{K}(\omega)]\nabla E_{rms}^2 \tag{1}$$

where a is the particle radius (m), ε_s is the permittivity of the suspension medium (F m^{-1}), E_{rms} is the root-mean-square electric field (V m^{-1}), and ∇ is the del vector operator. For silica particles, the frequency dependence of the induced DEP force is given by the real part of the Clausius–Mossotti factor, Re[$\underline{K}(\omega)$]

$$\underline{K}(\omega) = \frac{\underline{\varepsilon}_p - \underline{\varepsilon}_s}{\underline{\varepsilon}_p + 2\underline{\varepsilon}_s} \tag{2}$$

where $\underline{\varepsilon}_p$ is the permittivity of the particle. The underlined parameters denote the complex quantities. The complex permittivities of the particles and suspension medium are given by

$$\underline{\varepsilon}_p = \varepsilon_p - \frac{\sigma_p}{\omega} j \tag{3}$$

and

$$\underline{\varepsilon}_s = \varepsilon_s - \frac{\sigma_s}{\omega} j \tag{4}$$

respectively, where σ_p is the conductivity of the particle (S m^{-1}), σ_s is the conductivity of the medium (S m^{-1}), ω is the angular frequency ($=2\pi f$, where f is the applied frequency (Hz)) and $j=\sqrt{-1}$. In this model, it was assumed that the particles are spherical objects. The particle conductivity (σ_p) is thus given by the sum of the bulk conductivity and the surface conductivity (Arnold et al., 1987). The surface conductivity ($\sigma_{p,\ surface}$) is given by

$$\sigma_{p,surface} = 2\frac{K_s}{a} \tag{5}$$

where K_s is the surface conductance (S). It was also assumed that the bulk conductivity could be neglected because the particles that were used were composed of silica, which is an insulator. Curves a–e in Fig. 1 show the theoretical DEP spectra calculated from the different surface conductance of the particles. Nominal values were employed for the remaining parameters: $\varepsilon_p/\varepsilon_0=3.1$, $\varepsilon_s/\varepsilon_0=78$, and $\sigma_c=150$ mS m^{-1}. According to microscopic measurements, the average radius of the particles (a) was set at 2.5×10^{-7} m. The direction and magnitude of the DEP force acting on the particles depends on the Re[$\underline{K}(\omega)$] value. Particles subjected to an electric field with a positive Re[$\underline{K}(\omega)$] migrate toward the highest electric field region because of p-DEP, whereas particles subjected to an electric field with a negative Re[$\underline{K}(\omega)$] value migrate in the opposite direction because of n-DEP. The surface conductance predominately affects the low-frequency region, and particles with relatively low surface conductance experience n-DEP. However, an increase in the surface conductance results in an increase in the Re[$\underline{K}(\omega)$] value and a shift in the cross-over frequency to the high frequency region.

The theoretical DEP spectra calculated using different medium conductivities for cells have been previously reported (Yasukawa et al., 2012). The DEP spectrum calculated for HL-60 cells is shown in Fig. 1f. The nominal values of the parameters (Kaler and Jones, 1990): employed for this calculation were as follows: $C_m=0.015$ F m^{-2}, $\varepsilon_c/\varepsilon_0=60$, $\varepsilon_s/\varepsilon_0=80$, $\sigma_c=0.5$ S m^{-1} and $a=6.7\times10^{-6}$ m. In a solution with a conductivity of 150 mS m^{-1}, the Re[$\underline{K}(\omega)$] value was negative at lower frequencies (10–100 kHz) and positive at higher frequencies (1–10 MHz).

3. Experimental section

3.1. Fabrication of the device for accumulation of particles and cells

IDA electrodes were fabricated using photolithographic methods on glass substrates deposited with indium tin oxide (ITO). A positive

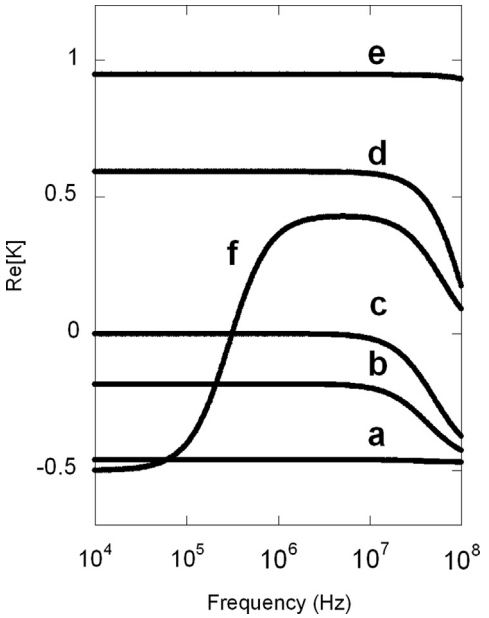

Fig. 1. Modeling of the Clausius–Mossotti factor spectra depicting (a – e) particles and (f) cells. Surface conductance: (a) 1.0×10^{-9} (b) 1.0×10^{-8}, (c) 1.9×10^{-8}, (d) 1.0×10^{-7}, (e) 1.0×10^{-6} S.