Dielectrophoresis in a Slanted Microchannel for Separation of Microparticles and Bacteria

Seong-Won Nam1, So Hyun Kim1, Je-Kyun Park2, and Sungsu Park1,*

1Department of Chemistry and Nano Sciences (BK21), Ewha Womans University, Seoul 120-750, Korea
2Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 305-701, Korea

Dielectrophoresis (DEP) is an effective method to trap, manipulate and separate various dielectric particles. To generate a DEP force, a spatially nonuniform electrical field has been generated by an array of electrodes, while electrodeless DEP has been accomplished by placing an insulating material between two electrodes. Here, we describe a new DEP method for generating a nonuniform electrical field using a slanted microchannel. The electric field gradient is induced due to a slope in the channel and can be used to move and separate particles. Based on the gradual electric field induced by three dimensional structure of the microchannel, our method enables particles of different sizes to be separated solely by DEP force without flow. The slanted microchannel was easily fabricated by a replica molding technique using a commercial UV-cured photopolymer (NOA 63) and bonded as an insulating layer between two indium-tin-oxide films. By applying the electrical field, polystyrene beads of different sizes (6–45 μm in diameter) were trapped and separated depending on the applied electric strength and frequency. Using this method, the opportunistic pathogen Pseudomonas aeruginosa attached to antibody-conjugated microbeads was successfully separated from Escherichia coli in a slanted microchannel.

Keywords: Dielectrophoresis, Slanted Microchannel, PDMS Stamp, NOA, Polystyrene Beads, Pseudomonas aeruginosa.

1. INTRODUCTION

Characterization and manipulation of micron particles are important for analyzing biomolecules. Laborious procedures such as filtration, analyzing and separation can be reduced by trapping particles. Moreover, reaction time and detection limit can be lowered by trapping particles. In this regard, dielectrophoresis (DEP) is a powerful tool to trap and separate particles.1 DEP refers to the force exerted on the induced dipole moment of an uncharged and conductive particle by a nonuniform electric field.2–5 Depending on the conductivity of the particles and the fluid, particles are attracted to higher electric field strength (called positive DEP) or to lower electric field (called negative DEP). The particles react to the electric field differently depending on the spatial distributions of the electric field, and the electric properties of the particles and of surrounding medium.1 In DEP migration, particles with different dielectric properties migrate differently to strong or weak electric field regions. In DEP retention, the DEP forces compete with other fluid-flow forces, and thus particles with large positive DEP forces are trapped. Meanwhile, particles are exposed to the field with different characteristic concentration profiles by field-flow fractionation (FFF), and moved at different velocities under the influence of fluid flow. Therefore, DEP force can also be generated from different types of electrodes, together with gravitational, magnetic and thermal fluids, etc. Several DEP-based separation techniques including DEP-FFF have been used to analyze various particles such as polystyrene beads,6–8 viruses,9 bacteria,10 malaria-infected blood cells,11 and other cells.12

The standard way to generate DEP is to generate an electric field gradient with an array of planar electrodes in the presence of an alternating current (AC) field. Although this method has been well characterized and commonly used, there have been some limitations in arranging electrodes; the fabrication of such electrodes is costly and technically challenging. Recently, electrodeless or insulator-based dielectrophoresis (IDEP) without patterned metal electrodes has been introduced as an alternative to conventional DEP.14–18 Electrodeless is defined as dielectrophoresis without metal surface at the position where the sample is manipulated, although electrodes are...
needed to apply the electric fields.\textsuperscript{14,15} Meanwhile, the insulating posts induced a gradual distribution of electric field in a channel, and a flow can be produced through the channel by applying of direct current (DC) field.\textsuperscript{15,16} Other IDEP methods exploit topographical structures such as ridges etched in glass or silicon substrates in order to generate a nonuniform electric field.\textsuperscript{14,17} Packing insulating materials such as oil in a microchannel induced a locally nonuniform DC field, which allows the particles to be separated by size.\textsuperscript{18}

In this paper, we report a novel method to generate a nonuniform electric field in a microfluidic channel with gradually increasing channel depths. The slanted channel was fabricated by a simple printing method in which a polydimethyl siloxane (PDMS) master mold fabricated by soft lithography was pressed against an UV-curable polymer such as Norland Optical Adhesive 63 (NOA 63). The method has many advantages over other DEP and IDEP methods. First, the printing method does not require any cumbersome steps for fabricating electrodes. Second, the channel is chemically inert and would not cause any problems, including troublesome steps caused by electrode fouling, filling materials and posts installed in microchannels. Third, particles can be separated without fluid flow, which can help to distinguish properties between particles. To demonstrate the feasibility of the method for bioanalysis, the method was used to separate microbeads and bacteria.

\section{2. Experimental Details}

\subsection{2.1. Design and Fabrication of a Microfluidic Device}

PDMS was obtained from Dow-Corning (Cortland, NY, USA) and used to fabricate a stamp with a microchannel pattern by soft lithography.\textsuperscript{19} In detail, SU-8 2035 (MicroChem, Newton, MA, USA) was first coated onto a silicon wafer. The wafer was inclined at 11° while exposed to ultraviolet (UV) through a film mask with the microchannel pattern. As a result, the wafer has a channel with different depths of 119.8 ± 6.2 \(\mu\)m and 95.6 ± 5.8 \(\mu\)m at the ends, as shown in Figure 1(a). The master was then passivated with vapor of tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-1-trichlorosilane for 15 min. Next, a 10:1 mixture of PDMS prepolymer and curing agent (Sylgard 184\textsuperscript{\text{\textregistered}}) was casted onto the Si master and cured for 2 h at 80 °C. The cured PDMS layer was peeled from the SU-8 master, producing a PDMS stamp with the microchannel pattern.

The pattern on the PDMS stamp was transferred onto NOA 63 (Norland Company, Cranbury, NJ, USA) by replica molding technique\textsuperscript{20–23} as shown in Figure 1(b). First, NOA 63 was poured over an indium tin oxide (ITO)-coated polyethylene terephthalate (PET; Sigma) film (60 \(\Omega/sq.ft\)). The PDMS stamp was then pressed into the NOA 63 layer. After the pressing step, both the PDMS stamp and NOA layer were cured under UV (\(\lambda = 365\) nm, 135 mW/cm\(^2\)) for 8 min. The cured NOA 63 layer was then peeled off the PDMS stamp. This short curing time is enough to prevent the patterned structure from collapsing but still enables the layer to remain sticky enough for bonding to another ITO film. The second ITO film with holes for the inlet and outlet was first attached to the NOA layer, and their bonding was then completed by curing them under UV-light for 2 h (Fig. 1(b)).

\subsection{2.2. Polystyrene Beads and Experimental Setup}

Bare polystyrene beads (Polysbead\textsuperscript{\text{\textregistered}}) with different sizes (6, 15 and 45 \(\mu\)m) were purchased from Polysciences (Warrington, PA, USA). Phosphate buffered saline (PBS) was made of 0.1 M phosphate and 0.15 M NaCl and its electrical conductivity was adjusted to 22 \(\mu\)Scm with deionized (DI) water to be used as a separation buffer (pH 7.2). 5\% bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) was added to PBS for inhibiting non-specific adsorption.

A slurry-bead mixture was introduced into a microchannel using a syringe. The electric field was produced from a function generator (MXG-9802A; METEX, Seoul, Korea). The movement of beads in microchannels was observed under a stereomicroscope (Nikon, Tokyo, Japan) and recorded by a CCD camera (SPOT INSIGHT\textsuperscript{\text{TM}}, Diagnostic Instruments, Sterling Heights, MI, USA).

\subsection{2.3. Bacterial Strain and Growth Condition}

\textit{Escherichia coli} O157:H7 43895 was obtained from the American Type Culture Collection (Manassas, VA, USA), and \textit{Pseudomonas aeruginosa} PA01 was obtained from Professor You-Hee Cho (Cha University, Korea). The strains were grown in LB (BD, CA, USA) at 37 °C in a shaker at 220 r.p.m. Their Growth was monitored by...
measuring the optical density at 600 nm using a spectrophotometer (Biowave CO8000, WPA Ltd., Cambridge, England).

2.4. Conjugation of Antibody to Polystyrene Microbeads and Separation of Bacteria

For capturing bacteria, 15 \( \mu \text{m} \) bare polystyrene beads in 0.1 M sodium phosphate buffer (pH 7.4) were reacted with anti-rabbit IgG (Sigma) in the same buffer by gentle shaking at RT overnight. *P. aeruginosa* PAO1 (O.D. = 0.44) in PBS was incubated with 20 \( \mu \)L of rabbit anti-*P. aeruginosa* IgG (AbCam, Cambridge, UK) at 1:100 dilution for 1 h by end-over-end rocking and then washed three times with PBS by centrifugation at 54,107 \( \times \)g for 1 min. Anti-rabbit IgG-conjugated beads were then added into the mixture and incubated for 1 h. The beads were then collected by centrifugation, and anti-rabbit IgG-TRITC (tetramethyl rhodamine isothiocyanate, Sigma) was added and incubated for 1 h. Then, the beads were washed three times with PBS by centrifugation and resuspended in PBS (22 \( \mu \text{S/cm})

To test if the dielectrophoretic characteristics of the beads depend on the binding of antibodies, the 15-\( \mu \text{m} \) polystyrene beads conjugated with anti-mouse IgG from goat (0.54 \( \times \)10\(^7\)/mL \~ 1.5 \( \times \)10\(^7\) IgG/particle) (Spherotech Inc., Lake Forest, IL, USA) were incubated with diverse ratios of rabbit anti-goat IgG-FITC (fluorescein isothiocyanate) (3.4 mg/mL, Sigma) for 1 h by end-over-end rocking at RT. Fluorescence was observed under an inverted fluorescence microscope (Nikon Eclipse TE2000-U) equipped with the CCD camera and analyzed by Image J Software (NIH, Bethesda, MD, USA).

3. RESULTS AND DISCUSSION

3.1. Simulation of Electric Field Distribution in the Slanted Channel

As shown in Figure 2(a), the channel was 47 mm long and 400 \( \mu \text{m} \) wide, and the depth difference between both ends was 24.2 \( \mu \text{m} \). The electric field distribution in the channel was simulated with a commercial computational fluid dynamics program (CFD-ACE\(^+\); ESI Group, Huntsville, AL, USA) (Fig. 2(b)). The electric field strength depends on the channel depth, and decreases from the lower part to the higher part of the channel. The gradual distribution of the electric field strength is the driving force for movement and separation of particles. When the electric field is generated between the two ITO films, the DEP force can be generated, and particles are affected by the DEP force, which is defined for the spherical particles as:

\[
F_{\text{DEP}} = 2\pi r^3 \varepsilon_m \text{Re}[K(\omega)] \nabla E^2
\]

where \( r \) is a radius of the particle, \( \varepsilon_m \) is the medium’s permittivity, \( E \) is the electrical field and \( K \) is the Clausius-Mossotti factor.

To move particles, the DEP force must overcome the drag force in a microchannel. For the special case of small spherical objects moving through a fluid with small Reynolds number,

\[
F_{\text{drag}} = 6\pi \eta a v
\]

where \( a \) is the stokes radius of a particle and \( \eta \) is the fluid viscosity.

Therefore, the velocity by DEP force is proportional to the square of the particle radius and electric field:

\[
v \propto r^2 \nabla E^2
\]

Since \( E = V/d \), where \( V \) is voltage and \( d \) is the distance of the conductive layers, the velocity of the particle is proportional to the radius of particles and inversely proportional to the distance of the conductive layers. Thus, the beads move differently in an electric field depending on their radius.

Another crucial factor for DEP force is the frequency applied to the conductive layers, as shown in Eq. (1). In this case, the DEP force can be modulated by changing the Clausius-Mossotti factor \( K \), which can be adjusted by changing the frequency of signals applied on the conductive layers. \( \text{20} \) Thus, the movement of beads depends on the applied frequency as well.

\[
v \propto \text{Re}[K(\omega)] \nabla E^2
\]

3.2. Separation of Micron Beads with DEP Force

Polystyrene beads with two different diameters (15 \( \mu \text{m} \) and 45 \( \mu \text{m} \)) were mixed in a test tube containing PBS buffer (permittivity = 22 \( \mu \text{S/cm} \)), and the mixture was loaded in the slanted channel. When 4 V and 100 kHz were applied to the ITO film, the 15 \( \mu \text{m} \) beads moved toward the deep side of the microchannel while the 45 \( \mu \text{m} \) beads did not move (Fig. 3(a)). When the applied voltage was increased slowly, the velocity of the 15 \( \mu \text{m} \) beads...
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When 7.4 V was applied at 10 kHz, the 6 μm beads congregated in the channel (Fig. 3(b)). When the same voltage was applied at a higher frequency (10 MHz), the heaps of beads dispersed and flowed through the channel (Fig. 3(b)). Dielectric interaction between two spherical particles in an electrolyte medium when applying a uniform electric field was described in previous reports.\(^2\)\(^{24}\) When particles were aligned parallel to the electric field, the lowest electric field occurred between the particles, and thus the interaction between the particles became attractive.\(^2\)\(^{24}\) The temporary aggregation of particles was analyzed as well, as shown in Figure 3(b), and the two-particle model could be adopted for explanation of aggregation of the particles.\(^2\)

To increase the separation efficiency, diluted PBS (permittivity = 11 μS/cm) was used. When 2.1 V was applied at 1 kHz, the 6 μm beads and 15 μm beads in the diluted PBS were successfully separated (Fig. 4). The separated equilibrium line depended on each bead’s radius, due to the nonuniformity of the electric field at y-position (Fig. 4(b)). In this condition, the dielectric force was exerted in three different directions, and the forces balanced each other depending on the radius of beads, applied field strength and frequency. When the dielectric force moved the beads in the direction of the x-axis, the 15 μm beads moved to the y-direction at the same time up to the lowest position of the gradually changing electric field strength of the y-axis. On the other hand, the 6 μm beads moved in the x-direction, were brought close to each other, and then aggregated.

3.3. Separation of Bacteria Cells with DEP Force

To demonstrate the feasibility of the DEP technique for bioanalysis, the technique was used to separate pathogenic bacteria such as P. aeruginosa and E. coli. When both P. aeruginosa and E. coli were incubated with anti-P. aeruginosa IgG (from rabbit), anti-rabbit IgG conjugated beads and TRITC-anti-rabbit IgG in a sequential manner, red fluorescence from TRITC was observed only on the beads. This result suggests that P. aeruginosa cells were specifically bound to the beads. When an electric field at 10.8 V at 11.8 kHz was applied into the electrode to separate P. aeruginosa-bound beads from E. coli cells, P. aeruginosa cells bound to the beads moved in the direction of higher electric field (Fig. 5(a)) while E. coli labeled with green fluorescent protein (E. coli-GFP) was confined to the channel wall (Fig. 5(a)). These results indicate that the DEP technique can be used to specifically separate P. aeruginosa from E. coli.
The dielectric characteristics of the particles can be altered by the surface properties. When the polystyrene beads were conjugated with IgG, the beads moved in the direction of higher electric field, while the beads without antibody did not move in any direction in the same conditions. This implies that the conjugated antibody affects the dielectric property of the beads. To test the effect of antibody on the mobility of beads, the beads were incubated with the secondary antibody (developed from a rabbit anti goat IgG) conjugated with fluorescein isothiocyanate (FITC) molecules. After incubation with the secondary antibody, the beads recovered the dielectrophoretic property, which was correlated to the number of secondary antibodies added. When the beads were incubated with 100- or 1000-fold secondary antibodies, the beads were moved by 7.4 V and 1 Hz (Fig. 5(b)). Meanwhile, the beads incubated with 10⁻¹- or 10⁻³-fold secondary antibodies needed 7.4 V with 10 Hz or 100 Hz to move. The increased molecules of the secondary antibody relative to the first antibodies on the bead needed higher frequency to produce movement, and relatively less electric strength. This implies that the dielectric properties of the beads are not directly correlated to the size of particles including the antibodies on the beads, but might depend on the surface properties of the beads, because more than one secondary antibody can bind on molecules of the first antibodies. In this range of frequency, the beads moved in the opposite direction, since positive DEP was exerted on the beads.¹

Our separation method using a slanted microchannel has several advantages in separating particles over other DEP-based methods. The slanted channel can be easily fabricated, and the method does not need patterned electrodes at the location of dielectrophoretic manipulation. Microbeads of different sizes can be separated from each other without the use of flow. Our results suggest that the method can be used for separation of micron-sized bioparticles, including microorganisms.

4. CONCLUSIONS

A slanted microchannel has been fabricated and tested for dielectrophoretic manipulation. In the channel, a gradual electric field was generated in parallel and perpendicular to the channel in which DEP forces were balanced to each other. Under this regime, beads with different size moved with different velocities and equilibrium positions and aggregated in the microchannel. Since the gradual electric field is distributed throughout the slanted microchannel, beads of different sizes can be separated without external flow, which offers certain advantages in mixing and separating bioparticles such as cells and microorganisms. Thus, the slanted microchannel-based DEP method has potential for bioapplications.

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References and Notes


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