Ripple structure-generated hybrid electrokinetics for on-chip mixing and separating of functionalized beads

I-Fang Cheng,¹ Sheng-Chuan Chiang,² Cheng-Che Chung,³ Trai-Ming Yeh,⁴ and Hsien-Chang Chang^{2,3,5,a)}

¹National Nano Device Laboratories, National Applied Research Laboratories, Tainan, Taiwan

²Department of Biomedical Engineering, National Cheng Kung University, Tainan, Taiwan

³Institute of Nanotechnology and Microsystems Engineering, National Cheng Kung University, Tainan, Taiwan

⁴Medical Laboratory Science and Biotechnology, National Cheng Kung University, Tainan, Taiwan

⁵Center for Micro/Nano Science and Technology, National Cheng Kung University, Tainan, Taiwan

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We present an electrokinetics-based microfluidic platform that is capable of on-chip manipulating, mixing, and separating microparticles through adjusting the interrelated magnitudes of dielectrophoresis and AC electroosmosis. Hybrid electrokinetic phenomenon is generated from an electric field-induced micro-ripple structure made of ultraviolet-curable glue. Size-dependent particle separation and selective removal over the ripple structure is demonstrated successfully. Varying the waveform from sine-wave to square-wave allows generating a fluid convection at specific positions to mix the antibody-functionalized beads and antigen. Potential application in the bead-based immunoassay was also demonstrated for immuno-reaction and subsequently separating the bead-bead aggregate and non-binding beads on-chip. © 2014 AIP Publishing LLC.

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INTRODUCTION

Immunoassay such as enzyme-linked immunosorbent assays (ELISA) is potentially an important technology for rapid, sensitive, and high accuracy detection of pathogens, protein, and biomarkers based on its specific antibody-antigen binding.^{1,2} In recent years, a new technology using functionalized silica, polystyrene, and magnetic beads, called bead-based assays has been found to reduce the detection time to within 0.5–1 h due to its high surface-to-volume ratio, which has been widely used to rapidly detect DNA, specific proteins, and biomarkers.^{3–7} However, in the case of a bead-based assay system, a big challenge is that removal of the non-binding beads is required in a bead-based immunoassay for precise analysis of the detection results. Many micro/nano particle manipulation techniques that have been used, such as using a magnetic field to manipulate magnetic beads,⁸ and centrifugal methods, pore-based filters, and electrokinetics to separate non-magnetic beads.^{3,9–11} However, the target-bound beads and non-binding beads are difficult to be separated using the conventional physical approaches.

Dielectrophoresis (DEP) provides a flexible manipulation of dielectric particles in a non-uniform electric field, which has good selectivity based on the size, sharpness, and dielectric properties of a particle.^{12–14} Unfortunately, the short-range manipulation mechanism of the pure DEP technique makes it difficult to transport particles with long range due to the exponential decay of the electric field gradient with the distance from the electrode. AC electroosmosis (ACEO) is capable of generating long-range fluid convection with proper design.^{15,16} Even

^{a)}E-mail: hcchang@mail.ncku.edu.tw

though ACEO can induce a net flow to convey liquid samples in a wide range, there is no selectivity for the particles in the fluid motion. By combining the DEP, which has separating capability, with ACEO, which has wide-range transporting capability, long-range selective concentration of bacteria from human blood can be achieved.¹⁷

In this research, we propose a novel approach that uses hybrid electrokinetics to mix the functionalized particles and antigens, and subsequently separate the non-binding beads and the aggregated beads for the purpose of on-chip immunoassay. The hybrid electrokinetics over the ultraviolet-curable made ripple structure can also generate the different ACEO and DEP strengths at the peak and valley, respectively (Fig. 1(a)). To show the potential application of this chip, DN5C6 functionalized silica beads were used for rapid capturing of the non-structure protein 1 (NS1) via electrokinetically mixing the sample and separating the bead-NS1-bead aggregate and non-binding beads on-chip (Fig. 1(b)). Both antibody (DN5C6) and antigen (NS1) are DENV serotype 2 specific. The experimental details are shown in the supplementary material (see Ref. 19).

RESULTS AND DISCUSSION

The resin-made ripple structure was produced by applying a non-uniform electrical field¹⁸ to the ITO-based interdigitated electrode (IDE) array. The undulated 3D ripple structure changes the electric field distribution and emphasizes specific AC electrokinetic phenomena at specific positions. Therefore, the highest electric field region locates at the peaks, and a second high electric field region locates at the valleys (Fig. 1(c)). The strongest field regions with a low ACEO effect locate at the electrode edges and also are the peak of the ripple structure where the positive DEP dominates the microparticle trapping mechanism. On the other hand, the secondary high electric field regions with a strong convergency ACEO effect locate in the valleys, which can effectively decrease the positive DEP influence at the electrode surface to effectively transport the smaller particles (Fig. 1(d)). Microparticle motion over the ripple structure is governed by the combination of frequency-dependent ACEO and DEP.

Two different sized polystyrene beads ($d = 4\ \mu\text{m}$ and $1\ \mu\text{m}$) suspended in deionized water ($\sigma \sim 2\ \mu\text{S}/\text{cm}$) were used to investigate particle manipulation and separation. The particles are distributed randomly when no electric field is applied to the electrodes. Initially, a voltage of $120\ \text{V}_{\text{p-p}}$ at the frequency of $200\ \text{kHz}$ was applied to collect particles on the peak surface by

FIG. 1. (a) The side view of ripple-structure on the interdigitated electrode. (b) Illustration of bead-based immuno-assay for NS1 detection. (c) Simulation result for the electric field distribution for the dielectric ripple-structure on the electrodes. (d) Illustration of particle concentration and removal of particles.

the dominated positive DEP trapping mechanism (above 100 kHz, the ACEO is relative weak).¹⁷ After collecting the particles for 1 min, the applied voltage was changed from a sine-wave to a square-wave at a frequency of 3 kHz. The balanced DEP and ACEO effects caused the 1 μm and 4 μm particles to be mixed at the peak regions of the ripple structure, as shown in Fig. 2(a). In the frequency range of 1–50 kHz, DEP influence is still present, but its effect is reduced compared to the ACEO flow. When the frequency was subsequently switched to 12 kHz, the induced ACEO drag on the ripple structure was lower than the positive DEP force on the bigger particles and higher than the positive DEP force on the smaller particles. The induced charges begin accumulating in the valleys of the ripple structure where the strong convergency ACEO effect dominated the particle motions. The effects caused the 1 μm particles to be convected into the valleys and transported toward the end-points by a lower pDEP force and a strong ACEO effect in the valleys, while the 4 μm particles were trapped on the peaks that had a relatively high pDEP force with weak ACEO drag which made these particles move along the peaks very slowly (Fig. 2(b)). The mechanism resulted in sized separation of particles and simultaneous removal of the smaller particles to the end-points (Fig. 2(b), see the detail phenomena in the supplementary video (Multimedia view)). The summary of the operation conditions related the induced functions, which is shown in the supplementary material (see Table I in Ref. 19).

NS1 protein is a specific biomarker that is produced by a dengue virus infection and is released into the blood stream at the early stage of this disease (within 48 h). Through detection of NS1, rapid screening of dengue infection could be carried out at the initial stage. Our proposed bead-based immunoassay was designed to demonstrate the possibility for rapid detection of NS1. The specific antibody, DN5C6 was modified on the 1 μm silica bead to form a functionalized particle for the purpose of specifically capturing the NS1 protein in the suspension medium. Initially, a voltage of 120 V_{p-p} at a frequency of 200 kHz was applied to collect the beads on the peak regions, and then the waveform was switched to a square-wave voltage for stirring and mixing of the DN5C6-functionalized beads and the suspended NS1 on-chip

FIG. 2. (a) The 1 and 4 μm particles were mixed at the peak regions of the ripple structure when applied a square-wave voltage. (b) When the applied voltage returns to sine-wave ($f=12\text{ kHz}$), 1 μm particles are transported toward the end-points along the valleys, while the 4 μm particles are trapped on the peaks. The sized separation and simultaneous removal of the smaller particles was achieved. (c) Experimental demonstration for detection of NS1 protein on-chip. The induced kinetics mixed the DN5C6-functionalized beads and the suspended NS1 using square-wave voltage. (d) When changing the square-wave to a sine-wave signal, the non-binding particles were moved from the peaks to the valleys and transported toward the end-points along the valleys. In contrast, the aggregated particles were trapped on the peak regions. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4905361.1>]

(Fig. 2(c)). Here, DEP and ACEO dynamics are balanced at the crests of the micro-ripple structure to generate fluid convection locally. The NS1-bound beads could bind with other functionalized beads forming bead-bead aggregation in which the total volume could be much bigger than a single bead in a free-bound state. Finally, the frequency was subsequently switched to 12 kHz; the lateral ACEO flows were generated to move the non-binding beads to the valleys and continuously transported them toward the end-points (Fig. 2(d)). The scaling dependence of the relative influence of DEP and ACEO on manipulating particles of different sizes can be expressed as $F_{ACEO} = F_{DEP} \sim 1/r^2$. The importance of ACEO increases for smaller particles, while DEP becomes the dominating dynamics for manipulating bigger particles. The bigger particle aggregates experienced a strong positive DEP and were trapped on the peaks, while the smaller non-binding particles experienced a lower positive DEP effect accompanied with a strong ACEO drag which made these particles transported along the valleys toward the end-points, as shown in Fig. 2(d).

CONCLUSIONS

Our proposed hybrid electrokinetics conducted on ripple-structures demonstrates the useful ability for a bead-based immunoassay that is capable of trapping the targeted particle aggregates and removing the non-targeted particles on-chip based on the sized difference. 1 μm and 4 μm particles were successfully separated by sine-wave voltage at 12 kHz on-chip. The on-chip immuno-reaction can be achieved via mixing NS1 protein and DN5C6-functionalized microbeads by applying a square-wave voltage (3 kHz) for 5 min. Through changing the magnitude of the tunable frequency-dependency ACEO relative to the size-dependency DEP force (sine-wave at 12 kHz), the bead-NS1-bead aggregates were trapped at the peaks, while the free NS1-functionalized beads can be removed due to the significant ACEO that is higher than the positive DEP force. The integrated immuno-processes could be accomplished by only the use of electrokinetic control that is useful for developing a wash-free and label-free automatic bead-based immunoassay. This rapid and portable positive-negative diagnostic platform would be a preliminary screening step for many field-use applications. Quantifying the antigen concentration is now in progress via electrokinetically arranging in order of size distribution based on their different magnitudes of aggregate DEP force.

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- ¹⁹See supplemental material at <http://dx.doi.org/10.1063/1.4905361> for fabrication methods, materials, sample preparations, and system configuration.