ULTRA-RAPID 3D-ACEO ELECTROKINETIC PRECONCENTRATION FOR VIRUS DETECTION
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ABSTRACT
High-sensitivity screening of biomarkers is a critical issue for early disease detection and diagnosis. Here the development of integrated electrostatic exclusion and AC electrokinetic flow control devices for rapidly attomolar detection of virus is reported. An insulated-CNTs electrode modified with monoclonal antibody for 80 nm bacteriophages exhibit close to a $10^4$ increase in sensitivity due to streaming ACEO and corresponding electrostatic contribution to the binding affinity after application of an AC electric-field. We demonstrate that bacteriophages can be dynamically positioned with an electric-field strength as small as $2 \times 10^{-3}-5$ V/m and define the operating parameters for increasing the concentration of phages by 4 orders of magnitude less than 10 minutes. These results show general applicability of this method, and could open up opportunities in early stage disease detection and the analysis of proteins from single cells.

KEYWORDS: 3D-AC Electroosmotic (3D-ACEO), Electrostatic Exclusion, MWCNTs-gate electrode

INTRODUCTION
The electrokinetic manipulation of particles has particular application in biotechnology. AC electrohydrodynamics are electrokinetic effects arising from the interaction of ions in the electrical double layer formed on a surface and the tangential component of the electric field inside the double layer [1]. AC electrohydrodynamics force has been used in colloidal crystal formation during electrophoretic or dielectrophoretic deposition. The application of an AC electric-field to manipulate bulk fluids and embedded objects for manipulating nanoscale and biological objects [2], AC electroosmotic (ACEO) [3] are ideal methods. It is reported that the electrokinetic trapping of nanofluidic filter could increase the protein concentration by 6 orders of magnitude in 100 min [4]. Choi et al. used dielectrophoresis (DEP) to concentrate DNA of Escherichia coli, and the enrichment of $10^3-10^5$ was obtained [5]. It is important to note that the above works suggested several mechanisms need special buffer reagents or waiting long time for concentration. To solve these problems, we developed an AC electrokinetic preconcentator integrated insulated MWCNTs-gate electrode to filtrate the charged-analyses by electrostatic exclusion enhances the concentration capability in a short time.

THEORY
A schematic of the integrated virus manipulation biochip is shown in figure 1a. A MWCNTs 3D electrokinetic-electrode pair was made by photolithography and metal deposition (Ti/Al/Ni), and it is also displayed by SEM image in figure 1b-c. After putting the poly(dimethylsiloxane) (PDMS) with a chamber on the functionalized chip for microfluidic sample delivery, an integrated 3D-ACEO chip was obtained.

In the case of ACEO, the induced EDL interacts with the tangential component of the electric field to induce bulk fluid motion. In an alternating electric field, the sign of the charges in the EDL and the direction of the tangential component of the electric field both change. Therefore, the direction of the resultant force on the fluid remains the same and generates a net fluid movement. We work with dimensionless variables appropriate for ACEO, set by the feature size L of the geometry, which is equal to the electrode spacing. The time of double-layer charging [1], $t = \frac{\lambda L}{D(1 + \delta)}$, where L is the Debye length, D is the electrolyte diffusion coefficient and $\delta$ is the ratio of the diffuse and electric double layer capacitances. Fluid velocity of ACEO speed is, $U = \varepsilon V^2/\eta L(1 + \delta)$, where V is the applied voltage and $\varepsilon$ and $\eta$ are the permittivity and viscosity of the solvent, respectively [3]. After the AC signal is applied, the AC electric field generated by coplanar microelectrodes produces a steady fluid flow pattern, moving out across the surface and dragging analytes regardless of size down to the gap of electrodes in electrolytic solution, as shown in Scheme 2.

In this paper, the three-dimensional geometries (3D-ACEO) can dramatically increase flow rates, compared to planar ACEO effect, at the same applied voltage and feature size.
EXPERIMENTAL AND RESULT

The polystyrene (PS) beads were diluted form 1 µM to 1 nM with DI water and adjusted to a conductivity of 2.56 mS/m. PS beads were carboxyl modified to prevent the aggregation. The electrode configuration for PS manipulation is shown in Figure 2. Figure 2a shows typical electrokinetic manipulation experiments by optimizing the applied voltage and frequency; we observed that the PS beads can be positioned at the center of the inner and outer electrode. Trapping of PS beads at the center of the inner electrode at higher frequency (10 Vpp, 100 KHz) and trapping of PS beads at the outer electrode at lower frequency (10 Vpp, 200 Hz). Figure 2b shows the capability of sample position control by this 3D ACEO-MWCNTs perconcentrator.

With ACEO for sample concentration, it would move out across the surface and dragging embedded analytes regardless of size down to the gap of electrodes in electrolytic solution was also shown in the Figure 3. The bacteriophages (phage M13k507) were diluted to 1 nM (10^{-15} moles in 5 µL) with 5mM HEPES puffer, and mixing with anti-M13-FITC monoclonal antibody for 1hr. Before experiment, the free anti-M13-FITC antibodies were washing by elusion buffer for 3 min. Figure 4 shows the fluorescence intensity of phage-anti-M13-FITC increased up to 10^4 folds of the initial concentrations at the concentration region in 10 mins.

For PS particles or virus manipulation, we provide a large region with the electric field strength of 2×10^{3.5} V/m, which is 2-3 orders of magnitude smaller than typical AC electrokinetic trapping conditions (1×10^{5.7} V/m). Our device have the potential to applied for the manipulation of other nanoscale entities by the effectiveness of our device which is a result of the combination of the long-range ACEO and the electrostatic exclusion preconcentration.
CONCLUSION

In this research, we developed an integrated electrostatic exclusion and AC electrokinetic device to push the detection limit while overcoming the harmful effect of changing electrokinetic parameters on signal measurements. An insulated-CNTs electrode modified with monoclonal antibody for bacteriophages (M13-phage) detection are shown to exhibit $10^4$ increase in sensitivity due to streaming ACEO and corresponding electrostatic contribution to the binding affinity after application of an AC electric-field. The electrostatic increases the protein concentration in less than 10 min at the sensor surface and the induced electrostatic interaction enhances protein association after the electrokinetic application.

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REFERENCES


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