Applications of dielectrophoretic/electro-hydrodynamic "zipper" electrodes for detection of biological nanoparticles

Yvonne Hübner[1]
Kai F Hoettges[1]
Martin B McDonnell[2]
Michael J Carter[3]
Michael P Hughes[1]

[1]Centre for Biomedical Engineering, School of Engineering, University of Surrey, Guildford, Surrey UK; [2]DSTL, Porton Down, Salisbury, Wiltshire, UK; [3]School of Biomedical and Molecular Sciences, University of Surrey, Guildford, Surrey, UK

Abstract: A major problem for surface-based detection techniques such as surface plasmon resonance and quartz crystal microbalances is that at low concentrations, diffusion is an insufficient driving force to bring colloidal submicron-scale particles to the detection surface. In order to overcome this, it has previously been demonstrated that a combination of dielectrophoresis and AC-electro-hydrodynamic flow can be used to focus cell-sized particles from suspension onto a large metal surface, in order to improve the detection capabilities of such systems. In this paper we describe how the combination of these two phenomena, using the so-called "zipper" electrode array, can be used to concentrate a wide range of nanoparticles of biological interest, such as influenza virus, dissolved albumin, and DNA molecules as well as latex beads of various sizes. We also demonstrate that the speed at which particles are transported towards the centre of the electrode pads by dielectrophoresis and electro-hydrodynamic flow is not related to the particle size for colloidal particles.

Keywords: electro-hydrodynamic flow, dielectrophoresis, influenza, DNA

Introduction

Detection technologies such as evanescent light scattering or surface plasmon resonance use surface interactions to detect the presence of particles on a metal surface (Perkins and Squirrell 2000). In order to be an effective method of pathogen detection, such a system is required to capture pathogens covering a wide range of sizes, from nanometers to micrometers in diameter, on the detection surface. However, the detection of bacteria or viruses is often hampered because such particles are small enough for Brownian motion to prevent them collecting onto the sensor surface; that is, they are *colloidal*.

In order to enhance the effectiveness of these detection systems, AC-electrokinetic forces have been employed to pull particles from suspension and concentrate the particles on a sensor surface (Hoettges et al 2003a, 2003b; Wong et al 2004). The method outlined in these papers exploits two phenomena that can be observed in cells suspended in a liquid medium and exposed to a low-frequency (approximately less than 1 kHz), non-uniform electric field. The first, *dielectrophoresis,* is the motion of suspended particles caused by polarization effects induced by an inhomogeneous electric field (Pohl 1978). Depending on the properties of the particle and the surrounding medium the particle can be attracted to (positive dielectrophoresis) or repelled from (negative dielectrophoresis) the electrodes (Pohl 1978). In addition to dielectrophoresis, *electro-hydrodynamic* (EHD) flow of the suspending liquid due to electroosmosis is also observed above adjacent electrode surfaces. As a result of the combination of these phenomena, a vortex of liquid is formed over the electrode edge that pushes particles onto the electrode surface (Gonzáles et al 2000; Green et al 2002).

Correspondence: Michael P Hughes
Centre for Biomedical Engineering, School of Engineering, University of Surrey, Guildford, Surrey GU2 7XH, UK
Tel +44 1483 686775
Email m.hughes@surrey.ac.uk

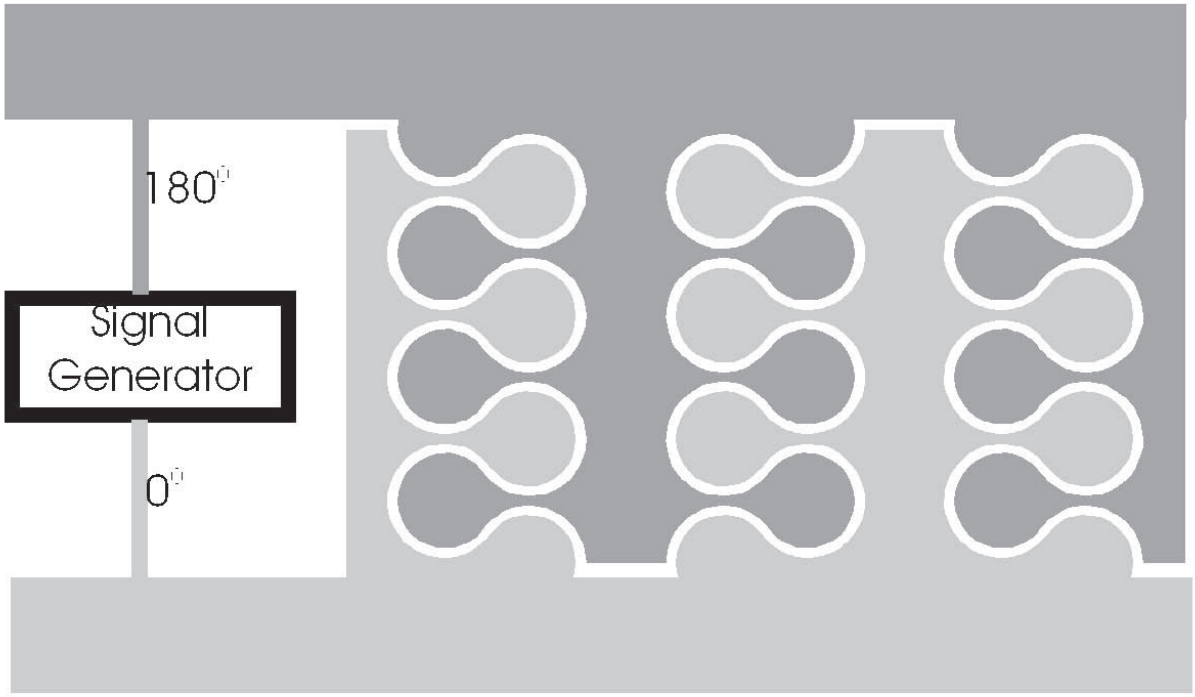

Figure 1 Schematic of the zipper electrode geometry used; interlocking circles cause particles to be pushed into the centre of the electrode "pads".

Careful selection of the electrode geometry allows these phenomena to be exploited for the collection of particles at a specific point. One such geometry is the so-called "zipper" electrode, consisting of interlocking circular electrode pads such as those shown in Figure 1 (Hoettges et al 2003a, 2003b). The advantage of such an electrode design is that the vortices formed around the edges of the electrodes act to push particles orthogonally to the electrode edge and hence focus particles in one spot in the middle of the electrode surface, rather than in a band as observed with interdigitated electrodes. Zipper electrodes have previously been used for focusing yeast cells, bacterial spores, latex beads (Hoettges et al 2003) and DNA (Wang et al 2004). In this paper we demonstrate that these effects can be used to concentrate a range of particle sizes from micron-scale particles to those with effective diameter 4 nm, including bioparticles such as viruses and proteins. We demonstrate that the particle collection of zipper electrodes is independent of the particle size, and can therefore be used to concentrate virtually any particle with a size between macromolecules and large cells.

Material and methods

Particle preparation

Influenza virus particles, A-WS strain, were cultured in allantoic cavity of a fertile 12 day old hen's egg (Tetra Poultry, Dunsfold). The virus was clarified and snap frozen. To perform experiments, virions were defrosted and fluorescently labeled with NBD-dihexadeclamine (Molecular Probes, Oregon). The dye was dissolved in DMF at a concentration of 2 mg mL^{-1}. To this solution the virus was added at a 1:100 dilution. Particles were incubated for 20 min at room temperature, pelleted at 14,000 rpm for 50 min and resuspended in 1 mL mannitol solution (280 mM). These cells were then used to perform dielectrophoretic experiments. The fluorescently labeled protein Albumin, conjugated to flourescein isothiocyanate (Sigma-Aldrich, UK) was dissolved in 280 mM mannitol solution at a concentration of 2.1 mg mL^{-1}. Lambda DNA was fluorescently labeled using YOYO-1 iodide dye (Molecular Probes, Oregon). DNA and dye were mixed at a ratio of 9:1 and the sample was incubated at 8 °C overnight. The fluorescently labeled latex beads (FluoSpheres® carboxylate-modified microspheres from Molecular Probes, UK) of a range of sizes between 0.02 μm and 0.5 μm diameter were suspended in distilled water.

Experimental setup

Zipper-shaped electrodes were manufactured by photolithography and wet etching on 100 nm thick gold coated microscope slides with a 10 nm titanium seed layer. The electrodes had a diameter of 300 μm and were separated by an interelectrode gap of 100 μm. Between 10 μL and 40 μL of the solution was pipetted onto the electrode array and

covered with a cover slip. A sinusoidal signal of magnitude of 10 V_{pk-pk} and frequencies in the range 500 Hz to 10 kHz were applied to the electrodes using a Thurlby-Thandar signal generator (TG120, 20 MHz Function Generator). Experiments were observed using a Nikon Eclipse 400 fluorescent microscope and images using a Photonic Science chilled CCD camera were taken every 0.1 s for each experiment. The distance of the particles from the electrode edge was measured for each time frame. For experiments with latex beads, a 10 V_{pk-pk} sinusoidal signal was applied to the electrode array with four different frequencies ranging from 500 Hz to 1400 Hz.

Results and discussion

When the signal was applied to the electrodes, fluid vortices were observed to form adjacent to the electrode edges. Particles moved first from this vortex onto the electrode edge, and then towards the electrode centre where they collected as shown in Figures 2a (latex beads) and 2b (virions). All latex beads, virions and albumin at 10V collected rapidly in the centre of the electrode structures within 2 s. At low voltages, albumin molecules were pushed into the middle of the electrode pad across the electrode gap similar to the behaviour shown by latex beads in Figure 2a. When the voltage was increased, the dissolved albumin molecules were initially pushed into the middle of the electrodes as before. However, after about 10 s, some molecules had also collected at the electrode edges by conventional dielectrophoresis began to precipitate from the electrode edges in lumps approximately 1 μm in diameter. These agglomerates were then pushed into the centre of the electrode and collected in the same manner as other particles. DNA strands were observed to collect close

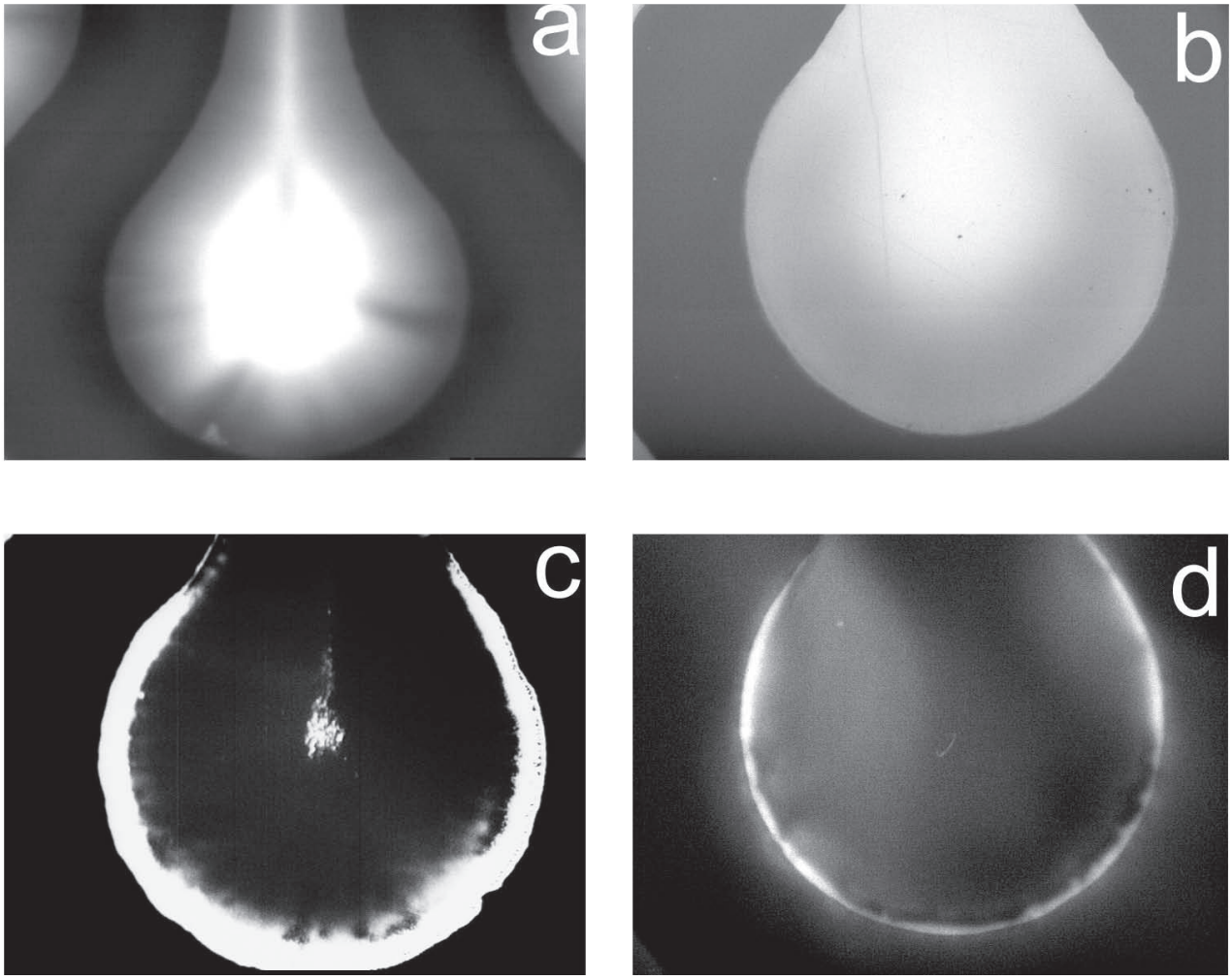

Figure 2 Images of particles trapped using the zipper electrode. **a**) Latex beads are pushed into the middle of the electrode pad, as are influenza virions; **b**). Albumin molecules **c**) collect both at the centre of the pad and at the electrode edge, with single molecules being pushed towards the electrode middle; **d**) DNA only collects close to the electrode edge.

to the electrode edge rather than at the centre of the electrode pad, forming regions of higher particle concentration as shown in Figure 2d. This may be due to surface charges on DNA molecules, or to a stronger dielectrophoresis influence being exerted on the molecules due to their length producing an exceptionally strong dipole.

Such collection is driven by a combination of dielectrophoresis and hydrodynamic flow (Green and Morgan 1998; Green et al 2000). While positive dielectrophoresis causes particles to move towards the region of highest field inhomogeneity, which forces particles to collect on the electrode edges, particles in these experiments collected on the electrode surface due to the electrode design and experimental conditions. However, the balance between the two phenomena has not yet been studied; in order to investigate this, we examined the effect of particle size on collection activity. Dielectrophoresis depends on the volume of particles and the force becomes weaker with decrease in particle size, whilst fluid flow should show much less size dependency beyond the effects of viscous drag; the balance of forces should thus be observable in the size dependency of induced particle motion.

In order to assess the relationship between the force on the particle and its size, measurements were taken of the distance between the particle and the electrode edge at a range of frequencies (0.5 kHz, 0.8 kHz, 1.1 kHz, 1.4 kHz). Experiments were repeated three times. It was found that there was a strong frequency dependency in the distance moved, in line with the results of Green et al (1998), but that there was no statistically significant relationship with particle size. Although the distances moved by the different particle types were different for different frequencies, the trend was common across all frequencies. Figure 3 shows the values for average measurements at 0.8 kHz as a representative sample; error bars have been omitted for the sake of clarity, but error margins were typically less than 10%. No results were taken for DNA as the molecules collected at the electrode edge.

At a frequency of 500 Hz all latex particles, regardless their size, were pushed to 71–102 μm from the electrode edge after a period of 1s, while at frequency of 800 Hz particles moved only 59–63 μm after 1s. Further increase of the frequency to 1100 Hz and 1400 Hz did not change this significantly. Small variations between the collection rates were probably down to Brownian scatter. Since the homogeneous latex beads are of different sizes (varying over more than one order of magnitude) but identical in their

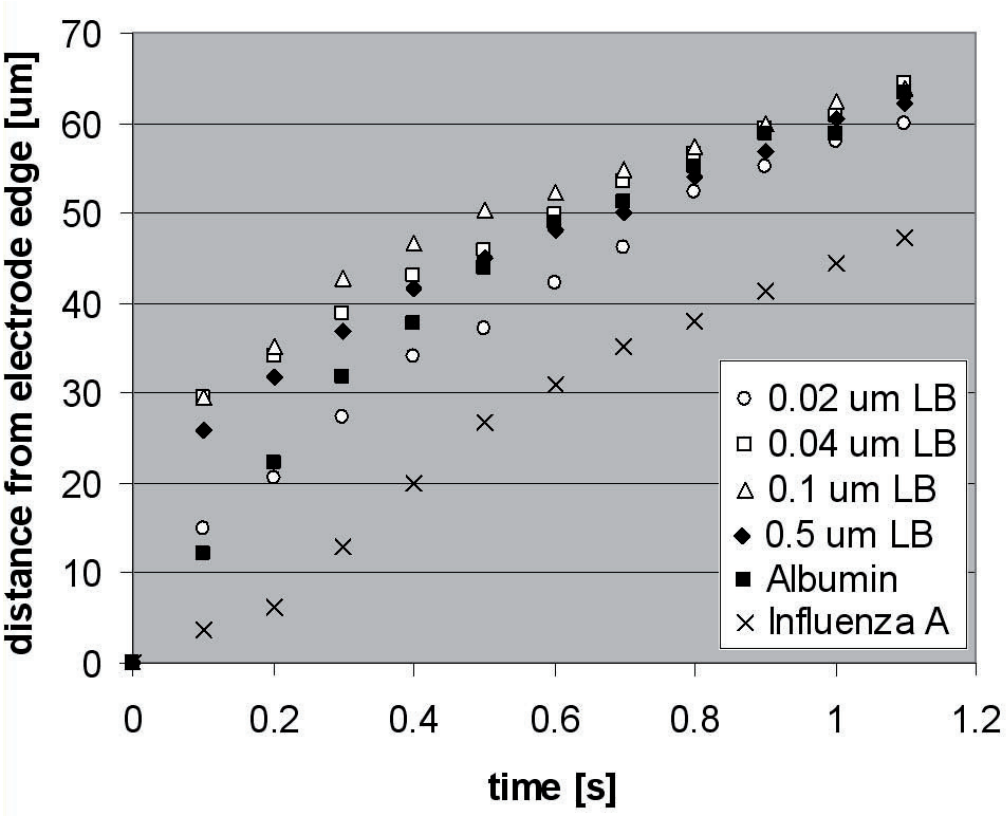

Figure 3 A graph showing the distance covered from the electrode edge after 1 second of applied electric field at 800 Hz, by different particle types of different sizes.
Key: LB = latex bead, figure quoted is diameter in μm; albumin molecules and influenza virions are approximately 0.04 μm and 0.1 μm diameter, respectively.

composition, it can be seen that the collection behavior was independent of size, which indicates a strong influence of the electro-hydrodynamic flow on the collection behavior. When comparing the results of the latex beads with bioparticles after 1 s it can be seen that albumin with a distance of 59 μm correlated well with the results of the latex beads. They move more slowly in for the first 0.7 s, but increase velocity after the first 0.7 s until they reach as far as latex beads. However, Influenza virions moved more slowly and only traveled 44 μm in the same time period. We speculate that this may imply that the dielectrophoretic force is pulling against the electro-hydrodynamic flow and thereby slowing the collection of larger particles; whereas all the other particles exhibited a generally exponential velocity curve, the virions exhibit a slightly s-curve shaped profile with velocity being suppressed near the electrode edge before accelerating at about 0.3 s. This would be expected if dielectrophoresis were retarding the motion of particles, as the dielectrophoretic force is far more strongly distance-dependent and would only have significant effect within a short distance of the electrode edge. This may indicate a stronger positive dielectrophoretic force on the virus than the other particles, possibly due to its relatively large size and greater surface charge.

In addition to this observation, the frequency dependence of the collection of particles highlights the influence of dielectrophoresis. The fact that the collection on top of the electrode pads is slower at higher frequencies where the influence of dielectrophoresis may be stronger, shows that dielectrophoresis worked to counteract the motion due to electro-hydrodynamic flow. Alternatively, as colloidal particles exhibit positive dielectrophoresis at low frequencies due to surface charge effects (Hughes 2002) whereas the larger particles may experience negative dielectrophoresis at low frequencies; it is possible that this combination of forces may put the particles into a different flow layer and correspondingly different velocity.

Conclusion

In recent years attempts have been made to combine dielectrophoresis and AC-electro-hydrodynamic flow to focus particles on a metal surface in order to improve detection systems such as surface plasmon resonance and quartz crystal microbalances. In this paper we have demonstrated that it is possible to use "zipper"-configuration electrodes for focusing and concentrating DNA and for the first time virus particles and proteins on a 300 μm-diameter metal surface. We have also demonstrated that the velocity at which particles are pushed towards the centre of the electrode pads by dielectrophoresis and electro-hydrodynamic flow is not generally related to the particle size for colloidal particles.

Acknowledgments

This work was funded by the UK Ministry of Defence.

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