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Insulator-based dielectrophoresis for the selective concentration and separation of live bacteria in water

Insulator-based dielectrophoresis (iDEP) was utilized to separate and concentrate selectively mixtures of two species of live bacteria simultaneously. Four species of bacteria were studied: the Gram-negative *Escherichia coli* and the Gram-positive *Bacillus subtilis, B. cereus*, and *B. megaterium*. Under an applied direct current (DC) electric field all the bacterial species exhibited negative dielectrophoretic behavior. The dielectrophoretic separations were carried out in a glass microchannel containing an array of insulating posts. The insulating posts in the microchannel produced nonuniformities in the electric field applied along the channel. Mixtures of two species of bacteria were introduced into the microchannel and the electric field was applied. The bacterial species exhibited different dielectrophoretic mobilities under the influence of the nonuniform field. From these experiments a trapping order was established with *E. coli* trapping at the weakest applied electric field, while the *Bacillus* species were trapped at different characteristic threshold fields. At stronger applied electric fields, the two different species of bacteria in the microchannel were dielectrophoretically trapped into two spatially distinct bands. The results showed that iDEP has the potential to selectively concentrate and separate different species of bacteria.

Keywords: Dielectrophoresis / Electrokinesis / Electroosmosis / Insulators / Trapping
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1 Introduction

1.1 General aspects

Many of the current bacteriological methods for water analysis involve lengthy culture steps to obtain concentrated samples, which lead to undesirable delays in analysis. For example, detecting coliform organisms in water requires presence-absence, membrane filter, and multiple tube fermentation methods that employ incubation for 24–48 h on lactose-based and/or defined-substrate media [1]. Dielectrophoresis (DEP), an electrostatic transport mechanism with a nonlinear dependence on electric field, can be used to concentrate and separate microorganisms [2, 3], motivating its use as an alternative to culturing in water monitoring systems. A nonuniform electric field produces an unbalanced electrostatic force on the charge in a particle. The motion resulting from this force is called DEP [3–5]. DEP can occur in either direct (DC) or alternating (AC) electric fields [6]. There are two regimes of DEP than have the potential for particle concentration. The first regime, "streaming dielectrophoresis," occurs when DEP dominates diffusion, but does not overcome electrokinetic flow, so particles remain mobile. The second DEP regime is called "trapping dielectrophoresis." Trapping DEP occurs when DEP overcomes diffusion and electrokinesis. Under this regime, particles are dielectrophoretically immobilized and can be significantly concentrated, nearly to solid density [2].

A number of studies have focused on the application of DEP for concentration, separation, transport, and identification of bacteria [4, 7–14]. The majority of DEP studies reported in the literature employ AC electric fields and closely spaced electrode arrays to produce the nonuniform fields. Microfabrication techniques enable the construction of such arrays of microelectrodes [7, 15]. However, microelectrode array-based DEP systems generally face performance-limiting issues such as electrode fouling. An alternative to electrode-based DEP is the technique called insulator-based DEP (iDEP). Insulators are

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Abbreviations: DC, direct current; **CM**, Clausius Mossotti factor; **DI**, deionized; **IDEP**, insulator-based dielectrophoresis; **EK**, electrokinetic

less sensitive than electrodes to fouling. In addition, insulative materials such as plastics have excellent malleability and can be mass replicated, providing for inexpensive, high-throughput, and large-volume devices [2, 16]. Additionally, electrokinetic flow can be used to deliver and convey the sample to the device; an advantage for automation and integration of a concentrator with other analytical methods such as electrophoresis and related electrokinetic techniques.

Cummings and Singh [2, 17] introduced the concept and initial characterization of a so-called "engineered" iDEP device consisting of an array of insulating posts in a microchannel. In their experiments a DC electric field was applied across this microchannel. Only two electrodes were present, in reservoirs at the fluidic inlet and outlet. The insulating posts created electric field intensity gradients. They successfully demonstrated dielectrophoretic manipulation and trapping of 200 nm fluorescent polystyrene particles [2]. Cummings and Singh [2, 17, 18] demonstrated iDEP with polystyrene particles using DC electric fields. Chou *et al.* [19] demonstrated iDEP trapping of DNA molecules using insulating structures and AC electric fields. Zhou *et al*. [20] and Suehiro *et al*. [21] used a channel filled with insulating glass beads and AC electric fields for separating and concentrating yeast cells in water. In their system, the direction of the water flow was normal to the applied electric field. Lapizco-Encinas *et al.* [16] demonstrated selective iDEP trapping of polystyrene particles, live *E. coli*, and dead *E. coli* in arrays of insulating posts using DC electric fields. Selective iDEP trapping of different types of live bacterial cells has not yet been reported.

This article describes the application of iDEP for selective trapping and concentration of four different species of bacteria, including the Gram-negative *Escherichia coli* and the Gram-positive *Bacillus subtilis, Bacillus cereus* and *Bacillus megaterium*. Aliquots of the live bacteria were mixed two bacterial species at a time and introduced into the inlet reservoir of a glass microfluidic channel containing an array of circular insulating posts. An electric field was applied *via* electrodes placed in inlet and outlet reservoirs. The behavior of the bacteria depended on the magnitude of the applied electric field. Selective dielectrophoretic trapping was observed even for mixtures of two different *Bacillus* species. These results demonstrate the great potential of iDEP in front-end devices for water analysis.

1.2 Background

1.2.1 Mathematical background of DEP

Cummings and Singh [2] provide a detailed description of the different regimes of DEP at DC fields. As stated in their publication [2], DEP has to overcome diffusion and electrokinetic (EK) flow (as well as pressure-driven flow if present) in order for the particles to be dielectrophoretically trapped by a DC electric field. The EK velocity, which is proportional to the electric field, comprises the effects of electroosmosis and electrophoresis [18]. The dielectrophoretic velocity, which is a second order effect of the electric field, can be expressed as [18]

$$\mathbf{u}_{DEP} = -\mu_{DEP}\nabla(\mathbf{E}\cdot\mathbf{E}) \tag{1}$$

where $\mathbf{u}_{DEP}$ is the dielectrophoretic velocity, μ_{DEP} is the dielectrophoretic mobility, and $\mathbf{E}$ is the electric field. For dilute, creeping flow in insulating, impermeable channels, the flux of particles, $\mathbf{j}$, including diffusion, pressure-driven flow, EK flow and DEP is [18]

$$\mathbf{j}\cdot\mathbf{n} = 0 \qquad \text{on the channel boundaries} \tag{2}$$

$$\mathbf{j} = -D\nabla C + C(\mathbf{u} + \mathbf{u}_{EK} + \mathbf{u}_{DEP}) \qquad \text{in the channel} \tag{3}$$

where $\mathbf{n}$ is the normal to the surface, D is the diffusion coefficient, C is the concentration of particles, $\mathbf{u}$ is the velocity of the pressure-driven flow (nonelectrokinetic component of the velocity).

The EK velocity ($\mathbf{u}_{EK}$) is related linearly to the electric field

$$\mathbf{u}_{EK} = \mu_{EK}\mathbf{E} \tag{4}$$

where

$$\mu_{EK} \equiv \mu_{EP} - \mu_{EO} \tag{5}$$

defines the electrokinetic mobility from a superposition of the electrophoretic mobility and the electroosmotic mobility, μ_{EO}, of the opposing flow generated at the liquid/channel interface. Normally, immersed bacterial cells have a negative surface charge, thus μ_{EP} has the same sign as μ_{EO} when a substrate having a negative surface charge (*e.g.*, glass) is employed. It is possible to obtain a simplified version of Eq. (3) at trapping where the flux along the electric field lines is equal to zero ($\mathbf{j}\cdot\mathbf{E} = 0$) and for cases where diffusion and pressure-driven effects are negligible, the flow of particles is controlled by the electrokinetic and dielectrophoretic velocities

$$\mathbf{j}\cdot\mathbf{E} = 0 \approx C[(\mu_{EP} - \mu_{EO})\mathbf{E} - \mu_{DEP}\nabla I]\cdot\mathbf{E} \qquad \text{in the channel} \tag{6}$$

where $I \equiv \mathbf{E}\cdot\mathbf{E}$ is the local field intensity.

Thus, a condition for dielectrophoretic trapping in a given region is

$$\frac{\mathbf{u}_{DEP}\cdot\mathbf{u}_{EK}}{\mathbf{u}_{EK}\cdot\mathbf{u}_{EK}} = \frac{\mu_{DEP}}{(\mu_{EP} - \mu_{EO})}\frac{\nabla I}{I}\cdot\mathbf{E} > 1 \tag{7}$$

Equation (7) shows that particles having a smaller μ_{EK} can be trapped at lower $\mathbf{E}$. The dielectrophoretic force acting on an insolated spherical particle is [3, 5].

$$F_{DEP} = 2\pi\varepsilon_0\varepsilon_m r^3 f \nabla I \quad (8)$$

where ε_0 is the permittivity of free space, ε_m is the relative permittivity of the suspending medium, r is the radius of the particle, and f is the Clausius-Mossotti (CM) factor.

$$f = \left[\frac{\tilde{\sigma}_p - \tilde{\sigma}_m}{\tilde{\sigma}_p + 2\tilde{\sigma}_m}\right] \quad (9)$$

where $\tilde{\sigma}_p$ and $\tilde{\sigma}_m$ are the complex conductivities of the particle and the medium, respectively. The complex conductivity is related to the real conductivity and dielectric constant by $\tilde{\sigma}_p = \sigma + i\omega\varepsilon$, where $i = \sqrt{-1}$, and ω is the angular frequency of the applied electric field. For frequencies below 100 kHz or when DC electric fields are applied, the imaginary part of the complex conductivity can generally be neglected [22, 23].

The DEP force acting on a particle can be positive or negative, depending on the sign of the CM factor. If the conductivity of the particle is greater than the conductivity of the medium, then the particle will exhibit positive DEP behavior, and it will be attracted to the areas of higher electric field strength. Particles whose conductivity is lower than that of the medium will exhibit negative DEP. At low frequencies, the applied electric field is primarily dropped across the outer cellular membrane, and the cells behave as poorly conductive spheres. At higher frequencies, the applied field is able to penetrate into the cells, and the cells behave as more conductive spheres having the conductivity of the cells interior [5, 9, 12, 24–28]. Different dielectrophoretic responses can be obtained from the same cells depending on the frequency and amplitude of the applied electric field applied. In the present study, all the experiments were carried out using DC electric fields. Therefore, from an electrical perspective, the CM factor contribution to the dielectrophoretic response of the different bacterial cells is dictated by the conductivity of the cell membranes. The other particle parameters that affect the dielectrophoretic response are geometrical: size, shape, cell morphology (*i.e.*, presence of a flagellum), surface charge, among others. These structural differences between the species of bacteria are responsible for the differences in dielectrophoretic behavior that was observed in these studies.

1.2.2 Differences between the cell surface of Gram-positive and Gram-negative bacteria

Gram-positive and Gram-negative bacteria have different surface properties. Both types of bacteria have a cell membrane and a cell wall. Most bacteria have cell walls that give them shape and protect them from osmotic lysis [29]. The cell wall in Gram-positive bacteria consists of a thick layer of peptidoglycan (20–80 nm) and teichoic acids, which give the wall a negative charge. The Gram-negative cell wall is much more complicated, composed of an outer membrane (7–8 nm) and a thin layer of peptidoglycan (1–3 nm) [29, 30]. The cell membrane of both Gram-positive and Gram-negative bacteria is a lipid bilayer composed of phospholipids, glycolipds, and proteins [30]. Cell membranes are thin (5–10 nm), and their main function is to retain the cytoplasm and serve as a permeable barrier. The membrane prevents the loss of essential components through leakage [29]. When the cell membrane has been compromised, the cytoplasm can leak out of the cell membrane increasing its conductivity [9, 31]. An intact cell membrane is a good insulator, being composed of lipids and proteins that present a simplified barrier to ions [27, 32]. The difference in conductivity between the cell wall and the cell membrane is significant; *e.g.*, Suehiro *et al*. [31] have reported that the conductivities of the cell wall and cell membrane of *E. coli* are 5×10^2 and 5×10^{-5} μS/mm, respectively.

Since the cell wall is highly conductive, electric fields can easily pass through the cell wall. At low frequencies, however, the cell interior is shielded by the highly insulating cytoplasmic membrane; *i.e.*, the membrane sustains the full electric potential applied to the cell [9, 12]. Burt *et al*. [27] state that at frequencies below 100 kHz, the low value of the bulk membrane conductivity prevents the applied electric field from penetrating the into the cytoplasm. As the frequency increases above 100 kHz, the membrane resistance is shunted by the membrane capacitance and the electric field is able to penetrate the cell [27]. These differences between Gram-positive and Gram-negative affect also the electrophoretic behavior of the cells [33]. Under physiological pH conditions both types of microorganisms have net negative charges [33–35]. Electrophoretic separations have been achieved between Gram-positive and Gram-negative bacteria [33, 34]. Sonohara *et al*. [33] found the Gram-negative bacterium *E. coli* to be charged more negatively and therefore to have a higher electrophoretic mobility (by a factor of 2) than the Gram-positive *Staphylococcus aureus*. In a similar study, Buszewski *et al*. [34] also reported higher electrophoretic mobilities (by a factor of 3) values for the Gram-negative bacterium *E. coli* than those for the Gram-positive bacterium *B. cereus*.

In the present study (performed at a pH of 8), the four species of bacterial cells, the Gram-negative *E. coli,* and the Gram-positive *B. subtilis*, *B. cereus*, and *B. megaterium* all were observed to have indistinguishable electrokinetic velocities, meaning that electroosmosis dominated over electrophoresis. In other words, the impact of the differences in μ_{EP} on the overall μ_{EK} of each bacterial species was negligible. According to the studies reported by Bus-

zewski *et al*. [34] and Sonohara *et al*. [33], the four species of bacteria have μ_{EP} opposed to the EOF when using a substrate with a negative surface charge (*e.g.*, glass). Since *E. coli* is Gram-negative, it has a higher density of negative charges on its membrane than the three Gram-positive *Bacillus* species and therefore has the most negative μ_{EP}. The overall EK mobility (μ_{EK}) of *E. coli* is therefore the lowest of the bacteria and consequently, in accordance with Eq. (6), *E. coli* should exhibit iDEP trapping at lower dielectrophoretic velocities than the three *Bacillus* species. However, since the EK velocities of the four species of bacteria are indistinguishable, the effect of differences in μ_{EP} on the trapping behavior is likewise negligible.

Other published results confirm that the electrophoretic mobilities of bacteria are relatively low. Buszewski *et al*. [34] found the μ_{EK} of *E. coli* did not differ much from the μ_{EO}. Armstrong and He [36] carried out CE of live and dead bacteria, and their results showed no significant difference between the migration times for the live and dead bacteria. Li and Harrison [37] mentioned that in uncoated glass, the EOF is greater than the μ_{EP} of cells. Armstrong's research group [38–41] has accomplished the separation of bacteria using CE and capillary isoelectric focusing by adding a polymer to the running buffer. Armstrong *et al*. [40] stated that without the addition of the polymer in the CE experiments, the microbes will elute near the EOF.

Thus, two major forces dominating the dielectrophoretic behavior of the bacteria in our system were drag from the EOF and the dielectrophoretic force. In order to trap dielectrophoretically, the condition in Eq. (7) simplifies somewhat to

$$\frac{\mu_{DEP}}{\mu_{EO}}\left(\frac{\nabla I}{I}\right)\cdot\mathbf{E} > 1 \qquad (10)$$

The bacteria strain specificity arises from differences in dielectrophoretic mobility, which are dominated by factors other than electrical properties in these DC experiments as discussed below.

2 Materials and methods

2.1 Apparatus

A schematic representation of the equipment used is shown in Fig. 1. Experiments were conducted in a microfluidic chip consisting of eight patterned channels isotropically etched in glass (Figs. 1a and b). The chip was reversibly sealed to a polydimethylsiloxane (PDMS) flow manifold *via* a vacuum chuck. The manifold provides 16 open reservoirs, and each reservoir has a volume of 0.1 mL. The manifold and chips are placed directly on an inverted epifluorescence microscope, model IX-70 (Olympus, Napa, CA, USA) using a filter set Chroma 51006 (Chroma Technologies, Brattleboro, VT, USA). A high-voltage power supply (PS350; Stanford Research Systems, Palo Alto, CA, USA) is used to apply electric fields to the microsystem *via* 0.508 mm diameter platinum-wire electrodes (Omega Engineering, Stanford, CT, USA) in the fluid reservoirs. Sequences of fluorescent images of the cells are recorded using a Sony digital camera (Sony, San Diego, CA, USA).

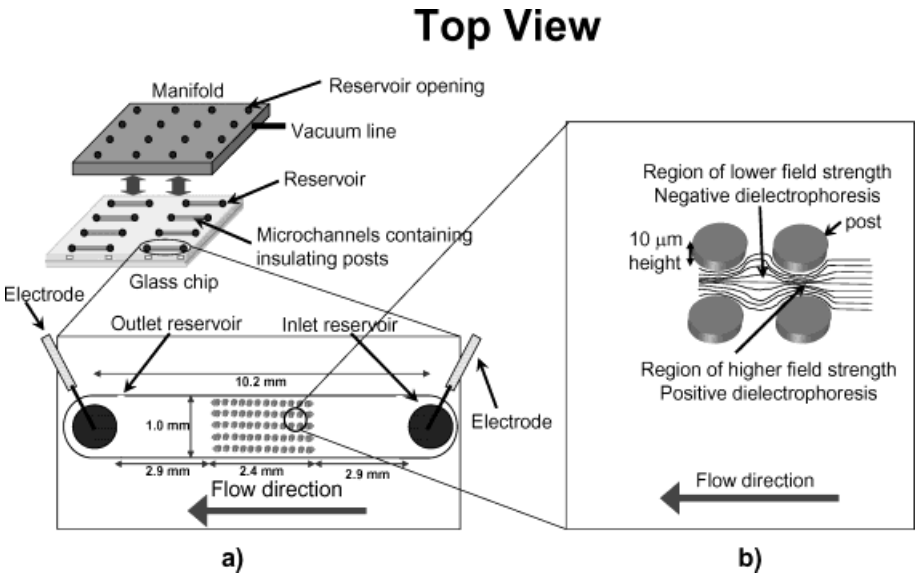

Figure 1. Schematic representation of the experimental setup. (a) Plan view, showing the manifold, glass chip, and an enlargement of the flow microchannels; (b) cartoon showing how the insulating posts modify the field distribution.

2.2 Microfluidic circuit fabrication

The microchips were fabricated from Schott D263 glass wafers (100 mm diameter, 1.1 mm thick; S. I. Howard Glass Company, Worcester, MA, USA) using standard photolithography, wet etch, and bonding techniques. The microfluidic chip contains eight independent microchannels. Each microchannel is straddled by two liquid reservoirs that have an approximate diameter of 1 mm and a depth of 1 mm. The distance between the reservoirs is 10.2 mm; the post-area is located in the middle of the microchannel, 2.9 mm from each *via* (Fig. 1a). The circular posts utilized are 150 μm in diameter and on a 200 μm centers square array that is aligned with the microchannel. The insulating posts transverse the entire depth (10 μm) of the microchannel (Fig. 1b).

2.3 Cell species/labeling protocols

Lyophilized *E. coli* (strain BL21) was obtained from Stratagene (La Jolla, CA, USA). *Bacillus subtilis* (strain ATCC # 6633), *B. cereus* (strain ATCC # 14579), and *B. megaterium* (strain ATCC # 10778) were obtained from ATCC (Manassas, VA, USA). Hydrodynamic diameters of the bacterial cells were estimated by dynamic light scattering (DLS) using a Zeta Plus Instrument (Brookhaven Instruments, Holtsville, NY, USA). In this technique, the time-dependent fluctuations of scattered light intensity are measured to determine the translational diffusion constant of a suspended particle, which in turn can be related to the hydrodynamic diameter. The device was calibrated by using a solution of 200 nm polystyrene particles. The values of the hydrodynamic diameters of the bacterial cells are shown in Table 1. All cell types were grown in 5 mL of Lennox L Broth (LB). Cultures of *E. coli* were grown at 37°C and cultures of the *Bacillus* species were grown at 30°C in an incubator for 12 h to achieve saturation conditions. A 1:20 volumetric dilution of each cell culture was then allowed to grow in the LB into late log phase to a cell concentration of 6×10^8 cells/mL, verified by optical density (OD) measurements at 600 nm [42]. Cells were centrifuged at 5000 rpm for 10 min, in order to eliminate the LB, and resuspended in deionized (DI) water (pH 8) utilizing a vortex mixer. The cells were then labeled with Syto® 11 (green) or Syto® 17 (red) bacterial stains (Molecular Probes, Eugene, OR, USA). Syto® 11 and Syto® 17 produce cells that will fluoresce green (excitation/emission 508/527 nm) and red (excitation/emission 621/634 nm), respectively. For every milliliter of cell culture present in the vial, 3 μL of the fluorescent nucleic acid stain was added. The cells were then incubated at room temperature for 15 min. The labeled cells were recovered by centrifugation at 5000 rpm for 10 min, washed three times with DI water to remove any free dye, and finally resuspended in DI water to the desired final volume to reach the desired cell concentration (typically 6×10^8 cells/mL). The labeled cell cultures were then used directly or mixed, and then 50 μL of this sample was added to the inlet reservoir in the flow manifold via pipette.

Table 1. Hydrodynamic diameters of the bacterial species utilized

Species	Hydrodynamic diameter (μm)[a)]	Flagella
E. coli	1.09 ± 0.32	Yes
B. subtilis	5.65 ± 1.23	No
B. cereus	4.01 ± 0.66	Yes
B. megaterium	3.15 ± 0.86	No

a) Measured using light scattering

2.4 Experiment preparation

Each experiment started with a clean glass chip. The ports in the chip were aligned with the flow manifold (Fig. 1), and the channel and corresponding reservoirs were filled with DI water. The background solution consisted of DI water, NaOH, and KCl. The pH of the solution was adjusted to a value of 8 by adding a 0.01 N NaOH solution. The conductivity of the DI water was adjusted by adding a 0.01 M KCl solution to values of either 2.2 or 10.4 μS/mm. The pH of the solution was measured at the inlet and the outlet reservoirs before and after running the experiments, and a pH change of 1 unit or less was observed during the experiment. Care was taken to eliminate pressure-driven flow produced by liquid-level differences in the reservoirs. A sample of labeled cells was introduced at the inlet reservoir. Electrodes were placed at the inlet and outlet reservoir and an electric field was applied across the 10.2 mm long microchannel containing the post array. The dielectrophoretic behavior of the cells and/or particles was recorded using the microscope and video camera.

3 Results and discussion

3.1 Lowest applied electric field required for iDEP trapping

Measurements of the lowest electric field applied required to achieve trapping were made for each bacterial species. In these experiments, only one bacterial species was used at a time. The goal of these experiments is to establish a trapping order or trend based on the minimum elec-

tric field required to achieve trapping. Sets of experiments were performed using two different solution conductivities: 2.2 and 10.4 μS/mm. The use of different solution conductivities helped to identify the mechanism of species specificity. If the membrane conductivity is the parameter controlling specificity, then increasing the medium conductivity should reduce specificity by reducing the relative differences in the CM factor. However, if the membrane conductivities are all much smaller than the medium conductivity, the CM factors of all species approach −0.5 regardless of the differences in conductivity and the species specificity cannot arise from electrical properties. Thus, if species specificity is observed not to change with solution conductivity, one can attribute specificity in these experiments to geometrical differences or other factors not included in conventional treatments of DEP. Based on the cell membrane conductivity of 5×10^{-5} μS/mm [31] of *E. coli* the CM factor in these experiments is practically equal to −0.5. In order to observe differential dielectrophoretic effects arising from differences in membrane conductivity, the other bacteria must have at least four orders of magnitude greater membrane conductivity than the *E. coli*, an unlikely difference that can be easily ruled out by repeating experiments at two different solution conductivities.

In order to monitor the minimum applied electric field required for DEP trapping, the bacteria sample was introduced into the microchannel and the electric field was increased until the bacteria began to be trapped. The results obtained are presented in Fig. 2. From the figure, it is possible to observe that the order of trapping of the four species of bacteria, from the lowest to the highest electric field required, is as follows: *E. coli* < *B. megaterium*< *B. subtilis* < *B. cereus.* The same order of trapping is observed at both values of solution conductivity,

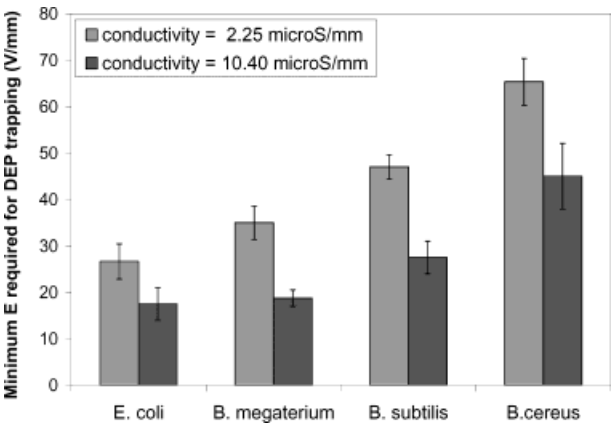

Figure 2. Minimum mean electric field required to achieve DEP trapping in the device that was studied.

but lower applied electric fields are required when using the 10.4 μS/mm solution. The differences between the cell membrane conductivities are not sufficient to account for the differences in the electrophoretic behavior between the bacterial cells. These results demonstrate that parameters other than the electrical properties of the cell control the differences in dielectrophoretic response of the bacterial cells. These parameters include the cell size, shape, morphological characteristics, and surface charge. In the case of surface charge, this parameter is directly related to the electrophoretic mobility of the cells, which, as discussed in the introduction, was not a significant factor in our experiments due to the presence of the EOF. If the EOF had been suppressed or eliminated, then the electrophoretic mobility would have been a significant parameter affecting the DEP trapping of the cells. These results are different from those obtained in our first dielectrophoretic study of bacterial cells [16]. In our previous report we analyzed the DEP response of live and dead *E. coli*. The live and dead *E. coli* cells had essentially the same size, shape, morphological characteristics, electrokinetic mobility, *etc.* The only significant difference between live and dead *E. coli* was the conductivity of the cell membrane, since dead cells, having compromised membranes, have a higher conductivity than live cells by ~4 orders of magnitude [9].

The results in Fig. 2 indicate that *E. coli* exhibits the strongest negative DEP behavior, since DEP trapping of *E. coli* was obtained with the lowest applied electric fields. *B. cereus* exhibited the least negative dielectrophoretic behavior, since the highest applied electric fields were necessary to achieve DEP trapping. This trend is not in agreement with the relative size of the bacteria (Table 1). According to Eq. (6), derived for spherical particles, the DEP force scales with the volume of a particle. The drag force exerted by the EOF scales with the particle size. Thus, generally it is expected that lower applied electric fields are needed to trap larger particles, other things being equal. However, Fig. 2 shows that *E. coli* have both the smallest size and strongest DEP behavior. Both the drag and dielectrophoretic forces depend on details of the particle shape. The long flagella of the *E. coli* and differing shapes of the species of bacteria are probably responsible for the poor agreement with the predictions of the simple sphere model used in Eq. (8), but further studies are needed. Figure 2 also illustrates the potential for the separation and concentration of different species of bacteria simultaneously. It is possible to concentrate a sample of different species of bacteria by applying a sufficiently high electric field to collect bacteria. Then each concentrated bacterial species can be selectively eluted by reducing the applied electric field in the manner of a conventional gradient elution.

The present publication is focused on the experimental results obtained for the separation of four different bacterial species. Selective separation and concentration of mixtures of bacteria were carried out. Due to the limitations of the excitation/emission filters, only two bacterial species were used at a time. The results obtained with each mixture of two bacteria are described below.

3.2 Separation of *E. coli* and *B. subtilis*

Figure 3 shows the dielectrophoretic behavior obtained when a mixture of equal amounts of *E. coli* (green) and *B. subtilis* (red) was introduced into the microchannel. *E. coli* was stained green using Syto® 11 and *B. subtilis* was stained red using Syto® 17. In Fig. 3a, when a lower electric field was applied (50 V/mm), mainly *E. coli* was trapped while *B. subtilis* flowed through the array of posts without trapping. In Fig. 3b, at a higher electric field (75 V/mm) it was possible to trap both species of bacteria in spatially separate bands. As expected, both bacteria exhibited negative dielectrophoretic behavior since trapping occurred upstream of the area where the field is most concentrated. The band of *B. subtilis* is located closer to the peak electric field concentration (see Fig. 1) than the band of *E. coli*. Thus, *E. coli* (green band) exhibited a greater negative dielectrophoretic mobility than *B. subtilis* (red band). These results agree with the trend presented in Fig. 2, where *E. coli* is shown to trap at a lower applied electric field than *B. subtilis*. The experiment was repeated using *E. coli* stained red with (Syto® 17) and *B. subtilis* stained in green (Syto® 11), and the same results were produced, *i.e.*, *E. coli* exhibited a more negative dielectrophoretic mobility than *B. subtilis*. This set of experiments was done in order to verify the assumption that the DNA-intercalating dyes were not affecting the dielectrophoretic or electrokinetic behavior of the bacteria.

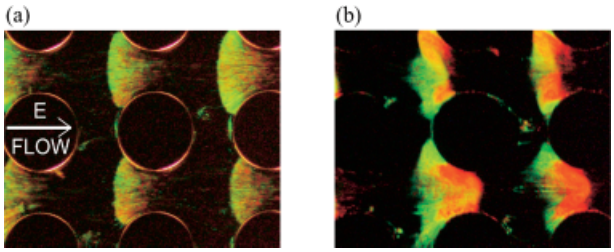

Figure 3. Epifluorescence image of selective trapping of *E. coli* and *B. subtilis.* The inlet cell concentration is 3×10^8 cells/mL *E. coli* and *B. subtilis* cells are respectively labeled green (Syto® 11) and red (Syto® 17) The flow direction is from left to right. The background electrolyte is deionized water whose pH has been adjusted to 8 by adding NaOH, and conductivity has been adjusted to 2.2 μS/mm by adding KCl. The circular posts in the flow-aligned square array are wet etched in glass 10 μm tall, 150 μm in diameter, and on 200 μm centers. Mean applied electric fields (a) 50 V/mm, (b) 75 V/mm.

3.3 Separation of *E. coli* and *B. cereus*

The dielectrophoretic separation and concentration of *E. coli* (green) and *B. cereus* (red) are shown in Fig. 4. The results are similar to those of Fig. 3. At an applied electric field of 50 V/mm (Fig. 4a), both cell species are trapped, but the majority of the trapped cells are *E coli.* By increasing the applied electric field to 75 V/mm (Fig. 4b) it was possible to trap both species of bacteria in separate bands. *E. coli* exhibited a more negative dielectrophoretic mobility than *B. cereus* since it was trapped further upstream of the peak field concentration. These results are in agreement with the trend shown in Fig. 2.

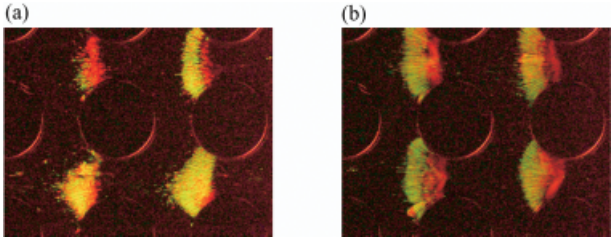

Figure 4. Epifluorescence image of selective trapping of *E. coli* and *B. cereus.* All conditions are as in Fig. 3 unless otherwise stated. *E. coli* and *B. cereus* cells are respectevely labeled green (Syto® 11) and red (Syto® 17). Mean applied electric fields: (a) 50 V/mm, (b) 75 V/mm.

3.4 Separation of *E. coli* and *B. megaterium*

The dielectrophoretic separation and concentration of *E. coli* (green) and *B. cereus* (red) are shown in Fig. 5. At an applied field of 50 V/mm (Fig. 5a) *B. megaterium* flowed while *E. coli* are trapped. When the electric field

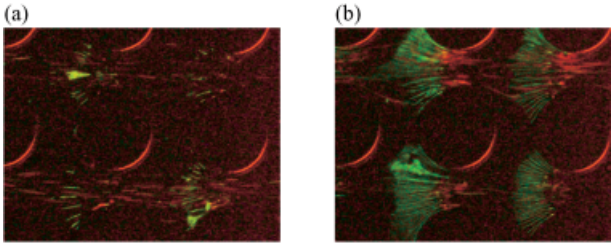

Figure 5. Epifluorescence image of selective trapping of *E. coli* and *B. megaterium.* All conditions are as in Fig. 3 unless otherwise stated. *E. coli* and *B. megaterium* cells are respectively labeled green (Syto® 11) and red (Syto® 17). Mean applied electric fields: (a) 50 V/mm, (b) 90 V/mm.

was increased to 90 V/mm (Fig. 5b) both types of bacteria were dielectrophoretically trapped. From the location of the bands of trapped bacteria, and the results in shown in Fig. 2, it was shown that *E. coli* has a greater negative dielectrophoretic mobility than *B. megaterium*.

3.5 Separation of *B. cereus* and *B. subtilis*

It was possible to selectively trap and concentrate a mixture of two *Bacillus* species. These results show that iDEP has potential for cell discrimination and identification, even when two different species of *Bacillus* are present. Figure 6a shows that at an applied field of 25 V/mm it was possible to selectively trap *B. subtilis* (red) while *B. cereus* (green) exhibited streaming DEP. At an electric field of 75 V/mm (Fig. 6b), both species of *Bacillus* were trapped. From the location of the bands of trapped bacteria it was found that *B. subtilis* has a greater negative dielectrophoretic mobility than *B. cereus*. This observation is consistent with the results in Fig. 2.

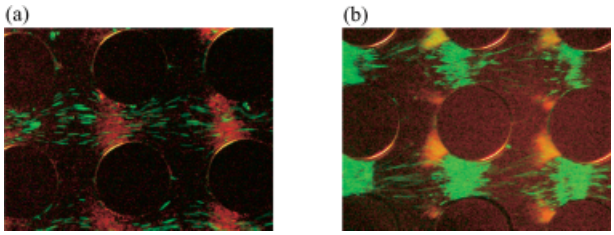

Figure 6. Epifluorescence image of selective trapping of *B. subtilis* and *B. cereus.* All conditions are as in Fig. 3 unless otherwise stated. *B. subtilis* and *B. cereus* cells are respectively labeled green (Syto® 11) and red (Syto® 17). Mean applied electric fields: (a) 25 V/mm, (b) 75 V/mm.

3.6 Separation of *B. megaterium* and *B. subtilis*

In these experiments *B. megaterium* (green) and *B. subtilis* (red) were not able to be trapped in two spatially distinct bands. At an electric field of 50 V/ mm (Fig. 7a), both bacteria are trapped. At a higher electric field of 75 V/mm (Fig. 7b), both of the *Bacillus* species were dielectrophoretically trapped. The bands of bacteria, while offset, are not distinctly separated. From the location of the area of green cells, it can be said that *B. megaterium* on average exhibit a more negatively dielectrophoretic behavior than *B. subtilis*. Again, in agreement with Fig. 2, *B. megaterium* exhibits a greater dielectrophoretic mobility than *B. subtilis*.

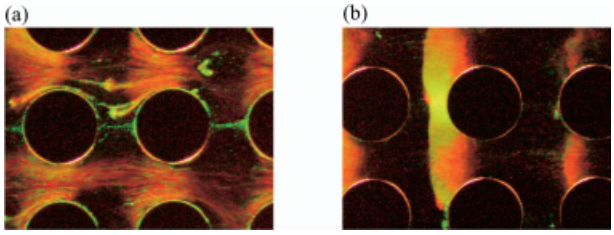

Figure 7. Epifluorescence image of selective trapping of *B. megaterium* and *B. subtilis.* All conditions are as in Fig. 3 unless otherwise stated. *B. megaterium* and *B. subtilis* cells are respectively labeled green (Syto® 11) and red (Syto® 17). Mean applied electric fields: (a) 50 V/mm, (b) 75 V/mm.

3.7 Separation of *B. cereus* and *B. megaterium*

The selective concentration and separation between *B. cereus* (green) and *B. megaterium* (red) was observed at an applied electric field of 30 V/mm (Fig. 8a). At this applied electric field, *B. megaterium* was dielectrophoretically trapped while *B. cereus* flowed through the array of posts. At a higher electric field of 75 V/mm (Fig. 8b), both *Bacillus* species were dielectrophoretically trapped in spatially distinct bands. *B. megaterium* exhibited a greater negative dielectrophoretic mobility than *B. cereus*. In agreement with the trend shown in Fig. 2, these results and are very encouraging, since they show that iDEP has the potential to clearly distinguish between different *Bacillus* species.

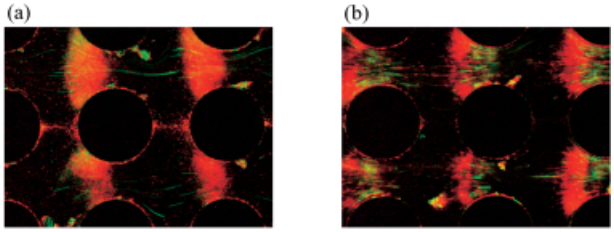

Figure 8. Epifluorescence image of selective trapping of *B. cereus* and *B. megaterium.* All conditions are as in Fig. 3 unless otherwise stated. *B. cereus* and *B. megaterium* cells are respectively labeled green (Syto® 11) and red (Syto® 17). Mean applied electric fields: (a) 30 V/mm, (b) 75 V/mm.

4 Concluding remarks

The application of iDEP for the separation of live bacteria has been demonstrated. This is the first report of iDEP separation of live bacteria using only DC electric fields. Insulating posts were utilized in the etched microchannel to generate a nonuniform electric field to drive DEP. Labeled bacterial samples were introduced into the water-filled microchannel, and an electric field was applied along the channel. All four types of bacteria (*E. coli, B. subtilis, B. cereus,* and *B. megaterium*) exhibited negative dielectrophoretic behavior, *i.e.*, the bacteria were trapped in areas upstream of the peak electric

field concentration. The threshold applied electric field required to trap each bacterial species was different for each species. The order of trapping, from lower to higher electric field threshold was: *E. coli* < *B. megaterium* < *B. subtilis* < *B. cereus* in both single-bacteria and mixed-bacteria experiments. It was demonstrated that the membrane conductivity is not the parameter controlling the differences in DEP behavior of the cells. Therefore other factors or parameters such as cell size, cell shape, and other morphological characteristics are responsible for differences in the dielectrophoretic response of the bacteria. Further investigations of these geometrical effects on the DEP response are needed to be able to predict trapping thresholds and understand what limits exist for the specificity of DC DEP.

Selective trapping was demonstrated when mixtures of two bacterial species were introduced into the microchannel. At lower applied electric fields, it was possible to trap one of the bacterial species selectively. At a higher applied electric field, it was possible to trap both bacterial species. Generally, the bacteria were trapped in spatially offset bands that were fully resolved in all but one case. It was possible to separate Gram-negative from Gram-positive bacteria: the Gram-negative *E. coli* had a greater negative dielectrophoretic mobility than the three Gram-positive *Bacillus* species utilized in the study. The dielectrophoretic mobility of the different of *Bacillus* species were observed to be different enough to separate them easily at a DC applied voltage. This selectivity allows to concentrate and elute populations of these cells by concentrating with a high applied electric field, and selectively eluting zones of different cell types by gradually lowering the electric field.

While these results indicate the considerable promise of iDEP for bacterial concentration, separation and identification, the immersion electrolyte, background particle composition (*e.g.*, organic or inorganic particles), and growth stage of the bacteria can complicate the behavior of such devices. The impact of these parameters on practical device performance requires future study. Insulator-based DEP is currently being developed for use in filter/concentrator "front ends" to sensor systems for bacterial identification and high-throughput devices that collect bacteria from large volumes of drinking water.

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5 References

[1] Greenberg, A. E., Clesceri, L. S., Eaton, A. D., *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC 1992.

[2] Cummings, E., Singh, A., *Anal. Chem.* 2003, *75*, 4724–4731.

[3] Pohl, H., *Appl. Phys.* 1951, *22*, 869–871.

[4] Brown, A. P., Betts, W. B., Harrison, A. B., O'Neill, J. G., *Biosens. Bioelectron.* 1999, *14*, 341–351.

[5] Jones, T. B., *Electromechanics of Particles*, Cambridge University Press, New York 1995.

[6] Pohl, H., *Appl. Phys.* 1958, *29*, 1182–1188.

[7] Betts, W. B., *Trends Food Sci. Technol.* 1995, *6*, 51–58.

[8] Markx, G., Dyda, P., Pethig, R., *J. Biotechnol.* 1996, *51*, 175–180.

[9] Pethig, R., Markx, G. H., *Trends Biotechnol.* 1997, *15*, 426–432.

[10] Betts, W., Brown, A., *J. Appl. Microbiol.* 1999, *85*, 201S–213S.

[11] Archer, G., Render, M., Betts, W., Sancho, M., *Microbios* 1993, *76*, 237–244.

[12] Markx, G. H., Huang, Y., Zhou, X. F., Pethig, R., *Microbiology-UK* 1994, *140*, 585–591.

[13] Medoro, G., Manaresi, N., Leonardi, A., Altomare, L., Tartagni, M., Guerrieri, R., *Proc. IEEE Sensors*, Orlando, FL, USA 2002, pp. 472–477.

[14] Medoro, G., Manaresi, N., Tartagni, M., Guerrieri, R., *Proc. IEDM*, San Francisco, CA 2000, pp. 415–418.

[15] Malyan, B., Balachandran, W., *J. Electrostatics* 2001, *51*, 15–19.

[16] Lapizco-Encinas, B. H., Simmons, B. A., Cummings, E. B., Fintschenko, Y., *Anal. Chem.* 2004, in press.

[17] Cummings, E., Singh, A., *Proc. SPIE*, Santa Clara, CA 2000, pp. 164–173.

[18] Cummings, E. B., *Proc. 32nd AIAA Fluid Dynamics Conference and Exhibit 2002*, St. Louis, MO 2002, pp. 2002–3193.

[19] Chou, C., Tegenfeldt, J., Bakajin, O., Chan, S., Cox, E., Darnton, N., Duke, T., Austin, R., *Biophys. J.* 2002, *83*, 2170–2179.

[20] Zhou, G., Imamura, M., Suehiro, J., Hara, M., *Proc. 37th Annual Meeting of the IEEE-Industry-Applications-Society*, Pittsburgh, PA 2002, pp. 1404–1411.

[21] Suehiro, J., Zhou, G., Imamura, M., Hara, M., *Proc. IEEE Annual Meeting of the Industry-Applications-Society*, Pittsburgh, PA 2003, pp. 1514–1521.

[22] Markx, G. H., Talary, M. S., Pethig, R., *J. Biotechnol.* 1994, *32*, 29–37.

[23] Van den Wal, A., Minor, M., Norde, W., Zehnder, A. J. B., Lyklema, J., *J. Colloid Interface Sci.* 1997, *186*, 71–79.

[24] Li, H., Bashir, R., *Sens. Actuators B* 2002, *86*, 215–221.

[25] Carstensen, E. L., *Biophys. J.* 1967, *7*, 493–503.

[26] Carstensen, E. L., Cox, H. A., Jr., Mercer, W. B., Natale, L. A., *Biophys. J.* 1965, *5*, 289–300.

[27] Burt, J., Pethig, R., Gascoyne, P. R. C., Becker, F., *Biochim. Biophys. Acta* 1990, *1034*, 93–101.

[28] Yunus, Z., Mason, V., Verduzco-Luque, C. E., Markx, G. H., *J. Microbiol. Methods* 2002, *51*, 401–406.

[29] Prescott, L. M., Harley, J. P., Klein, D. A., *Microbiology*, Wm. C. Brown Publishers, Dubuque, IA 1993.

[30] Moat, A. G., Foster, J. W., Spector, M. P., *Microbial Physiology*, Wiley-Liss, New York 2002.

[31] Suehiro, J., Hamada, R., Noutomi, D., Shutou, M., Hara, M., *J. Electrostatics* 2003, *57*, 157–168.

[32] Markx, G. H., Davey, C. L., *Enzyme Microb. Technol.* 1999, *25*, 161–171.

[33] Sonohara, R., Muramatsu, N., Ohshima, H., Kondo, T., *Biophys. Chem.* 1995, *55*, 273–277.

[34] Buszewski, B., Szumski, M., Klodzinska, E., Dahm, H., *J. Sep. Sci.* 2003, *26*, 1045–1049.

[35] Torimura, M., Ito, S., Kano, K., Ikeda, T., Esaka, Y., Ueda, T., *J. Chromatogr. B* 1999, *721*, 31–37.

[36] Armstrong, D., He, L., *Anal. Chem.* 2001, *73*, 4551–4557.

[37] Li, P., Harrison, D., *Anal. Chem.* 1997, *69*, 1564–1568.

[38] Armstrong, D., Schneiderheinze, J., Kullman, J., He, L., *FEMS Microbiol. Lett.* 2001, *194*, 33–37.

[39] Schneiderheinze, J. M., Armstrong, D. W., Schulte, G., Westenberg, D. J., *FEMS Microbiol. Lett.* 2000, *189*, 39–44.

[40] Armstrong, D., Schulte, G., Schneiderheinze, J., Westenberg, D., *Anal. Chem.* 1999, *71*, 5465–5469.

[41] Girod, M., Armstrong, D. W., *Electrophoresis* 2002, *23*, 2048–2056.

[42] Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., Struhl, K., *Short Protocols in Molecular Biology*, Wiley, New York 2002.