INSULATING POST ELECTRODELESS DIELECTROPHORESIS FOR THE SELECTIVE CONCENTRATION OF BACTERIA

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Abstract

Live and dead *E. coli* were concentrated and selectively released using a DC voltage across a microchannel filled with insulating-posts etched in glass. The only electrodes present were platinum wires that were placed in the inlet and outlet reservoirs. Electric fields of up to 2 kV/cm were applied. A DC voltage controlled cell trapping and release. The dielectrophoretic responses of the bacteria cells were observed as function of the post size, geometry and spacing. In addition to analyzing live and dead cells, cells were selectively trapped in the presence of 1 and 0.2 μm rhodamine labeled inert polystyrene particles.

Keywords: insulator dielectrophoresis, bacteria, concentration

1. Introduction

Water surety in the face of natural contaminants such as *Cryptosporidium parvum* and newly realized security threats such as bioterrorism presents a significant analytical chemistry challenge. Pathogenic bacteria can contaminate water at concentrations as low as 1 bacterium/liter. Even if a sensitive detection method is employed, the entire liter must be sampled. By selectively isolating and concentrating the pathogenic bacteria from large volumes a detectable amount of material could be delivered to an analytical device in a greatly decreased sample volume.

Insulating post structures fabricated in glass were used to concentrate and separate live *E. coli* in the presence of dead *E. coli* and inert particles using DC fields. To the best of our knowledge, this the first report of DC insulative (electrodeless) Dielectrophoresis (iDEP) for the concentration and separation of bacteria. Cummings *et al.* described the theory and proof of concept of iDEP using 200-nm polystyrene beads (1-3). Here, an electric field gradient is produced along an array of insulating posts in a microchannel with electrodes present only in the entrance and exit reservoirs. The flow is driven electrokinetically. The point where the dielectrophoretic mobility exceeds the electrokinetic mobility is the point at which species trap. The post geometry, spacing and size affect the strength of the dielectrophoretic traps (1, 3).

Polarizable particles can be moved in a non-uniform electric field by dielectrophoretic force (4). Separation of live and dead cells has been demonstrated by other investigators using pressure-driven flow with AC field in an electrode array

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(5-7). There are a number of drawbacks to this method including fabrication complexity and charging effects, fouling, and gas bubbles at the electrode surface (7). iDEP avoids these problems by creating the heterogeneous electric field using the geometry of posts etched in channels made in insulating materials. Because no thin film electrodes are required, a much larger working area can be fabricated. This could lead in the future to a highly parallel iDEP concentrator for bacteria that is capable of concentrating large volumes such as those required for water analysis.

2. Theory

The dielectrophoretic force acting on a spherical particle can be described by Equation (1).

$$F_{DEP} = 2\pi \in_0 \in_m r^3 \mathrm{Re}\left[\frac{\sigma_p^* - \sigma_m^*}{\sigma_p^* + 2\sigma_m^*}\right]\nabla E^2 \qquad (1)$$

where: $\in_0$ is the permittivity of free space, $\in_m$ is the relative permittivity of the suspending medium, r is the radius of the particle, ∇E^2 defines the local field strength, σ_p^* and σ_m^* are the complex conductivities of the particle and the medium respectively. Equation (1) shows that the dielectrophoretic force acting on a particle can be positive or negative. If the conductivity of the particle is greater than the conductivity of the medium, then the particle will exhibit positive dielectrophoretic behavior, and vice versa. In the present study only DC electric fields were used, therefore the dielectrophoretic response of the cells depends essentially on the conductivity of the cells membranes.

3. Experimental

Apparatus. Experiments were conducted in a microfluidic chip consisting of patterned channels isotropically etched 10-μm deep in glass with a thermally bonded glass cover. The microfluidic chip is reversibly sealed to a test fixture via a vacuum chuck. This PDMS fixture provides 16 open reservoirs. A high-voltage power supply (Stanford Research Systems, PS350, Palo Alto, CA) is used to apply the DC electric field. The dielectrophoretic behavior of cells and inert particles were imaged by an inverted fluorescence microscope (Olympus, Napa, CA) using a live/dead assay filter set (Chroma Technologies Corp, Brattleboro, VT) and a Sony digital camera (Sony, San Diego, CA). The cells were labeled using SYTO® 9 and propidium iodide, two nucleic acid stains (Molecular Probes, Eugene, OR). Rhodamine labeled polystyrene microspheres (Molecular Probes, Eugene, OR) of different sizes (200-nm and 2-μm) were utilized without further modification.

Microfluidic Circuit Fabrication. The microfluidic chip contains 12 sets of independently addressable subcircuits. Each subcircuit is straddled by two liquid reservoirs and consists of 6 separate patterned microchannels. The length of the microchannels is 1.25 cm. The microchannels studied have uniform square arrays of circular posts at different angles with respect to the applied electric field.

Experiment Preparation: The reservoirs openings in the chip were aligned with the manifold and the desired channel and corresponding reservoirs were filled with deionized water. A sample of labeled cells and/or inert polystyrene rhodamine-labeled particles was introduced at the inlet reservoir. Electrodes were placed at the inlet and outlet reservoir and an electric field was applied across the microchannel (1.25 cm in length) containing the insulating-post structures. The dielectrophoretic behavior of the cells and/or particles was recorded by employing the microscope and video camera.

Cell lines/labeling protocols: Lyophilized Escherichia coli (cell line BL21) was obtained from ATCC (Manassas, VA) and grown in LB nutrient broth using standard cell culturing techniques. Cells were centrifuged at 5000 rpm for 10 minutes in order to eliminate the LB nutrient broth. Live cells were re-suspended in DI water utilizing a vortex mixer. Dead cells were obtained by re-suspending live cells in a 70% v/v isopropanol solution at 37 ^{o}C and incubating them for 1 hour. After incubating the dead cells, the isopropanol was eliminated by centrifugation. Dead cells were then re-suspended in DI water. Live and dead cells were then labeled with the SYTO® 9-propidium iodide live/dead BAClight® bacterial stain (Molecular Probes, Inc., Eugene, OR) kit instructions. For live cells the SYTO® 9 labeling technique was utilized, whereas for the dead cells propidium iodide was used. This produces live cells that will fluoresce green and dead cells that fluoresce red. For every mL of the cell culture, 3 μL of the appropriate staining solution was added. The cells were then incubated at room temperature for 15 minutes. Both cell types were then concentrated by centrifugation at 5000 rpm for 10 minutes. The labeled cells were recovered by centrifugation at 5000 rpm for 10 minutes, washed three times with DI water to remove any free dye, and finally re-suspended in DI water to the desired final concentration (typically 6 x 10^{8} cells/mL). These two cell cultures were then mixed to give varying concentrations of live/dead cells. 50 μL of the resulting mixed live/dead cell cultures were added to the inlet reservoir in the flow manifold via pipette.

4. Results and Discussion

Figure 1 shows the separation and concentration of live and dead *E. coli,* a gram negative bacteria. The cells, diluted 1:10 in deionized water, were observed to have a negative dielectrophoretic mobility in a deionized water background, which is consistent with observations in conventional AC methods (6). When particles are negatively dielectrophoretic, the field-induced dipole in the particles is less than that of the background electrolyte, resulting in the particle being repelled from regions of high electric field (6). The dead bacteria have a leaky membrane resulting in a smaller difference between the cell conductivity and the background electrolyte conductivity. This results in a smaller relative DEP potential for dead bacteria, which is observed (Figure 1). Because of the potential of this method as a front end for bacterial analysis in water, it was important to determine the behavior of *E. coli* in the presence of inert, non-cellular particles that could be in sample background. Therefore, the behavior of bacteria under DEP field was evaluated in presence of

polystyrene particles. 1-μm polystyrene beads coated with rhodamine were added to a sample of live *E. coli*. The polystyrene beads exhibit positive dielectrophoresis, in contrast to the DEP behavior for live cells (Figure 2). Preliminary results indicate that for the geometries given below (Figures 1, 2), live cells trap and release at lower voltages than either dead cells or inert particles.

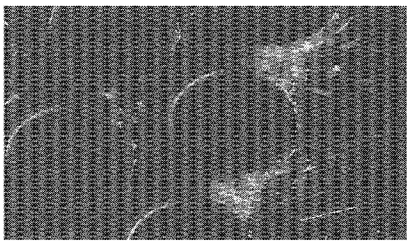

Figure 1. Image of iDEP Simultaneous Concentration and Separation of Live and Dead *E. coli*. 10 X magnification. Live cells are green, dead cells are red. A field of 1.3 kV/cm was applied.

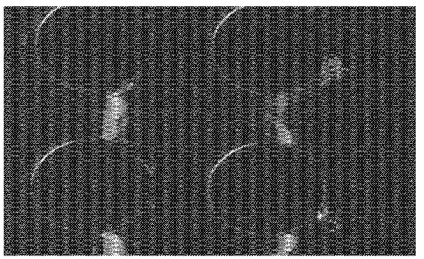

Figure 2. Image of Simultaneous Concentration and Separation of Live *E. Coli* and Inert 1-μm Particles. All conditions were as in Figure 1 except live cells were at a 1:100 dilution while the 1 μm rhodamine labeled polystyrene beads were diluted 1:10. The applied voltage was 2.5 kV over 1.25 cm. The post offset is 0° , and the spacing is 15 μm.

5. Conclusions

The application of electrodeless dielectrophoresis (iDEP) for the concentration of bacteria in the presence of inert particles has been demonstrated. The separation of live and dead bacteria in water has been achieved. This demonstrates the feasibility of iDEP as a selective bacteria concentrator. Potential applications include a front-end device for large volume applications such as water analysis and small volume applications such as medical diagnostics.

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