Polymeric Insulator-based Dielectrophoresis (iDEP) for the Monitoring of Water-borne Pathogens

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ABSTRACT

We have successfully demonstrated selective trapping, concentration, and release of various biological organisms and inert beads by insulator-based dielectrophoresis within a polymeric microfluidic device. The microfluidic channels and internal features, in this case arrays of insulating posts, were initially created through standard wet-etch techniques in glass. This glass chip was then transformed into a nickel stamp through the process of electroplating. The resultant nickel stamp was then used as the replication tool to produce the polymeric devices through injection molding. The polymeric devices were made of Zeonor® 1060R, a polyolefin copolymer resin selected for its superior chemical resistance and optical properties. These devices were then optically aligned with another polymeric substrate that had been machined to form fluidic vias. These two polymeric substrates were then bonded together through thermal diffusion bonding. The sealed devices were utilized to selectively separate and concentrate a biological pathogen simulant. These include spores that were selectively concentrated and released by simply applying D.C. voltages across the plastic replicates via platinum electrodes in inlet and outlet reservoirs. The dielectrophoretic response of the organisms is observed to be a function of the applied electric field and post size, geometry and spacing. Cells were selectively trapped against a background of labeled polystyrene beads and spores to demonstrate that samples of interest can be separated from a diverse background. We have implemented and demonstrated here a methodology to determine the concentration factors obtained in these devices.

Keywords: Insulator-based dielectrophoresis, polymer microfluidics, pathogens, separation, concentration, sample management

INTRODUCTION

Insulator-based DEP (iDEP), first developed by Masuda et al.\(^1\) and revisited by Lee et al.\(^2\) employs spatially non-uniform insulating structures to produce electric field non-uniformities that are created by energized remote electrodes. Devices for iDEP can be made purely from insulating materials (e.g., plastics),\(^3\) which can be replicated inexpensively, facilitating high-throughput and large-volume applications. This paper demonstrates the effectiveness of such polymer-based devices. Chou et al.\(^4\) has previously demonstrated iDEP of DNA molecules, E. coli cells, and red blood cells using insulating structures and AC electric fields. Recently, Zhou et al.\(^5\) and Suehiro et al.\(^6\) used a channel filled with insulating glass beads and applied AC electric fields for the separation and concentration of yeast cells in water.

We have previously demonstrated both the theory and application of iDEP with D.C. electric fields using arrays of insulating posts inside a glass-based microfluidic device to trap polystyrene particles\(^7\) and to separate live and dead bacteria.\(^8\) The effective separation between live and dead cells arose from differences between the membrane conductivities of the two classes of cells. When a cell dies, the cell membrane becomes permeable, and its conductivity can increase up to a value of 10 \(\mu\)S/cm; whereas the conductivity of the membrane of a live cell tends to be \(~\times 10^3\) \(\mu\)S/cm. These differences in membrane conductivity dramatically change the well-known Clausius-Mossotti factor, thereby producing significantly different dielectrophoretic trapping thresholds for the live and dead particles. While both exhibited negative DEP, the lower trapping threshold of the live cells allowed their selective collection, demonstrating the potential of iDEP for rapid cell viability analysis.\(^8\)

We have also reported the insulator-based dielectrophoretic separation between different species of live bacterial cells.\(^9\) In this case, parameters other than membrane conductivity play an important role in the separation process. These
parameters include cell size, cell shape, and other morphological characteristics of the cells, such as the presence of a flagellum. While the theory is not yet complete enough to predict the relative trapping thresholds of different bacteria, we empirically showed that these thresholds are typically different enough between species of bacteria to allow selective collection. The threshold (minimum) applied electric field required to achieve dielectrophoretic trapping of the four species of bacteria in the study, from lowest to highest threshold, was *Escherichia coli* < *Bacillus megaterium* < *Bacillus subtilis* < *Bacillus cereus*. These results demonstrate that iDEP can effectively and efficiently separate similar species of vegetative and viable bacterial cells.

Polymer microfluidic devices have been shown for several years in the fields of separation and other lab-on-a-chip applications. The main appeal of these polymeric devices is that they are relatively cheap in terms of mass fabrication, through standard techniques such as injection molding, compared to other microsystem fabrication platforms. This publication focuses on and presents the capabilities of polymer-based iDEP devices for the concentration and removal of water-borne bacteria, spores and inert particles using our micro-iDEP device. The dielectrophoretic behavior exhibited by the different particles of interest (both biological and inert) was observed to be a function of both the applied electric field and the characteristics of the particle, such as size, shape, and conductivity. The performance of the polymeric iDEP device was characterized in terms of concentration factor and selectivity. The results obtained illustrate the potential of polymer-based iDEP devices to act as a concentrator for a front-end device with significant applications for analysis of bacteria, spores, and viruses. The polymeric devices exhibit the same iDEP behavior and efficacy in the field of use as their glass counterparts, but with the added benefit of being easily mass fabricated and developed in a variety of multi-scale formats that will allow for the realization of a truly high-throughput device.

**EXPERIMENTAL PROTOCOLS**

**Device Fabrication**

The microfluidic chips were fabricated from silicon wafers, (100 mm diameter, 1.1 mm thick) using standard Bosch etching processing. The microfluidic chip contains eight independent microchannels that are etched to 80 µm deep. The insulating posts traverse the entire depth of the microchannel. The etched glass substrates were then sputter coated with an electroplating base material, in this case 500 Å of titanium (adhesion promotion) and 1500 Å of copper. The silicon substrates were then placed into a Digital Matrix commercial electroplating machine. The bath chemistry utilized was a standard nickel sulfamate with controlled pH to minimize stresses. Electroplating occurred at 37º C for a total of 40 amp-hours and produced nickel films with thicknesses typically on the order of 1mm. The nickel was then planarized and machined to the dimensions for use in our custom in-house fabrication facilities and the glass and metal seed layers dissolved. The nickel stamp was then thoroughly categorized through metrology, visual inspection, and electron microscopy.

Injection molding was carried out utilizing a 60-ton Nissei® (Nissei® America, Los Angeles, CA) TH-60 vertical injection molding machine. Pellets of Z1060R resin were dried at 40º C for at least 24 hours before use. The resin was then fed to the machine through a gravity-assisted hopper connected externally to the injection molding barrel. Injection molding operational conditions were empirically determined by the operators based on resin supplier recommendations utilized as a starting point for molding. Cross-polarized optical interrogation of the replicated substrates was employed to determine the presence of, and minimize residual stresses in, the injection molded parts. 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. These 1 mm vias were drilled in the corresponding top polymer substrate that, when aligned and bonded with the microfluidic channels, would provide a watertight seal and fluidic connections to and from the chip. The arrays of insulating posts have circular posts with 200-µm diameters spaced 250 µm center-to-center. The upper and lower discs were then thermally bonded using a Carver (Carver, Inc., Wabash, IN) press. Bonding conditions were held constant at the following: the press was heated to 190º F with a constant applied load of 750 psi and a corresponding cycle time at temperature of 60 minutes. The bonded assembly was then cooled to 75º F under constant load and then removed from the press. All bonded assemblies were checked for flow and channel blockage before use.

**Experimental Apparatus**
Microfluidic channels were arranged on bonded discs of Zeonor® 1060R resin (Zeon Chemicals, Tokyo, Japan), a cyclic copolymer polyolefin. The bonded chips are then reversibly sealed to a vacuum chuck at the base of a custom PDMS manifold. The manifold is ported with 16 holes spanning its thickness that coincide with the inlet and outlet vias of each channel. Each opening can accept a slip tip syringe, and forms a watertight seal with both the syringe and the drilled channel via. The channels were primed by gently forcing background solution through the channel. Bubbles were removed with suction as necessary. Three milliliter luer lock syringes with their plungers removed were loaded with approximately 1.5 ml of the desired particle suspension and inserted at the upstream and downstream ports of the manifold. The particles were then gently forced into the channel with a plunger until the expected concentration of particles could be visualized. The 0.508-mm-diameter platinum-wire electrodes (Omega Engineering Inc., Stanford, CT) were inserted directly into the syringes. The positive electrode always corresponds to the upstream direction, since Zeonor® 1060R produces a stable electroosmotic flow from the positive to negative electrode at pH ≈ 8. A programmable high voltage sequencer, Labsmith HVS 448 (Livermore, CA) was used to apply voltage sequences up to 1500 V. A manually controlled power supply, Bertran ARB 30 (Valhalla, NY) was used for higher voltages. The apparatus was visualized with an inverted epifluorescence microscope, model IX-70 (Olympus, Napa, CA) equipped with a fluorescence burner to serve as the illumination source. Different sets of fluorescence filters are employed: Chroma 51006, Chroma 51004 (Chroma Technologies Corp, Brattleboro, VT) and Olympus 41012 (Olympus, Napa, CA) to allow for direct visualization of the particles of interest.

Determining Concentration Factor: Methodology and Equations

After the bead suspensions were loaded, the liquid level in the syringes was balanced to ensure quiescent flow. Data required to calculate the baseline concentration of tracers was then gathered by recording the flow of tracer suspension through an unobstructed region of channel for 30 to 60 seconds with the Optronics camera. These baseline measurements were taken with an applied DC voltage of 19.6V/mm, which is sufficient to produce a steady EOF, but well below the observed iDEP trapping threshold of 100V/mm. After the baseline data was acquired, DC voltage was increased to the specified trapping voltage. During this period, iDEP trapping around the posts could be clearly observed. Once the trapping voltage had been applied for the specified time, the voltage was discontinued, and trapped particles were allowed to flow to the front of the posts under the backpressure generated by electroosmotic flow during the trapping run. This release plug was filmed for later counting.

The maximum concentration of polystyrene beads was determined by a manual count of the frame of the release plug video in which the plug is most dense. The concentration factor, CF, represents the ratio of the maximum observed concentration of beads in the plug to the average concentration observed in the baseline video. This was calculated with the formula:

$$CF = \frac{1}{L} \frac{N_p f}{N_b \tau}$$

where $L$ is the length of the plug as a fraction of the total frame length (set equal to one in this paper), $N_p$ and $N_b$ are the maximum count of beads in the release plug and the bead count for the entire baseline video segment, respectively. The parameter $\tau$ is the period in frames required for a flowing particle in the baseline video to cross the frame length, and $f$ is the total duration of the baseline video in frames.

RESULTS AND DISCUSSION

To evaluate the post geometry and replication fidelity, scanning electron microscopy (SEM) images were taken of the polymer chips before bonding. Figure 1 shows a representative image of the as-replicated post array.
The iDEP response of a suspension of *B. thuringiensis* spores is presented in Figure 3. As the applied field is increased 72 V/mm we observe a transition to dielectrophoretic trapping of the spores.

In addition to the ability to selectively trap and isolate a biological particle of interest, whether it is a spore, vegetative cell, and/or protein, the iDEP device must also be capable of delivering a concentrated sample to another microfluidic device component capable of detecting it. This may be in the form of a lysis unit followed by a LIF-based detection method on-chip, impedance measurements conducted while the particles are trapped, and/or some off-chip analytical device. One of the critical metrics for such a device is the effective concentration factor it can produce during operation. We have developed a protocol (see above) to aid in the determination of concentration factors and implemented it to evaluate the polymer-based iDEP chips. These results were collected for the concentration of 2 µm fluorescently labeled polystyrene beads. We operated the device at set applied fields for set time intervals and evaluated the resultant sample plug produced by the device when the field is removed. The results obtained are presented in graphical format in Figure 4.
The impact of increased applied field voltage and increased time follows the expected monotonic linear trend. As the device is operated for longer period of times and at higher voltages, the concentration factor of the iDEP devices should increase until the device is saturated and reaches a set limit. At 500V, the concentration factor increase from a value of ~14 after 1 minute of operation to ~50 after 4 minutes of operation. The maximum concentration factor of ~236 is realized after operating the device at 1000 V for 3 minutes. These numbers indicate the potential for these iDEP devices to release a selective and concentrated sample to further on- and/or off-chip sample processing devices.

CONCLUSIONS

We have demonstrated that polymeric iDEP devices are effective for selectively manipulating bacteria and inert particles. A nonuniform electric field was generated by applying a DC electric field across a microchannel filled with polymeric insulating posts. Regions with high field intensity were generated in the narrow spaces between the insulating posts. The performance and efficiency of the polymer-based iDEP devices appears to be comparable to that obtained in glass-based iDEP chips. We have demonstrated the selective trapping of spores from an inert background. We also carried out an evaluation of the polymer-based iDEP devices in terms of obtaining concentration factors under various applied field strengths at set time intervals. This experimentation showed a linear relationship between the applied-field duration and concentration factor at various fields suggesting that the traps had not fully saturated in these experiments. These results illustrate the great potential of polymer-based iDEP devices for concentrating and sorting bacteria and particles. An iDEP device can be employed as a front-end device to enhance bacterial analysis and detection. This work is being extrapolated to develop a high-throughput device capable for processing liters of sample per hour for real-time monitoring of pathogens in water.

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