The Development of Polymeric Devices as Dielectrophoretic Separators and Concentrators

Blake A. Simmons, Gregory J. McGraw, Rafael V. Davalos, Gregory J. Fiechtner, Yolanda Fintschenko, and Eric B. Cummings

Abstract

Efficient and reliable particle separators and concentrators are needed to support a wide range of analytical functions including pathogen detection, sample preparation, high-throughput particle sorting, and biomedical diagnostics. The advent of lab-on-a-chip devices based on the phenomenon of dielectrophoresis offers advantages that can meet several of the challenges associated with cell sorting and detection. The majority of the devices presented in the scientific literature have used glass-based devices for these applications, but there has been recent activity that indicates that polymer-based devices can operate as effectively as their glass progenitors. Processing and operational advantages motivate the transition from glass and silicon to polymer microdevices: mechanical robustness, economy of scale, ease of thermoforming and mass manufacturing, and the availability of numerous innate chemical polymer compositions for tailoring performance. We present here a summary of the developments toward, and results obtained from, these polymeric dielectrophoretic devices in the selective trapping, concentration, and gated release of a range of biological organisms and particles.

Keywords: *biomedical, dielectrophoresis, fluidics, microscale, polymer.*

Introduction

Efficient cellular and subcellular particle separation and sorting is an important field of science and technology development for numerous lab-on-a-chip and biomedical applications. For example, recent national and global events have drawn attention to the need for rapid and accurate monitoring of water distribution networks for safety and quality. To detect pathogens at low concentrations in raw water or other liquid samples, it is vital to develop selective techniques that collect, concentrate, and deliver such particles for further testing and identification. Similar operational requirements are present in the biomedical and analytical chemistry fields, where the delivery of a concentrated and purified sample is of paramount importance to eliminate the contribution of background interference and thereby minimize errors.

Dielectrophoresis (DEP) is the motion of particles driven by conduction effects in a nonuniform electric field.[1] It has been shown that DEP can be used to transport suspended particles using either oscillating (ac) or steady (dc) electric fields.[2] This phenomenon is attractive for differentiating biological particles (e.g., cells, spores, viruses, DNA), because it can collect specific types of particles rapidly and reversibly based on their size, shape, and intrinsic properties such as conductivity and polarizability.

Many device architectures and configurations have been developed to sort a wide range of biological particles by DEP. Early DEP experiments, carried out by Pohl et al., utilized pin–plate and pin–pin electrodes to differentiate live and dead yeast cells and collected the yeast cells at the surface of the electrode.[3,4] Currently, the typical dielectrophoretic device employs an array of thin-film interdigitated metallic (typically gold or platinum) electrodes deposited on a glass or silicon substrate located within a flow channel to generate a nonuniform electric field that interacts with particles near the surface of the electrode array.[5] The nonuniform electric fields are typically generated by a single-phase ac source, but multiple-phase sources can trap and sequentially transport particles in a technique called traveling-wave dielectrophoresis.[6] These electrode-based DEP devices have been shown to be effective for separating and concentrating cells,[7] proteins,[8] DNA,[9] and viruses,[10] and for manipulating carbon nanotubes.[11] There are devices that combine traveling-wave dielectrophoresis with electro-rotation for the manipulation of cancer cells[12] and to separate autotrophic from heterotrophic algae.[13]

Another technique is focused on insulator-based dielectrophoresis (iDEP), which uses insulating obstacles—instead of electrodes—within the device to produce spatial nonuniformities in an electric field that is applied through the suspending liquid. The elimination of metallic electrodes in contact with liquids allows for the use of ac fields at frequencies below 1 kHz and also dc current, both of which remove the threat of gas evolution through electrochemistry. The iDEP technique was first presented by Masuda et al.[14] and subsequently developed further by Lee et al.[15] as a means of separating particles. It has also been previously demonstrated that iDEP using glass elements can separate DNA molecules, *Escherichia coli* cells, and red blood cells using insulating structures and ac electric fields.[16] Zhou et al.[17] and Suehiro et al.[18] used a channel filled with insulating glass beads and applied ac electric fields for separating and concentrating yeast cells in water. Recent work involving glass nanopipettes demonstrated iDEP to be effective in the trapping of proteins.[19] Our group has reported the trapping and separation of latex particles,[20] the differentiation of live and dead bacteria,[21] the separation of species of different viable

prokaryotic cells,[22] and the trapping and concentration of viruses.[23] In these examples, a dc electric field is applied across a channel that contains an array of 200-μm-diameter insulating posts with 50-μm spacings inside a borosilicate glass-based microfluidic device. In addition to posts, insulating ridges can also be effective particle separators.[24]

While glass-based iDEP microdevices perform well, sample throughput is generally low because of the geometrical limitations present in isotropically etched devices.[25] Typical sample flow rates for glass-based devices are in the range of 10 μl per hour. In contrast, polymer-based iDEP devices can be easily scaled to handle much larger sample volumes using commercially available and inexpensive techniques that produce much deeper features and larger channel volumes.[26] Other polymer-based microfluidic devices have been developed and utilized for liquid/liquid and particle separation[27] and other lab-on-a-chip applications including capillary electrophoresis, miniaturized polymerase chain reaction (PCR) chambers, nucleic acid analysis, protein analysis, and fluidic mixers.[28,29] The main appeal of these polymeric devices is that they are relatively inexpensive and can be produced by standard mass-fabrication techniques such as injection molding and hot embossing instead of the costly per wafer technique of microlithography.[30] Our group has reported that polymeric iDEP elements can be made from cyclic olefin copolymers such as Zeonor®.[31] Cyclic olefin copolymers (COCs) have received a significant amount of recent interest in microfluidics owing to their low auto-fluorescence and high chemical resistance to a wide range of polar solvents; such properties support the use of this class of polymer for iDEP devices.[32] The following is a summary of the capabilities of polymer-based DEP and iDEP devices to separate and concentrate water-borne bacteria, spores, and particles.

Theory of Dielectrophoresis

As defined earlier, dielectrophoresis is the motion of a particle caused by the presence of a nonuniform electric field. The DEP force acting on a spherical particle in the presence of an applied dc electric field can be described by the following equation:[33,34]

$$F_{DEP} = 2\pi\varepsilon_0\varepsilon_m r^3 \mathrm{Re}\{f(\tilde{\sigma}_p, \tilde{\sigma}_m)\}\nabla E^2, \quad (1)$$

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, Re specifies the real part of the force term that follows, ∇E^2 is the local gradient of the electric field, and $f(\tilde{\sigma}_p,\tilde{\sigma}_m)$ is the Clausius–Mossotti factor:

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

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 the dielectric constant by $\tilde{\sigma} = \sigma + i\omega\varepsilon$, where σ is the real conductivity, $i = \sqrt{-1}$, and ω is the radian frequency of the applied electric field. For frequencies below 100 kHz, the imaginary part of the complex conductivity can generally be neglected. The dielectrophoretic force acting on a particle can therefore be positive or negative in magnitude. If the conductivity of the particle is greater than the conductivity of the medium, then the particle will exhibit positive DEP behavior and move toward regions of high electric field. If, as is typical for biological particles, the particle is less conductive than the suspending medium, the particle exhibits negative DEP and moves away from regions of high electric field. A comparison of the electric fields generated by these different types of architectures is presented in Figure 1. The arrays of electrodes (Figure 1a) produce electric-field gradients that decrease exponentially as a function of the height above the electrode. The arrays of insulating posts (Figure 1b) produce electric-field gradients that are present at any height relative to the channel surface with a maximum electric-field intensity felt at the areas between the posts.

Zeta Potential of Polymers: Comparison to Glass

Electro-osmosis is often employed to pump fluids through microchannels to prevent hydrodynamic dispersion and obviate the need for mechanical pumps.[35] The net charge density that spontaneously forms on glass[35] or polymer[36] surfaces in contact with water produces an electrical double layer. An electric field applied tangentially along the surface produces a net body force on the liquid within the Debye layer where there is a mobile net charge imbalance as a result of electrostatic attraction and repulsion from the charged surface. This electrostatic body force produces electro-osmosis: fluid motion within the Debye layer that viscously drags the bulk fluid. In straight channels having uniform surface charge and containing uniform liquid, electro-osmotic pumping produces no shear in the flow outside the double layer, resulting in flat velocity profiles in the bulk of the flow. Charged particles immersed in liquid generally also move electrophoretically under an applied field as a result of electrostatic forces on their screened net charge. The combination of electro-osmosis and electrophoresis, called electrokinesis, is the resultant motion of fluid and suspended particles in a stationary channel having only weak electrostatic forces.

Dielectrophoretic trapping occurs when dielectrophoresis overcomes the electrokinetic particle forces. Since the magnitude of electro-osmosis, and consequently electrokinesis, depends on the specific surface and liquid composition, glass and polymer devices will generally exhibit different trapping thresholds for a given particle type. The ratio of the electro-

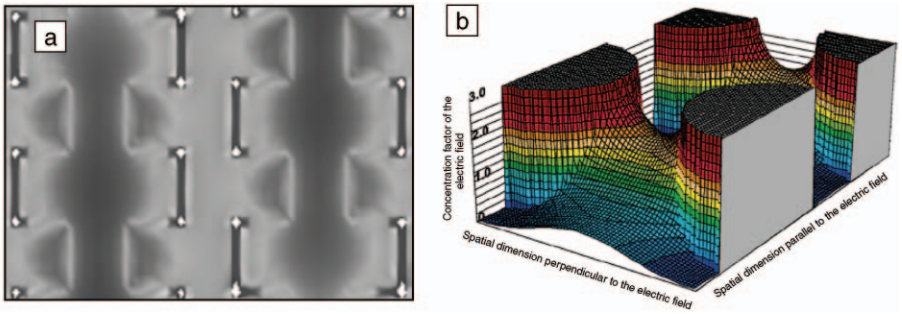

Figure 1. Images depicting (a) the calculated electric fields generated by an interdigitated array of electrodes with a characteristic dimension of 50 μm, where the electric-field gradient is maximum at the electrode edge (graph reproduced from Reference 43), and (b) the variation of the calculated electric-field intensity created by an array of insulating posts, where the electric-field intensity is maximum (by a factor of ~3) at the smallest distance between the posts (graph reproduced from Reference 21). The potential barrier between the posts traps the particles. The circular posts are 130 μm in diameter, 200 μm center-to-center, and at 0° with respect to the applied field.

osmotic flow velocity to the applied field is commonly related using an empirical parameter called the zeta potential, which is a measure of the effective surface charge density. There are numerous reports in the literature that present the zeta potential of common commercial thermoplastic polymers such as polycarbonate,[37] poly(methyl methacrylate),[38] polyethylene,[39] and polystyrene.[40] As mentioned previously, there has been recent interest in COCs, such as those available from Ticona (Topas) and Zeon Chemicals (Zeonor).

To illustrate the differences observed between glass and polymer surfaces, the zeta potential of the COC Zeonor is presented as a typical example in Figure 2.[31] It is observed that this polymer possesses a zeta potential whose magnitude exhibits the classical dependence on the pH of the solution and is relatively invariant with the type of buffer utilized.[31] The zeta potential of Zeonor has a value of zero located at pH = 4.8. In comparison, the zeta potential for glass reaches zero at pH = 2.7.[35] This implies that the direction of the electro-osmotic flow will be in opposite directions between the glass and polymer devices over a pH range constrained between these two values. The zeta potential of both the glass and polymer surface is negative in the pH range of interest for most biological samples (defined here as between 5 and 8). In a 10-mM phosphate buffer solution with pH = 7, for example, the zeta potential of glass is –52 mV, whereas the value for Zeonor is –38 mV.[31] This implies that for two channels of equal dimensions, one made from glass and the other from COCs, for a given electric field and using identical fluid conditions, particles of the same size and composition will be observed to move with a higher velocity, but in the same direction, in the glass channel as compared with the COC channel.

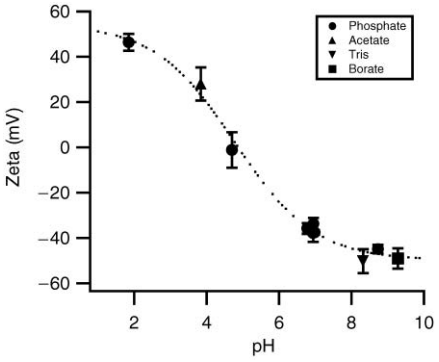

Figure 2. Graph of the measured zeta potential of Zeonor® 1060 as a function of buffer type (i.e., phosphate, citrate, tris, etc.) and pH. Graph reproduced from Reference 31.

Electrode-Based DEP

One of the earliest examples of a polymeric DEP device was developed by Pethig et al., who created arrays of electrodes through excimer laser ablation of thin gold on chrome metal films separated by polyimide layers to create traveling wave and electro-rotation DEP devices.[41] DEP devices manufactured with printed circuit board technology have been very effective in the separation of eukaryotic cells.[42] Other examples are based on arrays of electrodes that have been deposited on glass or silicon surfaces. These active DEP electrode layers can then be assembled into a device using layers of polymer films that form the channels and lids of the device. This approach can be employed to produce devices that can be disassembled after use if reversible seals are used. This reversible device assembly allows for periodic maintenance of the active elements and subsequently extends the overall operational lifetime.

One example of this approach consists of polycarbonate interlayers bonded by a pressure-sensitive adhesive film to a polyimide flip-chip active DEP layer containing platinum interdigitated electrodes.[43] This device was used to separate red blood cells, *Bacilus cereus*, *Escherichia coli*, and *Listeria monocytogenes* cells within distinct regions of the electrodes (Figure 3) that correlate to their respective DEP mobilities. The red blood cells are trapped in the recessed areas between the electrodes (Figures 3a–3d), *B. cereus* cells are trapped at the electrode edges and surfaces (Figure 3a), and *E. coli* cells and *L. monocytogenes* are trapped at the center of the electrodes (Figures 3b and 3c).[43] Another example of this hybrid approach is found in a traveling-wave DEP device that used the photoresist SU-8 or polyimide to form the channels on top of the deposited Ti/Au electrode surfaces.[44] This layered device was then bonded to a poly(dimethoxysilane) (PDMS) elastomer film that served as a lid, and the device

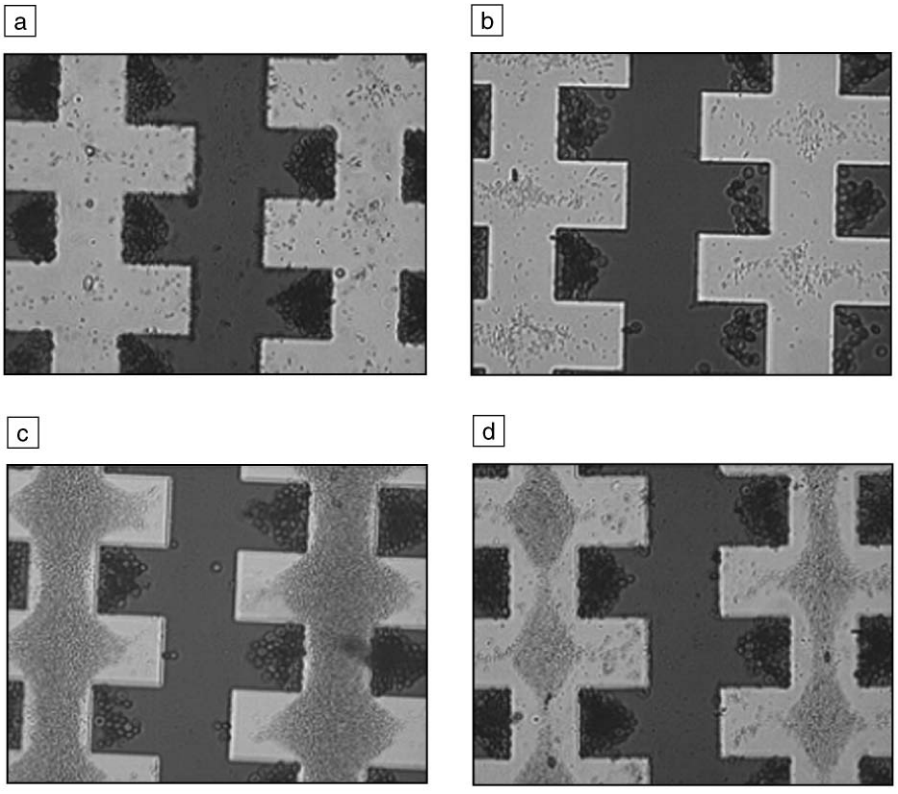

Figure 3. Images showing the separation of bacteria from blood with an electrode-based dielectrophoresis polymer laminate device operating at 10 kHz, 10 Vpp (volts peak-to-peak), 180 μS/cm for (a) B. cereus *(on electrode edge) and red blood cells (pyramids in dark region), (b)* E. coli *(on electrode surface) and red blood cells (pyramids), (c)* L. monocytogenes *(on electrode surface) and red blood cells (pyramids), and (d) all four types together. Image reproduced from Reference 43. The electrode and spacing dimension is 50 μm.*

was proven to be effective in the sorting of beads.[44] A similar hybrid material approach has been demonstrated using DEP-activated cell sorting in the differentiation and concentration of rare *E. coli* that possess a specific surface marker from other normal cells of the same species.[45]

Other hybrid glass–polymer systems deposit electrodes onto a glass or a polymeric membrane directly for use in DEP devices that are coupled to the phenomenon of field flow fractionation (FFF).[46,47] The separation channel is most often defined with a polymeric spacer (Figure 4).[48] FFF is a separation technique that employs the relative position of a particle in a fluid flow profile to achieve separation.[49] These DEP–FFF devices couple hydrodynamic focusing with DEP to create a three-dimensional flow-separation field capable of sorting particles such as human breast cancer cells from normal erythrocytes,[46] separating leukocytes,[48] and concentrating nanoparticles.[47] There has been some very recent work that has created arrays of three-dimensional carbon electrodes through the carbonizing of SU-8.[50] This approach is very intriguing, as it may combine the volumetric throughput of iDEP with the subtle particle manipulation obtained with electrode-based DEP.

Insulator-Based DEP

Arrays of three-dimensional insulating COC structures, described earlier, have been created through standard photolithography and replication processes including hot embossing and injection molding. These devices have been used in the separation of various biological particles and latex spheres. The channels are typically 10.2 mm long and 1 mm wide. The posts are typically 75 μm in height and 200 μm in diameter, with 250-μm center-to-center distances. The behavior of fluorescently labeled latex spheres was quantified to compare the polymeric device performance to that of glass microfluidic devices reported previously. As indicated by the COC zeta potential measurement results,[31] stable electrokinesis of particles toward the negative electrode is observed at low field strengths. As the field strength is increased, dielectrophoresis increases as the square of the field, according to Equation 1. Electrokinesis, which increases only linearly with the field, is overcome, and the 1-μm polystyrene spheres are observed to become trapped between the posts, as shown in the image in Figure 5a.[31] The value of the applied electric field when the dielectrophoretic force dominates the electrokinetic force is, therefore, termed the trapping threshold. This trapping of particles is reversible, and the spheres are released from the traps when the electric field is decreased. In addition to being able to trap a certain size of sphere, differential trapping, based on size, has also been observed.[32]

Trapping of *Bacillus thuringiensis* spores was also demonstrated (Figure 5b). The spores are trapped between the posts with the applied-field strength set to 80 V/mm. The spores differ from vegetative cells (de-

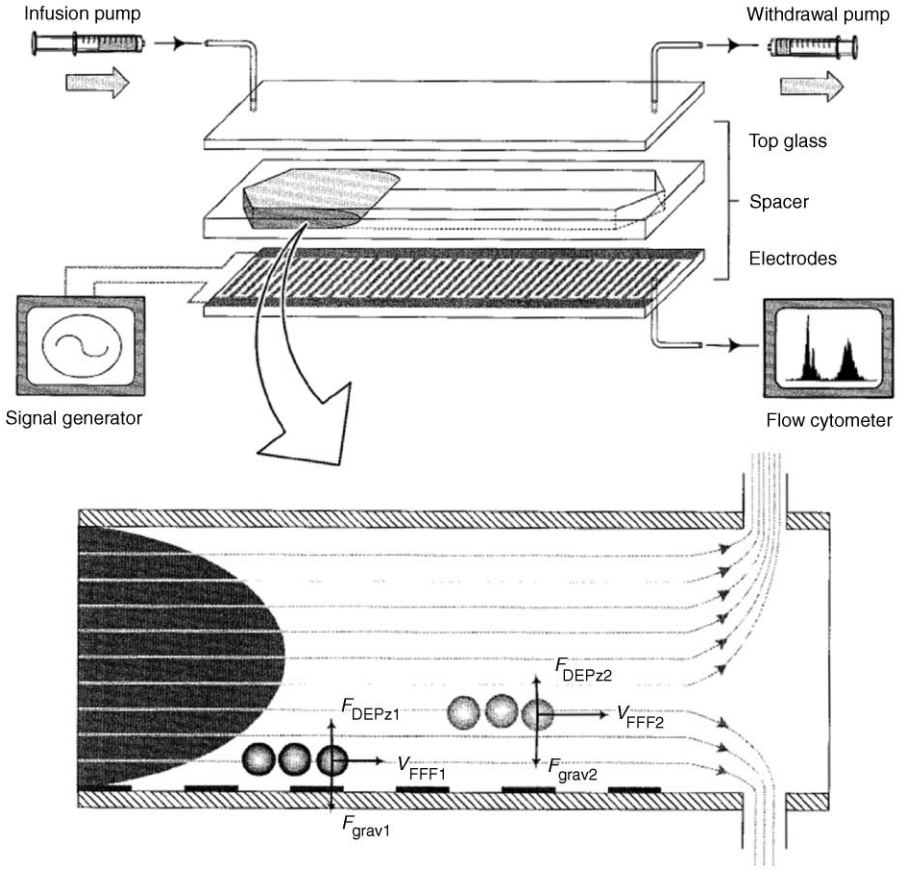

Figure 4. Schematic depictions of (top) typical device configuration and elements and (bottom) mechanism of dielectrophoresis (DEP) and field flow fractionation (FFF) separation fields and device performance utilized in the separation of particles. F_{DEP} is the dielectrophoretic force on the particle, F_{grav} is the gravitational force on the particle, and V_{FFF} is the velocity of the particle as a function of field flow fractionation, which is dependent on the particle location in a hydrodynamic flow field. Image reproduced from Reference 48.

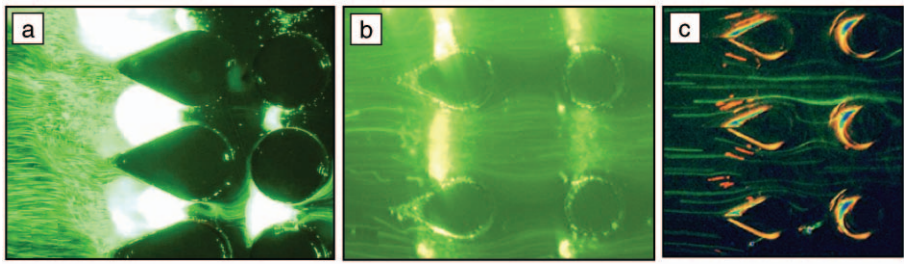

Figure 5. Images obtained from digital movies demonstrating polymer-based insulator-based dielectrophoresis (iDEP) trapping within an array of 200-μm-diameter posts of (a) 1-μm fluorescent polystyrene beads at an applied-field strength of 80 V/mm (adapted from Reference 31), (b) Bacillus thuringiensis *spores at an applied-field strength of 100 V/mm (adapted from Reference 32), and (c) separation of* B. thuringiensis *cells (red) from* B. thuringiensis *spores (green) at 74 V/mm (adapted from Reference 32). The net particle motion in all images is from left to right.*

fined here as those cells that are capable of actively growing) in that they are smaller and possess a different membrane compositional makeup. When the spores are mixed with vegetative cells, the vegetative cells are separated (Figure 5c) with an applied-field strength of 74 V/mm.[32] In the case of *Bacillus subtilis* vegetative cells, the trapping threshold in the glass device is 48.1 ± 2.3 V/mm,[22] whereas for the Zeonor devices, it is 40.8 ± 1.4 V/mm.[25] The higher trapping threshold in glass is expected, as the zeta potential of the glass is higher than that of Zeonor for identical fluid conditions (pH = 8, 10 μS/cm) and produces a higher electrokinetic force on the particle that must be overcome before trapping occurs. These results demonstrate that polymer-based iDEP devices can be used to trap and differentiate particles based on size and type in a manner equivalent to that observed in the glass iDEP devices. The major advantages to these polymeric iDEP devices are that they can be easily scaled to almost any dimension and can therefore effectively handle a large range of sample volume requirements.

Conclusions

Particle sorting and separation is a vital aspect in the performance of numerous lab-on-a-chip applications and will remain so for the foreseeable future. We have presented a review that indicates that polymer-based DEP and iDEP devices are effective for the selective trapping and concentration of a wide range of particles. The nonuniform electric field required to generate DEP can be generated by a variety of intrinsic structures and design methodologies. These include arrays of metallic electrodes contained within a polymer microfluidic device (DEP), or the application of a field across a polymeric microchannel containing posts, ridges, or other insulating obstacles (iDEP). Regions of high field intensity generated between these insulating posts repelled insulating particles dielectrophoretically by various degrees, producing selective and field-tunable particle traps. The performance of the polymer-based DEP microdevices at removing and concentrating cellular and subcellular particles selectively is similar to that obtained in the glass-based DEP microdevices. These results illustrate the great potential of polymer-based DEP and iDEP devices for use in the concentration and sorting of a wide variety of cells, particles, proteins, and DNA. We envision a role for these polymeric devices as cost-effective and disposable tools used in multiple front-end sample preparation applications that will enhance current detection techniques.

Acknowledgments

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