Control of Flow Direction in Microfluidic Devices with Polyelectrolyte Multilayers

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Electroosmotic flow (EOF) is commonly utilized in microfluidics. Because the direction of the EOF can be determined by the substrate surface charge, control of the surface chemical state offers the potential, in addition to voltage control, to direct the flow in microfluidic devices. We report the use of polyelectrolyte multilayers (PEMs) to alter the surface charge and control the direction of flow in polystyrene and acrylic microfluidic devices. Relatively complex flow patterns with simple arrangements of applied voltages are realized by derivatization of different arms of a single device with oppositely charged polyelectrolytes. In addition, flow in opposite directions in the same channel is possible. A positively derivatized plastic substrate with a negatively charged lid was used to achieve top−bottom opposite flows. Derivatization of the two sides of a plastic microchannel with oppositely charged polyelectrolytes was used to achieve side-by-side opposite flows. The flow is characterized using fluorescence imaging and particle velocimetry.

Plastic microfluidic devices can be easily and inexpensively fabricated; however, the chemical functionalities of the plastic surface differ from those of glass and vary from polymer to polymer. Electrically driven flow is commonly used in microfluidics, and the direction and rate of the electroosmotic flow (EOF) are determined by the substrate surface charge. Previous studies[1,2] have shown that various polymer substrates support very different EOF mobilities in microchannel devices. In addition, the distribution of surface charge in imprinted plastic microchannels has been shown to be nonuniform.[3]

Modification of the chemical functionalities on the substrate surface can control surface charge and uniformity and offers methodology, in addition to voltage control, for controlling flow rate and direction in microfluidic devices. Numerous surface modification techniques have been developed for electrophoresis applications in silica and glass substrates. Glass and quartz microchannels have been derivatized previously using covalent,[4,5] noncovalent,[6] and dynamic[7] coatings.

In contrast, little research has focused on surface derivatization of plastics for microfluidic applications. Plastic microdevices have been derivatized using dynamic coating;[8] however, dynamic coatings lack long-term stability and require addition of the coating material to the running buffer.

We have recently shown that the deposition of polyelectroyte multilayers[9] (PEMs) is a simple, reproducible method for derivatization of plastic microfluidic devices.[10,11] The multilayer is created by exposing the microchannel to alternating solutions of positively and negatively charged polyelectrolytes. Although the layers are adsorbed onto the substrate or previous layer by noncovalent interactions, the resulting multilayers have multiple electrostatic bonds and are stable and uniform. PEMs were used previously to derivatize polystyrene (PS) and poly(ethylene terephthalate) glycol (PETG) microchannels,[10,11] which resulted in comparable, reproducible EOF mobilities for the two substrates.

Here we describe the use of PEMs to control the flow direction in polystyrene and acrylic microfluidic devices in order to achieve complex flow patterning and flow in opposite directions within a single channel. We note that a similar method of flow patterning was recently reported by Stroock, et al.;[12] however, the details of that experiment, such as the substrate material and the derivatization methods, have not yet been published.

EXPERIMENTAL SECTION

Device Preparation: Sheets of polystyrene, PS, (Corning Costar Corp., Cambridge, MA)[13] were cut into 7.6 cm × 7.6 cm squares and rinsed with ethanol prior to use. UV-transparent polymethyl methacrylate, (PMMA) [Acrylite OP-4][13] was cut into 2.5 cm × 3.5 cm squares and rinsed with ethanol prior to use. Films of poly(dimethylsiloxane) (PDMS) were made according

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(13) Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsment by the National Institute of Standards and Technology, nor does it imply that the materials or equipment are necessarily the best available for the purpose.

to product information from a Sylgard 184 silicone elastomer kit[13] (Dow Corning, Midland, MI).

A silicon template, fabricated by photolithography,[14] was used to imprint channels in the plastic substrates by room temperature imprinting, as previously described,[11,15] or by hot imprinting. The channel design on the silicon template was either a "T" consisting of three arms, 1 cm in length, or a cross with four arms, each 2 cm in length. The PS was imprinted at room temperature with 6.1×10^6 Pa (890 psi) of pressure applied in a hydraulic press for 3 min. The PS channels were then covered with a PDMS lid, in which holes were made to serve as fluid reservoirs. PMMA devices were made with a hard plastic lid, rather than a PDMS lid, which was sealed using temperatures near the plastic softening temperature. With room-temperature imprinted channels, sealing at elevated temperature causes the plastic to flow and refill the channel;[16] therefore, hot imprinting was used in the fabrication of single-material (PMMA) devices. Channels were created in the PMMA by placing the plastic substrate on the silicon template between aluminum plates at 110 °C and 5.1×10^6 Pa (740 psi) for 1 h. The substrate plastic was then removed from the template and a second piece of the acrylic was placed on top. The two plastic pieces were clamped between glass slides and placed in a circulating air oven at 103 °C for 12 min. Typical channels had a trapezoidal cross section defined by the silicon template and were 30 μm deep, 20 μm wide at the bottom, and 75 μm wide at the top.

PEM Deposition: A 60 mM solution of poly(styrene sulfonate), sodium salt, [PSS] (Scientific Polymer Products, Ontario, NY, MW = 500 000)[13] solution was prepared using 18 MΩ·cm deionized water with 0.5 M NaCl adjusted to pH 9 with sodium hydroxide (NaOH). A 20 mM solution of poly(allylamine hydrochloride), [PAH] (Aldrich, Milwakee, WI, MW = 70 000)[13] was prepared using the same water, salinity, and pH. Polymer concentrations are based on the repeat unit.

The PEM deposition method used to deposit alternating layers of PAH and PSS was described previously.[11,17] Briefly, the channels were first treated with 1 M NaOH at 55 °C for 15 min. The channels were then rinsed with 18 MΩ·cm deionized water and dried with nitrogen. Alternating layers of PAH and PSS were then applied, with water rinses between each solution application, until the desired number of layers was deposited.

Modification of PS Room Temperature-Imprinted Devices: For the room temperature-imprinted plastics, only the substrate plastic, PS, was treated with the PEMs. The PDMS lid was not treated. Treatments were performed by pipetting solutions onto the plastic substrates, completely covering the channels. After PEM treatment, the PDMS lid sealed the channel.

Some room temperature-imprinted cross-designs were treated to have differing charges on the various arms. This was accomplished by treating the entire device with a desired number of layers, as described above, then pipetting the oppositely charged polymer solution onto the selected arms. The solution movement in the channels was observed with an optical microscope to verify that the solution only entered the desired channels.
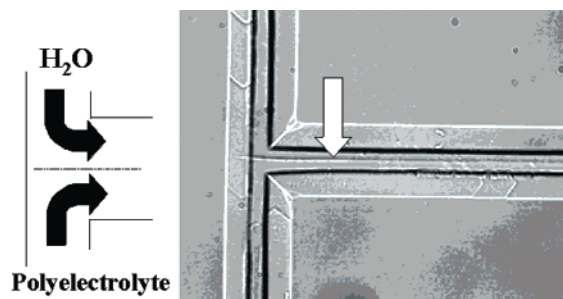

Figure 1. Laminar flow in a "T" chip used to selectively deposit the final polyelectrolyte layer in a single half of the channel. The contrast bisecting the channel-long axis, indicated by the white arrow, is due to the difference in the refractive indices of the water and the polyelectrolyte solution.

Modification of PMMA Hot Imprinted Devices: For the hot-imprinted PMMA channels, the entire device was assembled prior to PEM treatment and pressure-driven flow (syringe pump, Harvard,[13] Holliston, MA) was used to drive the solutions through the channels. The PMMA "T" devices were treated in the following manner: (1) the entire device was treated with either one or two layers, as described above; and (2) laminar flow patterning[18] was used to fill one-half of the channel with the oppositely charged polyelectrolyte solution and the other half with water, as shown in Figure 1. The polyelectrolyte solution input syringe was removed from the device prior to the removal of the water input, which allowed water to completely fill the channel and prevented the oppositely charged polyelectrolyte solution from contacting the other side of the channel. The device was then rinsed thoroughly with water.

Electroosmotic Flow Measurements: The EOF was measured using a current monitoring method,[19] and the experimental details were published previously.[2] In the method, EOF is determined according to the equation, $v_{eof} = Lt^{-1}$, where L is the channel length and t is the time required for a second buffer of different concentration to fill the microchannel. The electroosmotic mobility is given by the ratio of the EOF rate to the applied field strength, E. The field strengths were typically 300−400 V/cm.

Fluorescence Measurements: Fluorescent polystyrene beads, 1 μm in diameter, (Fluoresbrite)[13] were purchased from Polysciences, Inc.[13] (Warrington, PA). Beads with a different surface charge, carboxylate-derivatized polystyrene beads (Fluorospheres),[13] were purchased from Molecular Probes[13] (Eugene, OR). Fluorescein bis-(5-carboxymethoxy-2-nitrobenzyl) ether, dipotassium salt (CMNB-caged fluorescein), was purchased from Molecular Probes.[13]

Caged-dye fluorescence imaging techniques were similar to those of Paul et al.[20] Briefly, the channel was filled with a buffer solution containing a small amount of CMNB-caged fluorescein dye. The dye did not fluoresce until after it was uncaged by exposure to UV light. The output of a pulsed nitrogen laser at 337 nm (Laser Science, Inc.,[13] Franklin, MA) was focused into a narrow band perpendicular to the channel and a single pulse (duration < 4 ns) was fired, which marked a portion of the fluid

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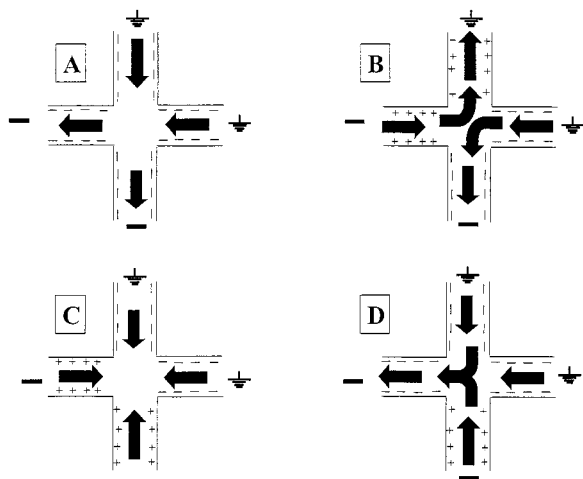
Figure 2. Four flow patterns achieved in cross-devices derivatized to have differing surfaces charges on the various arms. The applied voltages are the same in all cases.

in the channel with fluorescence. The flow in the channel was then determined by monitoring the motion of the marked portion of the fluid.

Fluorescence imaging of the fluorescent beads and of the uncaged dye was performed using a research fluorescence microscope equipped with a mercury arc lamp, appropriate filter sets, and a video camera (COHU,[13] San Diego, CA) for detection. Digital images were acquired using a frame grabber and imaging software (Scion, Inc.,[13] Frederick, MD).

RESULTS AND DISCUSSION

Flow Control in Multichannel Devices: Fluorescently labeled beads were used to image flow in the PEMs-derivatized PS microchannels with PDMS lids. The bulk flow in channels with a negative PSS top layer was from anode to cathode, while the bulk flow in channels with a positive PAH top layer was reversed and flowed from cathode to anode, as determined by particle flow. Cross-designs with four negative arms, three negative arms and one positive arm, or two positive arms and two negative arms were tested. Flow in the devices was controlled by either grounding or applying a constant voltage to each of the individual arms. The use of oppositely charged channels simplified the applied voltages needed for complicated flow patterns. For example, Figure 2 diagrams flow patterns achieved in four devices with differing charges on the various channels but with the same applied voltages. Flow patterns were determined by video imaging of fluorescent beads moving with the EOF in the channel.

Previously,[11] similar devices (PS/PDMS) were derivatized with PEMs consisting of different numbers of layers. The EOF mobility was determined, and the net EOF in channels with the negative PSS top layer was faster than, but in the opposite direction to, flow rates measured in channels with the positive PAH top layer. In channels derivatized with a PAH-terminated 13-layer PEM and a PSS-terminated 14-layer PEM, the EOF mobilities were -1.3×10^{-4} and 4.2×10^{-4} cm^2/V·s, respectively. In this study, mobilities measured in PAH-terminated or PSS-terminated arms of cross-devices were comparable to those measured previously in straight channels. Video imaging was used to determine that the slower net mobility in the PAH channels was due to flow in opposite
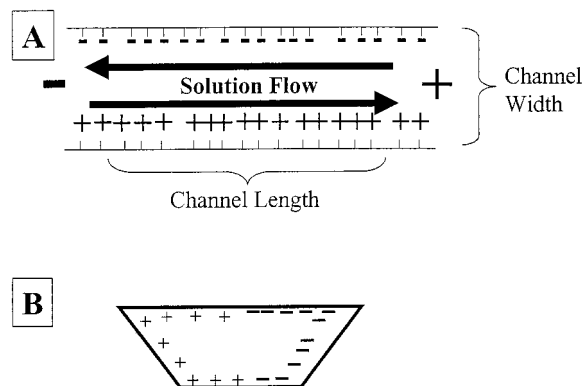

Figure 3. Schematic representations of a channel derivatized to have positive charges on one side of the channel and negative charges on the opposite side. A, top view; B, end-on view.

directions within the channel. No attempt was made to derivatize the channel lids, and, therefore, the lids retained the negative surface charge of the native PDMS. These negative charges on the channel lid induce flow in the opposite direction of the flow propagated by the positive PAH layer on the polystyrene channel walls and bottom. The beads stopped when the applied voltage was switched off, which indicated that flow was only electrically driven with no contribution from hydrodynamic flow.

Bi-Directional Flow in Single Microchannels: As described above, charge groups on the PDMS lids in the hybrid (PS/PDMS) channels contribute to EOF in the microchannels. Therefore, single-material acrylic devices were constructed and all channel surfaces (including the channel lids) were derivatized with PEMs. Such channels have similar EOF mobilities for both PAH- and PSS-derivatized channels. For example, in channels derivatized with a single PAH layer or a PSS-terminated 2-layer PEM, the EOF mobilities were -5.8 (±0.1) $\times$ 10^{-4} and 5.5 (±0.4) $\times$ 10^{-4} cm^2/V·s, respectively, as determined by the current monitoring method described above. Comparable EOF mobilities in PEM-coated acrylic channels were also measured using the caged dye method.[21]

Laminar flow patterning[18] in "T" devices was then used to derivatize acrylic channels so that they were positively charged on one side of the channel and negatively charged on the other, as shown in Figure 3. The flow in the derivatized channel was imaged using either a caged fluorescent dye or fluorescent beads.

The caged dye experiments were performed in channels derivatized with a two-layer PEM on one-half of the channel and a three-layer PEM on the other half of the channel. A PSS-terminated two-layer PEM was first deposited on all surfaces of the channel (including the channel lid), and the third layer (PAH) was then selectively deposited on one side of the channel.

To image flow, the channel was filled with the nonfluorescent caged dye in pH 7 buffer, 330 V/cm was applied to drive flow, and a plug of dye was uncaged with a pulse of focused UV light. The fluorescent plug motion was imaged in real time using a fluorescent microscope and video camera. Figure 4 shows time-step fluorescence images of the plug in the microchannel. The fluorescent plug separates due to the EOF in opposite directions in the two sides of the channel. Broadening of the dye plug is

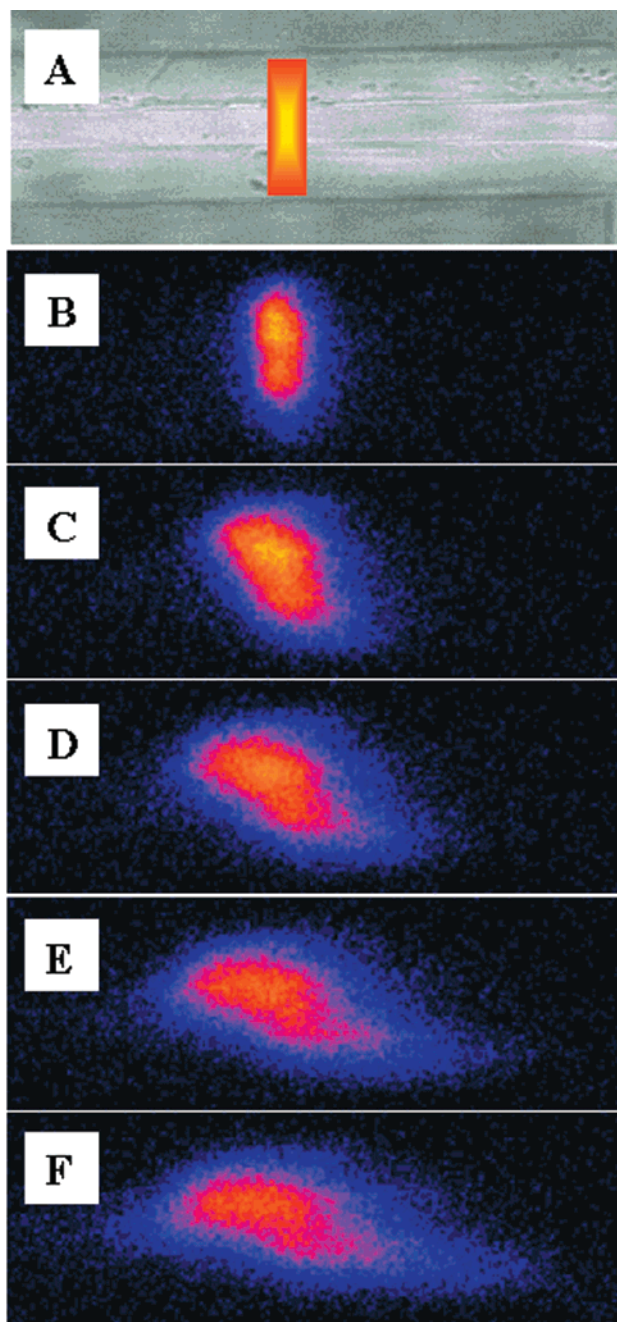(21) Ross, D. Unpublished results; NIST: Gaithersburg, MD, 2000.

Figure 4. Time-step images of an uncaged dye plug moving in a channel derivatized as shown in Figure 4. A, photomicrograph of the microchannel with schematic depiction of dye at instant of uncaging; B–F, sequential fluorescence images of the plug movement, acquired every 67 ms.

Figure 5. Velocity profile of beads moving in a channel derivatized as shown in Figure 3 with an applied voltage of 130 V/cm. Bead velocity (cm/s) vs distance (μm) across the width of the channel, where distance = 0 μm is the positively charged side of the channel, as shown in Figure 3A, and distance = 60 μm is the negatively charged side of the channel, as shown in Figure 3A. The bead velocity was determined by comparing two video images of the channel taken 1/60 s apart. A number of beads were stuck to the walls of the channel and did not follow the fluid flow; particles with velocities near zero were not included in the measurement in order to avoid counting these stationary particles.

due to diffusion. Control experiments, imaging an uncaged dye plug with zero applied voltage, were performed before and after the opposite flow experiments to verify the absence of hydrodynamic flow.

Bi-directional flow in single channels was also verified by imaging fluorescent beads. Experiments were performed in channels derivatized by coating the entire device with one polyelectrolyte layer (PAH) and then selectively depositing a second polyelectrolyte layer (PSS) on one-half of the channel. The opposite flow was then imaged using carboxylate-derivatized polystyrene beads in pH 3.5 phosphate buffer. This pH was chosen
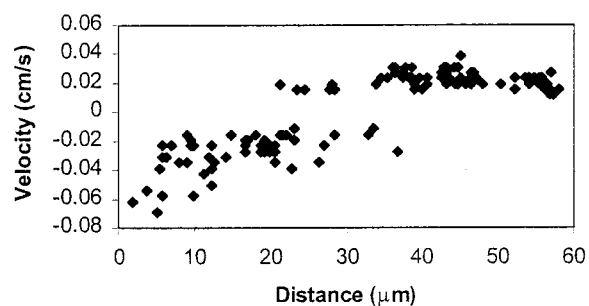
to minimize the electrophoretic bead mobility while maintaining EOF. Video imaging was used to observe the movement of the particles in the channel and to measure the particle velocities. Figure 5 is a graph of the particle velocities as a function of distance across the channel. Particles in the PSS-derivatized side of the channel move from anode to cathode, which is indicated by positive velocities, while particles in the PAH-derivatized side move from cathode to anode, which is indicated by negative velocities. The average velocity of the beads on the PSS side of the channel was 2.2 ($\pm$0.5) $\times$ 10^{-2} cm/s while that on the PAH side was -2.9 ($\pm$1.3) $\times$ 10^{-2} cm/s. The particle velocity standard deviation is 45% on the PAH side of the channel, but only 23% on the PSS side of the channel, which indicates a greater uniformity of charge in the PSS half of the channel. This result is consistent with the fact that the PSS coating is comprised of two layers, whereas the PAH coating is a single layer. It is generally accepted that a two-layer PEM will have greater uniformity than a single polyelectrolyte layer.[22] In addition, the PAH coating may have been subjected to contamination during the PSS deposition. These particle velocities are similar to those that were measured in separate, uniformly coated channels where the velocity in the PSS (2-layer) and PAH (1-layer) channels were 1.4 ($\pm$0.2) $\times$ 10^{-2} and -2.2 ($\pm$0.7) $\times$ 10^{-2} cm/s, respectively. Again, greater standard deviation is seen in the PAH channel.

We note that the particle velocities measured in both the PAH- and PSS-derivatized channels are slower than expected, as compared to the EOF mobility of the derivatized channels that was determined by current monitoring and caged dye experiments. The interactions responsible for the slower particle movement in the derivatized channels are under investigation.

One parameter which may contribute to this deviation is the bead electroophoretic mobility, which was determined to be -3.1 $\times$ 10^{-4} cm^2/V$\cdot$s (cathode to anode direction) in pH 3.5 phosphate buffer. In the PSS channel, if we assume that the particle measurement is the result of the combined electroosmotic and electrophoretic mobilities ($5.5 \times 10^{-4} + -3.1 \times 10^{-4}$ cm^2/V$\cdot$s),

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then the total particle mobility is 2.4×10^{-4} cm²/V·s, which is consistent with the particle flow measurements. The same calculation (electroosmotic mobility + electrophoretic mobility) is not consistent with the particle flow measurements that were made in the PAH-coated channel. In this case, it is possible that the negatively charged beads interact with the PAH-coated walls and become over-coated with the positively charged PAH. The expected bead electroophoretic mobility would then be 3.1×10^{-4} cm²/V·s (anode to cathode direction), resulting in a total mobility of -2.7×10^{-4} cm²/V·s, which is consistent with particle flow measurements.

Despite deviations in the particle velocities, bi-directional flow in a single channel was clearly achieved, and applications of this new methodology are under development.

Such applications include transport of analytes to separate detection systems, such as electrochemical and optical sensors positioned at opposite ends of the microchannel. In addition, studies of reactions at the interface of the two aqueous streams could be performed. Because the flow in microchannels is laminar, the introduction of mixing prevents reactions in the channels from being diffusion limited. Patterns of charge within microchannels have been demonstrated to affect electroosmotic flow,[12] and the opposite flows shown here could be used to create mixing loops in the channels.