

Plastic Microfluidic Devices Modified with Polyelectrolyte Multilayers

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Control of the polymer surface chemistry is a crucial aspect of development of plastic microfluidic devices. When commercially available plastic substrates are used to fabricate microchannels, differences in the EOF mobility from plastic to plastic can be very high. Therefore, we have used polyelectrolyte multilayers (PEMs) to alter the surface of microchannels fabricated in plastics. Optimal modification of the microchannel surfaces was obtained by coating the channels with alternating layers of poly(allylamine hydrochloride) and poly(styrene sulfonate). Polystyrene (PS) and poly(ethylene terephthalate) glycol (PETG) were chosen as substrate materials because of the significant differences in the polymer chemistries and in the EOF of channels fabricated in these two plastic materials. The efficacy of the surface modification has been evaluated using XPS and by measuring the EOF mobility. When microchannels prepared in both PS and PETG are modified with PEMs, they demonstrate very similar electroosmotic mobilities. The PEMs are easily fabricated and provide a means for controlling the flow direction and the electroosmotic mobility in the channels. The PEM-coated microchannels have excellent wettability, allowing facile filling of the channels. In addition, the PEMs produce reproducible results and are robust enough to withstand long-term storage.

Plastics have been used in place of silicon as substrates for lab-on-a-chip devices, because the use of plastics simplifies fabrication and reduces costs.^{1–7} However, the plastic surface chemical functionalities differ from those of glass and vary

from polymer to polymer.^{2,8} Differences in surface chemistry can have a dramatic effect on flow rates and separations in devices utilizing EOF. For example, various polymer substrates exhibit different EOF mobilities in microchannel devices.^{2,8} Therefore, the development of methods for controlling microchannel surface chemistry is crucial for reproducible and controllable separations.

The use of polyelectrolyte multilayers (PEMs) has been shown to be a simple, reproducible method for surface derivatization.⁹ Multilayers are created by exposing a surface to alternating solutions of positively and negatively charged polyelectrolytes. Although the layers are adsorbed on the substrate or previous layer by noncovalent interactions, the resulting multilayers have multiple electrostatic bonds and are stable and uniform. PEMs have been used to derivatize silica and plastics,^{10,11} to incorporate active antibodies,¹² to prevent adhesion of cells and proteins for tissue engineering applications,¹³ and to enhance capillary electrophoresis separations in glass capillaries.^{14–17}

Here we report the use of polyelectrolytes to alter the surface of microchannels imprinted in two plastics, polystyrene (PS) and poly(ethylene terephthalate glycol) (PETG). Controlling the surface chemistry with the PEMs allows the substrate plastic to be chosen for properties other than the surface charge, such as optical clarity or ease of imprinting. The efficacy of the surface modification has been evaluated using X-ray photoelectron spectroscopy (XPS) and by measuring the EOF mobility. Preliminary applications indicate that PEM-derivatized channels exhibit reproducible and stable EOF behavior.

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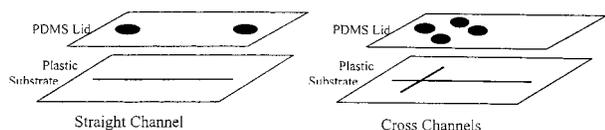


Figure 1. Schematic of microchannel devices consisting of imprinted plastic substrates with PDMS lids.

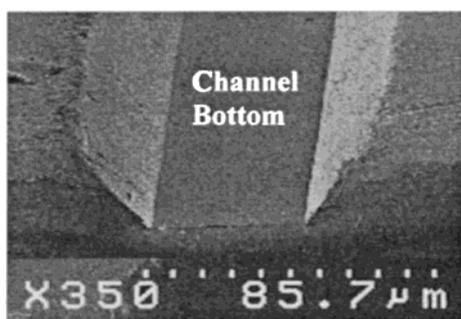


Figure 2. SEM of a microchannel imprinted in polystyrene.

EXPERIMENTAL SECTION

Device Preparation. Sheets of PS (Corning Costar Corp., Cambridge, MA),¹⁸ PETG (Vivak, DMS Engineering Plastic Products, Sheffield, MA),¹⁸ poly(methyl methacrylate) (PMMA; Lucite, ICI Acrylics, Memphis, TN),¹⁸ and polycarbonate (PC; Lexan, GE Co., Mount Vernon, IN)¹⁸ were cut into 7.6 cm by 7.6 cm squares and rinsed with methanol prior to use. Films of poly(dimethylsiloxane) (PDMS) were made according to product information from a Sylgard 184 silicone elastomer kit (Dow Corning, Midland, MI).¹⁸

Figure 1 shows a schematic of the plastic microfluidic devices used in these studies. A silicon template, fabricated by photolithography,⁶ was used to imprint channels in the plastic substrates as previously described.⁷ Each chip was imprinted with three or seven parallel channels, but only one is depicted in the figure for simplicity. A cross design (Figure 1) consisting of two perpendicular channels was imprinted for devices used with discrete sample injection, while straight, single channels (Figure 1) were used for all other experiments. Typical channels were 20 μm deep and 40 μm wide, as evidenced in Figure 2, which displays an SEM image of an imprinted PS substrate. All imprinted channels, including those modified with PEMs as described below, were sealed using a cured PDMS film. Holes 2 mm in diameter cut into the PDMS served as the fluid reservoirs for the channel. Two reservoirs, 2–4 cm apart were used in the straight channels, while four reservoirs, each 1 cm from the channel intersection were used in the cross design.

PEM Deposition. An aqueous 60 mM poly(styrene sulfonate), sodium salt (PSS) (Scientific Polymer Products, $M_w = 500\,000$)¹⁸ solution was prepared with 0.5 M NaCl and adjusted to pH 9 with NaOH.¹¹ A 20 mM poly(allylamine hydrochloride) (PAH; Aldrich, $M_w = 70\,000$)¹⁸ solution of the same salinity and pH¹¹ was also prepared. Polymer concentrations are based on the repeat unit. All water used in this study was deionized (18 M Ω ·cm).

The PEM deposition method, similar to previously published methods of Chen and McCarthy,¹¹ was used to deposit alternating layers of PAH and PSS. The substrate plastics, PS or PETG, were treated with 1 M NaOH at 55 $^\circ\text{C}$ for 15 min. The substrates were then rinsed with water and dried with nitrogen. The first PAH solution was pipetted onto the plastic substrate, completely covering the channels, and allowed to stand for 30 min. The PAH solution was removed by rinsing with water and dried. PSS was then pipetted on the plastic substrate, completely covering the channels, and allowed to stand for 30 min. The PSS was rinsed off with water and dried. Alternating layers of PAH and PSS were applied for 5 min with water rinses between each solution application until the desired number of layers was deposited. Therefore, channels with an odd number of layers had a positively charged top layer, corresponding to PAH, while those with an even number of layers had a negative PSS surface.

Electroosmotic Flow Measurements. The EOF was measured using a current monitoring method¹⁹ and the experimental details were published previously.⁸ In the method, EOF is determined according to the equation, $v_{\text{eof}} = L t^{-1}$, 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. Solutions containing 10, 20, and 40 mM phosphate buffer, pH 7, were utilized for flow measurements in the (1) native plastic, (2) NaOH-treated plastic, and (3) PEMs with the negative, PSS final layer. For measurements in channels with a final positive, PAH layer, 5 and 10 mM phosphate buffer solutions, pH 3, were used.

Separations. Discrete injections were made using a variable-volume sample fill method, described previously,²⁰ in a device made with a cross design consisting of two perpendicular channels. Fluorescein-labeled biotin (Sigma)¹⁸ and fluorescein-labeled morphine 3-glucuronide solutions were prepared in phosphate buffer. Fluorescence measurements were made using a research fluorescence microscope equipped with a mercury arc lamp, appropriate filter sets, and a photomultiplier tube (Hamamatsu, Bridgewater, NJ)¹⁸ for detection.

XPS. X-ray photoelectron spectroscopy was performed on the samples using one of two instruments. PS samples were evaluated using a Surface Science/Fisons 220il ESCA system¹⁸ equipped with a monochromatic aluminum X-ray source and an electron flood gun for charge compensation. PETG samples were analyzed using a previously described system and operating parameters.²¹ No X-ray-induced decomposition of the samples was observed during XPS data acquisition, as evidenced by the consistency of the peak areas and positions.

RESULTS AND DISCUSSION

EOF mobilities of the native plastics were investigated with hybrid devices composed of four different imprinted plastic substrates with untreated PDMS lids. The results are summarized in Table 1. For most of the plastics, the flow in these hybrid

(18) Certain commercial equipment, instruments, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement 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.

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Table 1. EOF Mobility ($10^4 \text{ cm}^2/\text{V}\cdot\text{s}$) of Channels Made in Various Substrate Plastics with PDMS Lids^a

substrate	EOF mobility
PETG	4.3 ± 0.4
PC	3.0 ± 0.3
PS	2.5 ± 0.4
PMMA	1.3 ± 0.4

^a Standard deviations determined from repeated measurements on multiple channels.

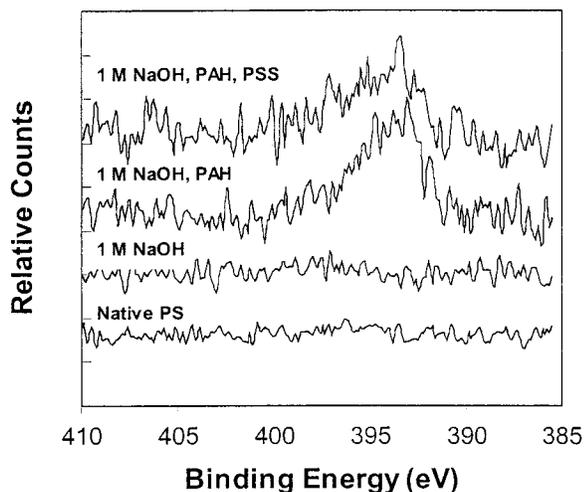


Figure 3. N 1s XPS of PS treated up through one of the following steps: (1) methanol rinse; (2) 1 M NaOH treatment at 55 °C; (3) one PAH layer deposition; and (4) one PSS layer deposition. Spectra offset for clarity.

devices was slower than the flow⁸ in wire-imprinted devices made from a single plastic. Although differences in surface chemistry may arise from the different imprinting techniques, the main contribution to the slower flow in the hybrid devices is probably the PDMS lid, as discussed below. PETG and PS were chosen as test substrates for the PEM derivatization, due to their significantly different chemical compositions and native flow mobilities.

Chen and McCarthy have shown XPS to be an effective tool for evaluation of PEMs on plastic substrates.¹¹ Therefore, the efficacy of the PEM deposition was assessed by XPS. Spectra were obtained of four discrete samples at different stages of the PEM deposition techniques. Channel dimensions preclude direct measurement of surface modification, so spectra were acquired from unimprinted substrates treated with the same protocols used to modify the channel walls. Figure 3 shows the nitrogen 1s region and Figure 4 shows the sulfur 2p region of the native PS, PS treated with NaOH, and PS with one or two deposited polyelectrolyte layers. As evidenced in the figures, the nitrogen peak appears after deposition of the PAH, while the sulfur peak is seen only after deposition of the PSS. Figure 3 shows the expected attenuation of the nitrogen peak intensity by the final PSS layer. Similar spectra were obtained for the PETG substrate.

The flow direction and the EOF mobility were used to characterize the PEM-treated channels. PETG and PS microfluidic channels were derivatized, and as expected,¹⁶ the flow in channels with a negative PSS top layer was from anode to cathode, as found in silica-based devices and in the native plastic devices.⁸

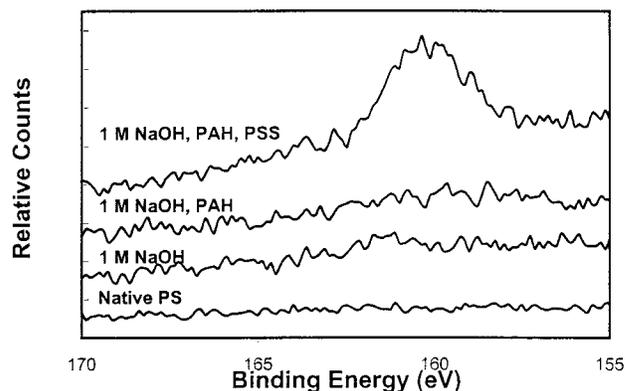


Figure 4. S 2p XPS of PS treated up through one of the following steps: (1) methanol rinse; (2) 1 M NaOH treatment at 55 °C; (3) one PAH layer deposition; and (4) one PSS layer deposition. Spectra offset for clarity.

Table 2. EOF Mobility ($10^4 \text{ cm}^2/\text{V}\cdot\text{s}$) of PEM-Derivatized Channels Made in PS or PETG with PDMS Lids^a

surface treatment	PETG/PDMS	PS/PDMS
1 M NaOH	5 ± 1	
3-layer PEM	-1.8 ± 0.3	
4-layer PEM	4.2 ± 0.2	
13-layer PEM	-1.7 ± 0.3	-1.3 ± 0.2
14-layer PEM	4.8 ± 0.5	4.1 ± 0.2

^a Standard deviations determined from repeated measurements on multiple channels.

The flow in channels with a positive PAH top layer was reversed and flowed from cathode to anode. The latter we designate as negative flow. In positive and negative channels, the flow direction was unaffected by buffer pH (pH 3 or pH 7). The EOF mobility was determined for a number of different layers, as shown in Table 2. In positively coated channels, studies have shown that faster flow is measured in channels run at acidic pH as compared with neutral pH, possibly due to phosphate adsorption on the positive surface at neutral pH.¹⁶ Therefore, for purposes of comparison with literature values, the EOF mobility was measured using pH 3 or pH 7 running buffer, for channels with a positive PAH or negative PSS top layer, respectively. Graul and Schlenoff¹⁶ reported slight drift in the EOF mobility toward slower values for PEMs prepared in fused-silica capillaries, if the capillaries were used continuously. Radiolabeling was used to determine that this drift resulted from conformational changes of the polyelectrolytes, rather than desorption from the substrate.¹⁶ Slight mobility drift toward slower values was also observed in our work, but only with PEMs consisting of three layers on either substrate material. Such mobility drift was not observed for PEMs consisting of more than three layers. Deposition of 13 or 14 layers (Table 2) resulted in similar mobilities in the PETG and PS channels, despite the differences in the mobility of the native plastics. This result indicates that modification with PEMs is a useful way of producing similar surfaces on different plastic materials, allowing greater flexibility in substrate polymer selection for a given application.

Using both the PETG and the PS substrates, the flow rates obtained for channels with the negative PSS were similar to previously published results for PEMs made with poly(diallyldimethylammonium chloride) (PDAMAC) and PSS on glass sub-

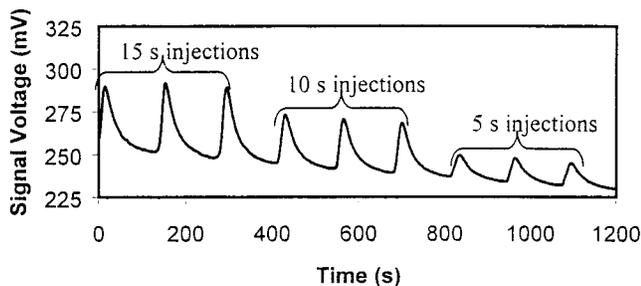


Figure 5. Three 15-s, three 10-s, and three 5-s injections of carboxyfluorescein using a PS microchannel treated with a 14-layer PEM.

strates.¹⁶ Slower flow rates were measured in channels with the positive PAH top layer using either substrate. This slower mobility was presumably due to charge contributions from the PDMS lid. To simplify fabrication, no attempt was made to derivatize the channel lids. Therefore, the lids retained the surface charge of the native PDMS.²² In devices made completely from PDMS, the measured value of the EOF mobility was $1.8 \times 10^{-4} \text{ cm}^2/\text{V}\cdot\text{s}$.²³ Since the native charges on the PDMS were negative, these charges increased the net flow in the channels with the negative PSS top layer but decreased the net flow in the channels with the positive PAH top layer.

One significant practical advantage of the PEM-coated channels is the ease with which the channels can be filled with aqueous buffers, as compared with untreated plastic channels. The hydrophilic character of the PEMs presumably facilitates the wetting of the microchannel walls. For experiments utilizing uncoated plastic substrates, the channels were primed with methanol, filled with water, and finally filled with buffer using vacuum to pull the liquid into the channel. Even if great care was taken during the filling procedure, air bubbles were often trapped in the channel. However, with the PEM-coated channels, the methanol priming step is unnecessary, and bubbles were never formed in the PEM-coated channels during our experiments. This greatly reduces the time required to prepare the channel for use and may facilitate the use of plastic microchannels for more widespread applications.

Channels with 14-layer PEMs on polystyrene were used to demonstrate preliminary applications of the derivatized channels. Figure 5 shows repeated injections of carboxyfluorescein in phosphate buffer. A series of three 15-, 10-, and 5-s injections were made. As can be seen, the peaks are quite reproducible. The standard deviation of the peak areas for such repeated injections is typically 3–5%, as compared to 5.6% standard deviation for experiments performed in bare plastic channels.⁶ Peak tailing is also noticeable in Figure 5. Previous studies have shown that small molecules are able to partition into PEMs.¹⁶ We speculate that hydrophobic interactions of the carboxyfluorescein with the PEMs may contribute to the observed tailing.

An additional advantage of the PEM-coated channels is the ability to regenerate the surface by flowing the final polyelectrolyte solution through the channel. For example, Figure 6A shows an attempt to separate fluorescein isothiocyanate labeled-morphine and fluorescein-labeled biotin (three injections) using a 1-cm PS

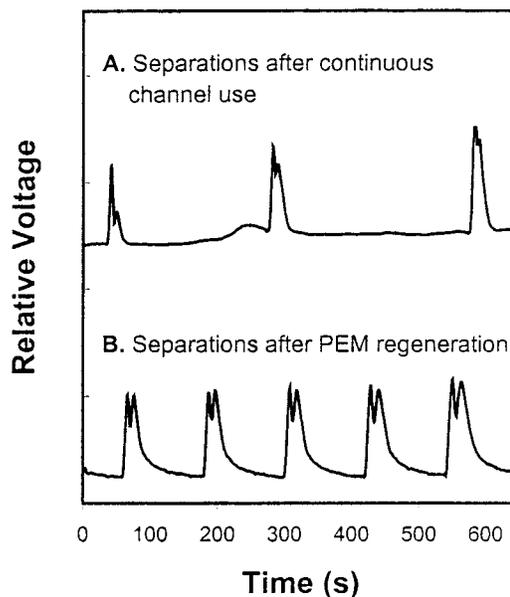


Figure 6. Multiple injections of a mixture of fluorescein isothiocyanate-labeled morphine and fluorescein-labeled biotin on (A) PS channel coated with a 14-layer PEM after a day of continuous use (three injections) and (B) the same PEM-coated PS channel after renewal of the fourteenth layer (five injections).

channel coated with a 14-layer PEM after a day of continuous use. Figure 6B shows the same separation (five injections) in the same 14-layer PEM-coated channel after regeneration of the top layer. The two compounds are now repeatedly separated with reproducible retention times of 30 and 42 s, respectively, thus demonstrating the ease with which the PEM coating can be renewed. We note that this separation was not optimized and the channel utilized was very short. Tailing of the biotin peak can be seen in Figure 6. Tailing was not observed for injections of morphine only (data not shown); however, the biotin does interact with the PEM, as evidenced by the peak tailing and fluorescence imaging.²⁴

In addition, the PEM-coated channels were found to be quite stable. After preparation, the derivatized channels were stored and reused over a one-month period. The chips were stored dry at room temperature for several days, with no adverse affect on the PEMs. For long-term storage, the channels were filled with water and stored at 4 °C although dry storage may be as effective.

CONCLUSIONS

The PEMs were found to be an effective method for controlling the surface chemistry of plastic microchannels. However, the undesirable interactions of certain molecules with the PEMs reduce the utility of the PEM-coated channel for some separations. Our future applications will focus on the salient features of the PEM-derivatized microfluidic devices, including facile channel filling and surface regeneration, and use of surface charge for control of flow direction.

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