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Comparing polyelectrolyte multilayer - coated poly(methylmethacrylate) microfluidic devices and glass microchips for electrophoretic separations

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Abstract

There is a continuing drive in microfluidics to transfer microchip systems from the more expensive glass microchips to cheaper polymer microchips. Here, we investigate using polyelectrolyte multilayers (PEM) as a coating system for poly (methylmethacrylate) (PMMA) microchips to improve their functionality. The multilayer system was prepared by layer-on-layer deposition of poly (diallyldimethylammonium) chloride (PDAD) and polystyrene sulfonate (PSS). Practical aspects of coating PMMA microchips were explored. The multilayer buildup process was monitored using EOF measurements, and the stability of the PEM was investigated. The performance of the PEM-PMMA microchip was compared to those of a standard glass microchip and a PEM-glass microchip in terms of electroosmotic flow and separating two fluorescent dyes. Several key findings in the development of the multilayer coating procedure for PMMA chips are also presented. It was found that, with careful preparation, a PEM-PMMA microchip can be prepared that has properties comparable - and in some cases superior - to those of a standard glass microchip.

Introduction

The concept of microchip capillary electrophoresis (MCE) systems was first introduced by Harrison et al. 1. What began as a demonstration of the feasibility of a MCE system has led to a drive to transfer standard analytical methods to a microchip platform. Initially the vast majority of the work in MCE was on glass microchips, as the surface chemistry of a glass chip is the same as the surface chemistry of a capillary used in traditional capillary electrophoresis 2. More recent research has focused on transferring the glass microchip systems to a polymer platform due to the high cost associated with the production of glass chips. In addition, the polymer platform would provide more flexibility in designing and fabricating the chip. To this end several approaches have been developed to produce polymer microchip devices including techniques such as photolithography, stereolithography, laser ablation, hot embossing, microthermoforming, injection molding, and plasma etching 3.

Not only is the type of method used for fabricating the polymer devices important but the type of polymer used is also a critical choice 3–5. Commonly used polymers include poly(methyl methacrylate) (PMMA), poly (carbonate) (PC), polyethylene terephthalate (PET), and poly(dimethylsiloxane) (PDMS). While polymer devices may be cheaper and

easier to manufacture, problems exist that prevent the devices from being replicated successfully and consistently. These can be related to inconsistencies in the bulk polymer between different batches as well as different suppliers. In a paper by Nikcevic et al.⁶, the differences in the performance of injection molded PMMA microchips were explored as a function of the fabrication conditions, thus highlighting the importance of careful characterization of the polymer used. That work suggests that PMMA microchips have a great potential for mass fabrication. In addition, some fabrication techniques require very precise conditions, and slight deviations from them can result in inconsistent performance of the device. Even with rigorous fabrication specifications and in-depth polymer characterization, modifying the surfaces of these polymer devices is usually unavoidable^{7, 8}.

Control of the electroosmotic flow (EOF) is crucial when developing capillary electrophoresis systems. In traditional capillaries and glass microchips the EOF is generated by the charged silanol groups on the microchip / capillary surface. It has been established that the EOF values for plastic materials are generally lower than glass due to the fewer charged surface groups⁹. This creates an additional problem in that the hydrophobic surfaces are often difficult to wet with the commonly used aqueous solutions. However, surface modifications can be used to control surface charge and EOF as well as prevent non-specific interactions of the analytes with the channel wall – all of which are crucial to electrophoretic methods. Common methods of surface modification include covalent and dynamic coatings, and are the subject of a recent review article¹⁰. Soper et al. covalently modified the surface of PMMA microchannels with an aminolysis reaction to yield an amine-terminated PMMA surface¹¹, and also developed several grafting procedures to attach different compounds to the aminated surfaces¹¹. Several methods using sol-gel technology have also been developed to modify the PMMA surface^{12–14}. Dynamic coating with positively or negatively charged surfactants or hydrophilic neutral polymers in the buffer systems has also been used^{9,15–17}. Recently a novel coating approach involving evaporation has been published¹⁸.

Another form of surface modification- polyelectrolyte multilayers (PEM)-has received much attention. Originally reported by Decher et al., the systems are generated by a layer - by - layer deposition technique^{19, 20}, where polyanions and polycations are deposited alternately on the capillary surface^{19–21}. Each deposition step effectively adds a reproducible quantity of material to the surface and reverses the surface charge to prepare it for the next deposition step. The alternating deposition steps result in a multilayered complex that is stabilized by strong electrostatic interactions²². Many studies have been done in the last decade to better understand the adsorption and formation of multilayer systems^{22–36}, the versatility of which allow a variety of compounds to be incorporated for a wide range of applications^{37–40}.

Although PEM has been used as a form of surface modification for polymer microfluidic devices, the use of PEMs on PMMA microchips has received only minimal attention⁴¹. In this work a multilayer film of poly (styrene sulfonate) (PSS) and poly (diallyl dimethyl ammonium chloride) (PDAD) was successfully applied to PMMA microchips. The coating procedure was evaluated using ACLARA 32 channel Lab Cards and then transferred to in-house fabricated PMMA devices. The multilayer buildup process was followed by monitoring the electroosmotic flow as a function of layer. The stability of the system as a function of pH and layer number was also determined and compared to standard glass microchips. Two model fluorescent dyes were separated successfully to demonstrate the utility of the PEM-PMMA system. Overall, the PEM-PMMA microchip was shown to be functionally similar to the more expensive glass microchip.

Experimental

Reagents and solutions

The components of the polyelectrolyte multilayer system PDAD, (average MW 500,000) and PSS, (average MW 250,000) were from Scientific Polymer (Ontario, NY). EOF measurements used 20 mM and 10 mM borate (pH 9.2), phosphate (pH 7.2 and 2.8), and acetate (pH 5.5) buffers prepared from sodium tetraborate and boric acid, sodium phosphate and phosphoric acid, and sodium acetate and acetic acid, respectively (Sigma-Aldrich, St. Louis, MO). Fluorescein sodium salt (FL) and fluorescein isothiocyanate isomer I 90% (FITC) were obtained from Sigma-Aldrich (St. Louis, MO). FL and FITC stock solutions were prepared fresh before use by dissolving appropriate amounts of each dye in methanol (Pharmco, Brookfield, CT), and appropriate aliquots of each stock solution were mixed and diluted with running buffer to give a combined standard solution containing 25 μ M FL and 50 μ M FITC. The running buffer for FL and FITC separations was 10 mM borate prepared from sodium tetraborate. All solutions were degassed offline and filtered with a 0.45 μ m syringe filter (Millipore Corp., Bedford, MA).

Instrumentation and methods

ACLARA 32 channel Lab Cards (ACLARA Biosciences, Mountain View, CA) were used to optimize the polyelectrolyte multilayer coating and have been described previously 42. PMMA (GE Polymerland, Pittsfield, MA) chips were fabricated in-house by injection molding (IM). Glass microchips were from Micralyne (Micralyne, Inc., Edmonton, AB, Canada). The microchip instrument setup has been described 43. Briefly, experiments were observed on a Nikon inverted microscope (Nikon Corp., Melville, NY). Fluorescence excitation was by a xenon arc lamp equipped with a 480 ± 20 nm bandpass filter. A 10x microscope objective focused the excitation beam on the microchip and collected fluorescence emission that was spectrally and spatially filtered with a 535 ± 25 nm bandpass filter and a 1 mm pinhole, respectively. Fluorescence was initiated 4 cm from the injection separation channel intersection and detected with a photomultiplier tube (Hamamatsu R3896, Bridgewater, NJ). Data were acquired at 20Hz with a PE Nelson Model 970A Intelligent A/D interface and Turbochrom software (PE Nelso, San Jose, CA). Voltages were applied to microchips through platinum electrodes using a high voltage power supply (Micralyne Inc., Edmonton, AB, Canada) controlled by a LabView (National Instruments Corp., Austin, TX) program by Micralyne.

Polyelectrolyte multilayer coating

The polymer coating method we used was published by Graul and Schlenoff 44 and was optimized for the current system. Polymer deposition solutions contained 10 mM polymer and 500 mM NaCl. The multilayer coating was deposited on the chip by rinsing the various solutions through the chip by applying a vacuum to the waste reservoir. The initial rinse was 5 min 0.1 M NaOH, followed by a 2 min water rinse to prepare the chip for the multilayer deposition. The initial PDAD layer was rinsed through the chip and allowed to remain in the channel for 30 min. This was followed by a 2 min water rinse. Subsequent polymer layers were deposited using a 5 min polymer rinse followed by a 2 min water rinse. The final coated microchip consisted of a 6.5 bilayer system with the negative PSS as the outermost layer. After each water rinse all liquid was removed from the microchip prior to the next polymer rinse.

Microchip fabrication—The microfabrication techniques used in this study have been described in detail 45. Briefly, microstructures were fabricated using the UV-LIGA process. The master mold for microchip replication was fabricated on a nickel (Ni) mold disk (3 in diameter, 1.6mm thick). The disk was lapped flat and a thick photoresist (SU-8 2075

negative photoresist, Microchem, Newton, MA) process was applied to the disk followed by Ni electroplating. A plating height of $\sim 100\ \mu\text{m}$ was obtained after 10 h of electroplating in a Ni-sulfamate bath with a $\sim 10\ \mu\text{m/h}$ plating rate. The electroplated pattern was polished mechanically after removing the photoresist, resulting in greatly improved transparency and a reduced number of channel imperfections in the chips.

Replicate plastic microchips were made by injection molding at high pressure (19,000 psi) after insertion into a custom-designed molding block. After replication, the chips were cleaned of particulate and organic matter using sequential rinses with isopropanol (IPA) (Pharmco, Brookfield, CT, USA) and deionized-water. An O_2 reactive ion etching (RIE) process was used to increase the wettability of the PMMA chips by generating on the chip surface new functional groups that contain oxygen ($-\text{CO}_x$, $-\text{OOH}$, and $-\text{C}=\text{O}$) 46. The O_2 RIE surface treatment was done by first cleaning the wafers with IPA and deionized-water, then applying the O_2 RIE plasma using a Technics Micro-RIE Series 85 (Technics, Dublin, CA, 20 sccm, 210 mTorr, 100 W, 30 kHz) instrument for 1 min, followed by a 30 s rest, and a 1 min etch. The patterned wafer (channel width, $100\ \mu\text{m}$ and depth $80\ \mu\text{m}$) was then bonded to a cover wafer (IM-PMMA, 0.9 mm) using thermoplastic bonding (86°C , 0.2 tons pressure). Reservoirs for loading analytes, and to which operating voltages could be applied, were made by drilling holes at appropriate locations on the fabricated wafers. The reservoir volumes were increased by attaching polypropylene pipette tips to the wafer holes with epoxy after the chips were bonded.

Results and Discussion

Optimization of multilayer coating on ACLARA chips

The PDAD/PSS multilayer coating system was initially evaluated using the ACLARA 32 channel Lab card (PMMA). The ACLARA lab card was used initially during the development of the in-house PMMA microchips. A schematic of the channel design is shown in Figure 1a. The initial coating procedure involved a sodium hydroxide rinse, followed by a water rinse, and then a rinse with the PDAD polymer solution, which was kept in the channel for 30 min. At the end of the deposition time the channel was rinsed with water and the PSS polymer solution, which was kept in the channel for 5 min. This process was repeated with alternating polymer solutions until a 6.5 bilayer system was generated with PSS as the outermost layer. However, this coating procedure resulted in clogs forming in the microchip channels. Figure 2 shows a microscopic view of a clog at the end of the channel next to the sample reservoir (labeled S in Figure 1). An investigation of clog formation showed that altering the composition of the polymer solution (both the polymer and the salt concentration) did little to diminish the occurrence of clogs in the microchip channels. The deposition process was also evaluated by examining the microchip for clogs after each rinsing step. The initial appearance of the clog was noted including the step in the deposition process and the location of the clog in the microchip. It was found that while clogs occurred in all areas of the microchip they were more prevalent in the long sample injection channel. It was also noted that there was no clog formation before both polymer solutions were introduced into the system.

A common problem experienced with polymer chips is the need for time consuming reconditioning steps 47. In the ACLARA chip the long sample injection channel presents a special problem with our method of fluid manipulation. The ACLARA chips were designed for the ACLARA microfluidic card reader system which uses a different method to manipulate fluids than in our in-house system. We condition chips with a vacuum pump applied to the buffer waste position (D) to pull liquid through the channels. The ACLARA system however, functions similarly to a traditional capillary electrophoresis system by pumping the liquid through the microchip. Further investigation revealed that after filling

the chip with liquid, simply applying the vacuum at the buffer waste position was insufficient to remove all the liquid from the chip, therefore we modified the deposition procedure. We ensured that all the liquid from the polymer solutions was removed from the microchip by rinsing the chip with water between depositing polymer solutions and then removing all the liquid. This procedure eliminated effectively the occurrence of clogs.

Performance of PMMA-coated microchips

The adjusted multilayer coating method was transferred from the ACLARA lab cards to the in-house fabricated PMMA microchips (Figure 1b) that were evaluated previously 6. We did this because, first, the long sample channel on the ACLARA chip made it difficult to develop an injection protocol; but more importantly, the cover plates used on the ACLARA chip were extremely thin allowing the electrodes to penetrate through the reservoirs.

Monitoring the multilayer buildup process—During the multilayer buildup process each polymer layer reverses the surface charge. Because the direction and magnitude of the electroosmotic flow of the system is related to the charge on the surface, the growth of the multilayer can be monitored by measuring the magnitude and direction of the electroosmotic flow after each layer is added. Figure 3 shows the electroosmotic flow of the system versus the layer added. The EOF of the PMMA chips was determined using the current monitoring method 47 and borate buffers (20 mM and 10 mM, pH 9.2). The EOF of the unmodified plastic is shown at the initial point (point 0), and prior to any modification of the PMMA surface. Under the test conditions the EOF of the unmodified PMMA chip is significant at $4.93 \times 10^{-4} \text{ cm}^2/\text{VS}$. This is related to the O_2 RIE treatment of the microchannels during the fabrication process. Previously, it has been found that the O_2 RIE treatment effectively doubles the EOF at high pH values compared to untreated PMMA microchips 6. The negative EOF values in Figure 3 indicate that the EOF is towards the anode due to the positive charge of PDAD on the microchip wall. The positive values indicate that the EOF is towards the cathode due to the negative charges on the capillary wall from either the bare PMMA or the negatively charged PSS. The average EOF for the positively charged PDAD layers was $-3.42 \times 10^{-4} \text{ cm}^2/\text{VS}$ with a %RSD between the layers of 3.86% (n=3). The average EOF for the negatively charged PSS layers was $4.22 \times 10^{-4} \text{ cm}^2/\text{VS}$ with a % RSD between layers of 6.33% (n=3).

Electroosmotic flow vs. pH for glass and PEM-PMMA devices—In capillary and glass microchip electrophoresis systems the magnitude of the EOF depends on the pH of the system due to the pK_a of the silanol groups on the surface. One advantage of PEM systems is that if the polymers chosen are strong polyelectrolytes, then the EOF is unaffected by pH since the surface charge doesn't change. Previous work using a PDAD/PSS multilayer in a traditional capillary has supported this 48. For a bare capillary above pH 7 the EOF value is high, but below pH 4 the EOF is virtually non-existent. Previous work with O_2 RIE treated microchips reveals a similar trend in that at low pH (pH 3) the EOF value is $1.28 \times 10^{-4} \text{ cm}^2/\text{VS}$ whereas at high pH (pH 11) the EOF is $4.84 \times 10^{-4} \text{ cm}^2/\text{VS}$ 6.

The EOF of the PMMA coated microchips was compared to that of a standard glass microchip. Table 1 shows the EOF value at four pH values (9.5, 7.2, 5.5, and 2.8) for a standard glass microchip and a PMMA microchip coated with PEM. It should be noted that channel dimensions vary slightly between the two chips. The EOF values in the glass microchip follow the same trend as predicted in previous studies 48. Thus, at high pH the glass chips have a high EOF, but at lower pHs the EOF was not observed within 120 s (three times the standard run time for EOF monitoring). An additional point to note is the high variability between the EOF measurements for the glass microchip. This is probably related to inconsistencies in conditioning the channel. However, the high variability between data

points is absent for the PMMA chip coated with PEM even though the same conditioning protocol was used. Further, the electroosmotic flow is independent of the pH of the system, unlike the glass microchip. The stability of the multilayer system was determined by repeating the EOF measurements on the PEM-PMMA microchip after approximately 100 runs under various conditions. It was found that the EOF values differed by 1.95% (n=100) from the original data set indicating that the multilayer system was stable.

Separation of fluorescent dyes—The separation performance of the PEM-PMMA microchip was evaluated using standard solutions of fluorescein (FL) and fluorescein isothiocyanate isomer (FITC) and compared to those on a standard Micralyne glass microchip, and a PEM-glass microchip. Injection and separation conditions were adjusted on each chip to give the best separation. The separation was also attempted on a bare PMMA chip; however, after the first run the analytes coated the bare PMMA channel rendering the chip useless. Table 2 shows the results of the separation of FL and FITC on all three microchips. Notably, all three performed similarly, with the PEM-PMMA microchip having the longest separation time because it required low injection and separation voltages due to the poor heat dissipation of the PMMA. This also had some affect on the overall separation performance. Figure 4 shows the separation of FITC and FL on a PEM-PMMA coated microchip. While the separations are comparable on the three chips it is important to note that vast differences in chip conditioning are required for the glass Micralyne microchip compared to the PEM coated glass and PEM microchip. A typical conditioning protocol for the bare glass microchip requires rinses with base, water, and buffer, and the overall conditioning time per microchip run is between 10 – 15 min. However, with the multilayer system, only minimal microchip conditioning was required (less than 2 min) consisting of only a buffer rinse between runs.

Conclusion

The utility of PEMs was investigated with polymer microchips made of PMMA. The multilayer buildup process was monitored by measuring the electroosmotic flow of the system as a function of the number of layers. The PEM-PMMA microchip was compared to a standard glass system and a PEM-glass system, yielding comparable separations of fluorescein and FITC for all three microchips. This is noteworthy since these compounds cannot be separated on a bare PMMA chip because they adsorb to the surface of the chip. The PEM-PMMA chip also has the advantages over the bare glass microchip that the EOF was independent of pH, and the requirements for microchip conditioning were significantly diminished. Overall, using the PEM on PMMA microchips increases their usefulness, making them a viable cost-effective substitute for the standard glass microchip.

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Fig. 1.

a. Schematic of a single lane on the ACLARA lab card. The reservoir abbreviations are sample (A), buffer (B), sample waste (C), and buffer waste (D). The distances from the reservoirs of the injection channel to the separation channel are 14 mm for A to E and 4 mm for C to F. The injection channel is a 0.25 mm offset T design (E to F). The distance from reservoir B to the intersection of E is 8 mm. The distance from reservoir D to the intersection of F is 44 mm.

b. Schematic of in-house PMMA microchip. The reservoir abbreviations are sample (A), buffer (B), sample waste (C), and buffer waste (D). The distances from the reservoirs to the center of the intersection (E) are 4 mm for A, B, and C and 39 mm for D (separation channel). Channel width and depth were 100 μm and 80 μm, respectively.

Fig. 2.
Image of typical clog formation at the sample reservoir in an ACLARA microchip.

Fig. 3.

Electroosmotic flow of the microchannel vs. the layer number. The positive EOF values indicate that the EOF is flowing toward the cathode (normal polarity) and the negative EOF values indicate that the EOF is flowing toward the anode (reverse polarity).

Fig. 4. Electropherogram showing separation of (1) fluorescein and (2) FITC on a PEM-PMMA microchip. Note that the time shown includes a 100 s gap between the run time and the time the detection system started. Separation conditions as detailed in the Experimental section.

Table 1

Comparison of EOF on bare and PEM microchips

pH	μ_{eof} Bare Chip (10^{-4} cm ² /Vs)	μ_{eof} PEM Chip (10^{-4} cm ² /Vs)
9.5	1.5 ± 0.7	4.2 ± 0.2
7.2	1.2 ± 0.3	4.6 ± 0.1
5.5	na	4.5 ± 0.3
2.8	na	4.4 ± 0.1

The EOF for standard glass and PEM-coated PMMA microchips. “na” indicates that there was no visible EOF for the first 120 s (three times the normal run time).

Table 2

Separation performance of microchip devices

Chip	Run Time (s)	FI (s)	FITC (s)	Resolution
Bare glass	45.0	23.8	28.8	1.0
PEM glass	45.0	30.3	35.2	1.0
PEM PMMA	75.0	44.8	50.1	0.9

The separation performance of a standard glass Micralyne chip, a PEM -coated glass Micralyne chip and a PEM-coated PMMA microchip.