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A general microchip surface modification approach using a spin-coated polymer resist film doped with hydroxypropyl cellulose

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Abstract

We have developed a simple and effective method for surface modification of polymer microchips by entrapping hydroxypropyl cellulose (HPC) in a spin-coated thin film on the surface. Poly(methyl methacrylate-8.5-methacrylic acid), a widely available commercial resist formulation, was utilized as a matrix for dissolving HPC and providing adherence to native polymer surfaces. Various amounts of HPC (0.1–2.0%) dissolved in the copolymer and spun on polymer surfaces were evaluated. The modified surfaces were characterized by contact angle measurement, X-ray photoelectron spectroscopy and atomic force microscopy. The developed method was applied on both poly(methyl methacrylate) and cyclic olefin copolymer microchips. A fluorescently labeled myoglobin digest, binary protein mixture, and human serum sample were all separated in these surface-modified polymer microdevices. Our work exhibits an easy and reliable way to achieve favorable biomolecular separation performance in polymer microchips.

Introduction

Polymer microfluidic devices are emerging as powerful tools with numerous applications in chemistry, biology, physics, environmental science, forensics, etc.^{1, 2} Interest in polymer microsystems arises from a combination of low cost, design flexibility and mass production scalability.^{3–5} Importantly, microfluidic device performance is dictated in part by surface properties. In microchip capillary electrophoresis (CE) of proteins and peptides, it is critical to minimize the nonspecific adsorption of analytes on the surface to achieve high separation efficiency. Hydrophobic, electrostatic, or other interactions between analyte and the polymer surface can lead to nonspecific adsorption, resulting in sample loss, band broadening, and poor separation reproducibility.^{6, 7} Hence, it is necessary to tailor polymer microchip surface properties to meet analysis demands with regards to wettability, biocompatibility and nonspecific adsorption.

Currently, there are several methods used to modify polymer microchip surfaces: dynamic coating, chemical modification and physical treatment.³ Several polymers for dynamic coating have been explored, including hydroxypropyl cellulose (HPC),⁸ poly(dimethylacrylamide-*co*-allyl glycidyl ether),⁹ polyacrylamide¹⁰ and poly(ethylene oxide).¹⁰ In dynamic coating a high molecular weight polymer is typically used as a buffer additive, which increases viscosity and can be problematic for filling microchannels; furthermore, a column conditioning process is usually needed before each separation. Chemical surface modification is also used frequently, and extensive research has been done on covalently grafting functional groups to polymer surfaces. For example, poly(methyl methacrylate) (PMMA) microchannels were modified by atom-transfer radical polymerization to attach a polymer with a dense poly(ethylene glycol)

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(PEG) coating.^{11, 12} Stable, hydrophilic, protein-resistant poly(dimethylsiloxane) surfaces were formed by electrostatic self-assembly of poly(ethyleneimine) and poly(acrylic acid) on top of a hydrolyzed poly(styrene-*alt*-maleic anhydride) base layer, followed by covalent attachment of PEG chains to the polyelectrolyte multilayers.¹³ These permanent surface functionalizations are stable, but the process is usually laborious and time consuming. Typical physical modifications involve exposure to radiation,¹⁴ plasma¹⁵ or an ion beam.¹⁶ These treatments can alter the surface chemically or physically, or can deposit a layer on top of the existing material. Plasma treatment is easy to do, and the surface remains hydrophilic in air for at least 15 days without significant contact angle change for PMMA. However, stability is more limited during separations, as surface-modified material can be dissolved or washed away.¹⁷ The development of a simple and general modification technique is challenging because of the wide variety of polymer materials available for microfluidic chips, which often require customized methods for derivatization.

Here, we spin coat a broadly available commercial resist, poly(methyl methacrylate-8.5-methacrylic acid) [P(MMA-MAA)], doped with HPC for polymer surface modification. P(MMA-MAA) is used as a high-resolution resist for electron beam as well as X-ray and deep-UV lithographic processes. We found that P(MMA-MAA) can dissolve HPC readily and has excellent adhesion to polymer substrates, which allowed spin-on thin-film modification of polymer microdevices, before channel imprinting and microchip bonding. We successfully applied this method in the fabrication of PMMA and cyclic olefin copolymer (COC) microchips. Contact angle measurements and X-ray photoelectron spectroscopy (XPS) showed a more hydrophilic surface and an increase in surface oxygen content after modification. Atomic force microscopy (AFM) data indicate an increase in surface roughness with higher HPC doping levels. Our devices were tested by separating a myoglobin digest, human serum albumin (HSA) and green fluorescent protein (GFP), and a human serum sample, all with improved performance relative to unmodified surfaces.

Experimental section

Reagents

P(MMA-MAA) ($T_g \sim 50^\circ\text{C}$, MW 50000, containing 8.5 mol% of acid groups) in ethyl lactate (11% solids) and 950PMMA were both obtained from Microchem (Newton, MA). Myoglobin from horse skeletal muscle (95–100%), trypsin from bovine pancreas, fluorescein isothiocyanate (FITC, 98%), immunoglobulin-depleted human serum, FITC-human serum albumin (HSA) and HPC (MW 10000) were obtained from Sigma (St. Louis, MO). Recombinant green fluorescent protein (GFP) was purchased from Clontech (Mountain View, CA). The run buffer (10 mM Tris, pH 8.2) and dimethyl sulfoxide (DMSO) were from Fisher Scientific (Pittsburgh, PA).

Sample preparation

The myoglobin digest was prepared by dissolving 10 mg myoglobin in 1 mL of 50 mM sodium bicarbonate buffer (pH 8.6). 0.5 mg trypsin in 50 μL HCl was then added, and the mixed solution was incubated at room temperature for 18 hours to complete digestion. The resulting digest was labeled by adding 0.6 mg FITC in 100 μL DMSO and storing for 2 days in darkness; lastly, the mixture was filtered and diluted to the desired concentration. A 50 μL human serum sample was mixed with 10 μL of 1 mg/mL FITC in DMSO; this solution was diluted to 100 μL in 100 mM bicarbonate buffer. The labeling reaction proceeded at room temperature for 2 hours, and then the sample was held at 4°C for 48 hours. Finally, the sample was filtered and diluted 100 fold before electrophoresis.

Surface modification and microfabrication

The surface modification and microfabrication process for polymer microchips is described schematically in Fig. 1 and outlined below, first for PMMA and then for COC. Briefly, PMMA sheets (Acrylite FF, CYRO Industries, Rockaway, NJ) were cut into 45 mm × 20 mm × 4 mm pieces (Fig. 1A). After cleaning with soapy water, rinsing, and drying with compressed air, the slides were spin-coated with the P(MMA-MAA) solution containing HPC at 3000 rpm for 1.5 min (Fig. 1B). Silicon templates for device imprinting were made as described previously.¹⁸ The elevated features in the Si template were embossed into the coated PMMA substrates in a convection oven at 140°C as described in earlier work.¹⁸ Channel heights were 10 μm, with a bottom width of 50 μm and a top width of 75 μm; these dimensions did not change noticeably during the bonding process. Each coated and imprinted substrate (Fig. 1C) was then bonded to a modified cover plate at 110°C for 30 min (Fig. 1D). Although the glass transition temperature of P(MMA-MAA) (~50°C) should allow bonding of surface-modified polymer pieces at lower temperatures, we performed this step at 110°C to provide a more uniform and robust coating. HPC is stable well above 150°C, and thus is unaffected by the imprinting/bonding procedures.

COC sheets (1020R, Zeon Chemicals, KY) were cut with a saw, and rough edges were trimmed using a sharp knife. After spin-coating the COC plates with P(MMA-MAA) containing HPC, surface imprinting similar to the PMMA procedure was done at 120°C for 30 min, followed by bonding at 105°C for 40 min.

Characterization of surface properties

Contact angles were measured using an Imagelite Lite Mite Series instrument (Stocker & Yale, Salem, NH). For each experiment, a drop of doubly distilled water was placed on the surface at room temperature, and contact angles were obtained by direct reading. The reported values are the average of three measurements performed on different regions of a given surface. An ESCA system XX-100 (Plymouth, MN) was used for XPS analysis. The XPS data presented in Table 1 are the average of four measurements taken from two batches of modified chips. The thickness of coated P(MMA-MAA)/HPC layers was measured with Alpha-step 200 metrology equipment (Tencor Instruments, Sunnyvale, CA). AFM tapping mode height images were obtained with a Multimode IIIa instrument (Veeco, Sunnyvale, CA); images were processed offline using the bundled software package.

Separation and detection

The microchip layout is shown in Figure 1E. Sample (10 μL) was placed into reservoir 1, while the other reservoirs were filled with run buffer. To load sample into the injection intersection, reservoirs 1, 2 and 4 were grounded, while 500 V were applied to reservoir 3. During separation, reservoir 2 was grounded, reservoirs 1 and 3 were maintained at 500 V, and 2000 V were applied to reservoir 4. The laser-induced fluorescence detection system has been described previously.^{18, 19} Briefly, excitation of fluorescent compounds was achieved using the 488-nm line from an air-cooled Ar ion laser (Laser Physics, West Jordan, UT), which was focused ~2 mm from the end of the separation channel with a 20× 0.45NA objective. Detector signal was both amplified and filtered using a SR-560 preamplifier (Stanford Research Systems, Sunnyvale, CA) and then digitized at a 10 Hz sampling rate with a PCI-6035E (National Instruments, Austin, TX) analog-to-digital converter interfaced with LabVIEW (National Instruments) software.

Results and Discussion

Contact angle measurements

To study the effects of surface modification, contact angle measurements were obtained for PMMA, P(MMA-MAA) treated PMMA, and various percentages of HPC-doped P(MMA-MAA) treated PMMA (Table 1). Native PMMA surfaces exhibited a mean contact angle of 65°, which is close to the 70° result reported by Schmalenberg et al.²⁰ Surfaces coated with pure P(MMA-MAA) had a contact angle of 60°, slightly lower than native PMMA. Surfaces coated with increasing concentrations of HPC-containing P(MMA-MAA) exhibited enhanced wettability and decreasing contact angles. A contact angle of 16° was obtained for the 2.0% HPC doped P(MMA-MAA) coated PMMA surface, which is significantly lower than the reported 36° of PEG-grafted PMMA,¹² and 37° of oxygen plasma treated PMMA.²¹ This small contact angle is consistent with the existence of hydrophilic hydroxyl-terminated HPC molecules on the surface. The hydrophilic surface resulting from the coating greatly reduced interactions between proteins and channel walls, and suppressed the electroosmotic flow, factors that can improve the electrophoretic separation. When the coated PMMA was soaked in water for up to 48 hours, there was a 4° or less contact angle change for HPC doping concentrations of 0.5% or lower, which indicates that at these concentrations HPC is entrapped stably on the surface. For PMMA pieces coated with P(MMA-MAA) having 1.0% or higher HPC doping, contact angles increased by about 20% after 4 hours of water exposure, which may be due to excess HPC on the surface that was readily dissolved. We also tried spin-coating films of HPC dissolved in 950PMMA, but the solvent (typically anisole or chlorobenzene) damaged the PMMA microdevices and resulted in cracks on the surface.

XPS analysis of modified PMMA surfaces

To quantify the atomic composition of the various surfaces and confirm HPC doping, we characterized these materials by XPS. Survey spectra showed two peaks, one at 282 eV and another at 528 eV, assigned to C1s and O1s, respectively. We used these peaks to determine C:O ratios for different surface modifications (Table 1). The C:O ratio of 2.5 we observed for native PMMA is in good agreement with the reported value of 2.8.²² Surfaces coated with P(MMA-MAA) containing increasing fractions of HPC had a gradual decrease in C:O ratio because of comparatively more hydroxyl groups on the HPC-modified surfaces. This is also consistent with the contact angle data, which indicate greater hydrophilicity with higher HPC doping levels.

Surface morphology

The surface morphology before and after coating was investigated with AFM (Figure 2), and the root mean square (RMS) surface roughness from these images is summarized in Table 1. Typical P(MMA-MAA) film thicknesses under our coating conditions were ~100 nm. For native and P(MMA-MAA) coated PMMA, the surfaces are very smooth with a roughness <1 nm (Fig. 2A–B and Table 1). For 0.1% HPC-modified surfaces (Fig. 2C), a small increase in roughness to 2.5 nm is observed. When the percentage of HPC doping goes to 0.5% and above, the treated surfaces show a significant increase in surface roughness to >20 nm. The greater roughness is due to both increased density and size of the recessed circular regions on the surface (Fig. 2D–F), which probably result from phase segregation or self-association in the spin-coated copolymer films with higher HPC concentrations.

Separation performance of surface-coated polymer microfluidic chips

The separation ability of copolymer-modified microdevices was tested by carrying out CE of a myoglobin digest, a mixture of FITC-HSA and GFP, and a fluorescently labeled human serum sample. PMMA microchips with HPC doped P(MMA-MAA) coated surfaces showed well-

resolved peaks with myoglobin digests (Figure 3). We evaluated the reproducibility of electrophoretic performance by carrying out consecutive separations without any conditioning procedure between each run. Fig. 3A shows CE of a myoglobin digest in a PMMA microchip that had both surfaces spin coated with P(MMA-MAA) having 0.5% HPC. Five consecutive injections and separations were done in 15 min, with each analysis taking <200 s. The relative standard deviations (RSDs) of migration time and peak height in five runs were 2.2% and 5.7%, respectively. A closer view of an individual CE analysis is shown in Fig. 3B. For the separation of the myoglobin digest, PMMA microchips modified with P(MMA-MAA) doped with various amounts of HPC (0.1%, 0.5%, 1.0%, 1.5% and 2.0%) all showed good separation. We further carried out five replicate myoglobin digest separations in four separate 0.5% HPC modified PMMA microchips and obtained a 2.5% migration time RSD and a 5.0% peak height RSD for the 20 runs. These results highlight the reproducibility and durability of the modified surfaces during CE.

We also tested the performance of native and modified PMMA for analyzing fluorescently tagged human serum (Figure 4). Separation in a device with unmodified PMMA yielded very poor results (Fig. 4A), with a sharp increase in signal followed by extremely severe tailing (due to analyte adsorption) and only a single peak (unconjugated FITC) in the electropherogram. Subsequent runs in devices with unmodified surfaces showed even lower signal for the FITC peak and higher background fluorescence, indicative of the extensive adsorption of fluorescently tagged serum components on the PMMA surface. On the other hand, CE of this same complex sample in a 1.0% HPC doped, P(MMA-MAA) coated PMMA device (Fig. 4B) showed a series of sharp, well-resolved bands.

For CE of the myoglobin digest, unmodified COC surfaces had a few sharp bands and one broadened peak in the electropherogram (Fig. 5A), most likely due to some sample adsorption on the channel walls. In contrast, a COC chip coated with P(MMA-MAA) doped with 0.5% HPC yielded excellent separation of the myoglobin digest (Fig. 5B). Similar to PMMA, uncoated COC had poor performance for protein separation (Fig. 5C), with a high, drifting background and no peaks when a mixture of HSA and GFP was run. However a COC microchip coated with P(MMA-MAA) having 1.0% HPC provided excellent separation performance for the HSA/GFP mixture (Fig. 5D).

In general, microchips with HPC doping concentrations of 1.0% or above had limited protein adsorption and good resolving ability. HPC doping concentrations as low as 0.1% yielded good separations of the myoglobin digest, which has reduced nonspecific adsorption compared to proteins. These results establish our method of spin coating prior to embossing or bonding as a general surface modification strategy for polymer microchips.

Conclusions

In conclusion, we have developed a simple and effective way to modify polymer microfluidic device surfaces, as an alternative to dynamic coating or direct chemical derivatization. The broad availability of commercial P(MMA-MAA) resists in cleanrooms should enable the widespread usage of our approach. The HPC-doped P(MMA-MAA) coating provided significantly improved performance in microchip CE of protein digests and protein mixtures, compared with uncoated devices. Moreover, due to the compatibility of this copolymer with glass, ceramics, and polymers, our modification technique should be readily transferable to other substrates. The surface derivatization methods presented herein show great promise to simplify microchip preparation and facilitate biomolecular separations.

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Fig. 1. Fabrication process for surface-modified microchips. Schematic side-on views of (A) unmodified polymer surfaces, (B) surfaces coated with a layer of P(MMA-MAA) containing HPC, (C) channels imprinted on a modified surface, and (D) imprinted and cover pieces bonded together. (E) Top view of the microfluidic chip layout (4 cm distance from reservoir 2 to 4; 1.4 cm distance from reservoir 1 to 3), and (F) photograph of a bonded microdevice.

Fig. 2. AFM images of native PMMA, and surfaces coated with P(MMA-MAA) and different concentrations of HPC. (A) Uncoated, (B) P(MMA-MAA) coated, (C) 0.1% HPC doped, (D) 0.5% HPC doped, (E) 1.0% HPC doped and (F) 1.5% HPC doped PMMA surfaces.

Fig. 3. Electropherograms of a myoglobin digest in PMMA microchips. (A) Replicate separations of a myoglobin digest in a microchip with a 0.5% HPC doped P(MMA-MAA) coating, and (B) expanded view of a single separation in (A).

Fig. 4. Electropherograms of human serum in PMMA microchips. (A) Separation of a fluorescently labeled human serum sample in an uncoated microdevice, and (B) in a microchip with a 1.0% HPC doped P(MMA-MAA) coating.

Fig. 5. Electropherograms of a myoglobin digest (A–B), and FITC-labeled HSA and GFP (C–D) in COC microchips. (A) CE of a myoglobin digest in an uncoated microchip, and (B) in a microchip with a 0.5% HPC doped P(MMA-MAA) coating. (C) Separation of FITC-HSA and GFP in an uncoated microchip and (D) in a microchip with a 1.0% HPC doped P(MMA-MAA) coating.

Table 1

Characterization of surface-modified microdevices: contact angle, XPS and RMS AFM roughness measurements for PMMA, P(MMA-MAA) spun on PMMA, and P(MMA-MAA) doped with various percentages of HPC and spun on PMMA.

Surface	Contact angle	XPS C:O ratio (std. dev.)	AFM surface roughness (nm)
Native PMMA	65°	71.4:28.6 (1.0)	0.94
P(MMA-MAA)	60°	70.0:30.0 (0.8)	0.87
0.1% HPC	49°	69.0:30.9 (1.4)	2.5
0.5% HPC	45°	68.4:31.6 (0.6)	21
1.0% HPC	32°	67.0:33.0 (0.7)	28
1.5% HPC	23°	66.9:33.1 (1.1)	97