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Research Article

Surface modification of poly(methyl methacrylate) for improved adsorption of wall coating polymers for microchip electrophoresis

The development of rapid and simple wall coating strategies for high-efficiency electrophoretic separation of DNA is of crucial importance for the successful implementation of miniaturized polymeric DNA analysis systems. In this report, we characterize and compare different methods for the chemical modification of poly(methyl methacrylate) (PMMA) surfaces for the application of wall coating polymers. PMMA surfaces coated with 40 mol% diethylacrylamide and 60 mol% dimethylacrylamide are compared to the PMMA surfaces first oxidized and then coated with hydroxypropylmethylcellulose or poly(vinyl alcohol) (PVA). PMMA oxidation was accomplished with UV/ozone or an aqueous solution of HNO_3 to yield hydrogen-bond donors for the spontaneous adsorption of the coating polymers. Contact angle measurements of UV/ozone exposed PMMA surfaces indicate increase in hydrophilicity, and polymer coated surfaces show a strong dependence on the coating polymer and the oxidation method. Fast and repeatable electrophoretic separations of a 10-base and 20-base DNA ladder were performed in PMMA micro CE devices. All analyses were completed in less than 10 min, resulting in the number of theoretical plates as high as 583 000 in a 7.7 cm long separation channel. The duration of UV/ozone treatment was found to have a considerable impact on separation performance. The microchips irradiated with UV for 10 min and coated with PVA as well as the microchips treated with HNO_3 and coated with HPMC were found to have the best separation performance. These results demonstrate facile and robust methods for the surface modification of PMMA enabling low-cost single use devices for electrophoretic DNA separations.

Keywords: Microchip DNA electrophoresis / Microfluidic device / Physically adsorbed coating / Surface modification / Wall coatings DOI 10.1002/elps.200600118

1 Introduction

Micro total analysis systems or microfluidic devices have generated significant interest in the last two decades because of reduced analysis times, reduced sample and reagent consumption and their ability to be fabricated into high-density arrays to perform high-throughput parallel analyses [1]. In microfluidic devices, hydrophobic, electrostatic, or other interactions can cause undesired, nonspecific analyte adsorption onto the microchannel walls leading to asymmetry in analyte zones, increased peak widths, and sample loss [2]. Furthermore, changes in the surface due to analyte adsorption from the running buffer can create a nonuniform distribution of the ζ-potential over the length of the microchannel. This results in nonuniform liquid flow profiles and local variation of EOF, leading to additional band broadening and poor reproducibility of electrophoretic separations [3, 4]. Therefore, the development and optimization of surface coating strategies is of crucial importance for reliable, repeatable, and high-efficiency electrophoretic DNA separations [5, 6].

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Abbreviations: DEA, *N*,*N*-diethylacrylamide; **DMA**, *N*,*N*-dimethylacrylamide; **EVA**, ethylene vinyl acetate; **HPMC**, hydroxypropylmethyl-cellulose; **LDPE**, low-density poly(ethylene); **LPA**, linear polyacrylamide; **PC**, poly(carbonate); **PMMA**, poly(methyl methacrylate); **PVA**, poly(vinyl alcohol); **TTE**, Tris-TAPS-EDTA buffer

Conventionally, inorganic substrates such as glass and silica have been used for the fabrication of microfluidic devices, and numerous silane-based chemistries originally developed to passivate fused-silica capillary surfaces were relatively simple to transfer to silica-based microfluidic devices [7]. However, the devices made from glass and silica substrates are not as economical as polymeric microfluidic devices for mass production. Moreover, the low cost of mass manufactured polymeric microfluidic devices could make the production of microfluidic devices feasible for applications in which cross contamination can yield unacceptable results (*i.e.* single-use disposable devices) [8, 9].

The development of robust and simple surface modification techniques for polymeric substrates is challenging due to the wide variety of polymeric materials available for microfluidic device fabrication. To date, there have been numerous reports on surface modification techniques of various polymer surfaces, such as UV/ozone graft polymerization [10], graft copolymerization [11], laser activation [12], vacuum UV irradiation [13, 14], oxygen plasma treatment [15, 16], and ion bombardment [17]. Henry *et al.* have demonstrated chemical modification of poly(methyl methacrylate) (PMMA) substrates *via* aminolyses of surface esters to yield amine-terminated PMMA surfaces [18]. The same group also studied chemical modification of poly(carbonate) (PC) surfaces by treatment with sulfur trioxide gas, yielding hydrophilic sulfonated surfaces [19, 20]. Further, Johnson *et al.* reported that the surfaces of PMMA microchannels can be modified by pulsed UV excimer laser irradiation (KrF, 248 nm) resulting in carboxylate groups at the PMMA surface [21, 22]. Although considerable work has been carried out on the modification of various polymeric materials, simple, fast, and automatable techniques that enable high efficiency biopolymer separations in microfluidic devices have yet to be fully developed.

In this report, we compare a few methods for the application of wall coating polymers to PMMA microchannels. Whereas our experiments explore a subset of the PMMA and coating polymer parameter space, the results identify several important parameters of the coating polymer protocols necessary to improve the aggregate separation performance. Specifically, the PMMA microchannels were: (i) flushed with an aqueous solution of poly (diethylacrylamide-*co*-dimethylacrylamide) (40 mol% diethylacrylamide and 60 mol% dimethylacrylamide) (40DEA-60DMA); (ii) oxidized with an aqueous solution of HNO_3 or UV/ozone, and subsequently flushed with an aqueous solution of the coating polymer, hydroxypropylmethylcellulose (HPMC), or poly(vinyl alcohol) (PVA).

Oxidation of the PMMA surface creates hydrogen bond donors that aid in the spontaneous formation of polymer coating on the surface of the microchannel. The low pressure mercury vapor lamp used in this study for UV/ozone treatment at room temperature in air generates over 95% of the UV energy at 254 nm and the balance at 185 nm. The presence of these two wavelengths in air causes continuous formation and destruction of ozone resulting in the generation of atomic oxygen [23]. In particular, the radicals react with O_2 molecules as well as with activated species produced by photolysis, resulting in oxidation of the polymer surface [14]. Therefore, UV irradiation of PMMA causes scission of various chemical bonds in polymer chains including C—C, C—H, C==O, and results in the formation of oxygen-containing species such as carboxylate and hydroxylate groups [24]. HNO_3 is also a rapid oxidizer and likely introduces nitrate groups on the surface of the microchannel. In either case, oxidation of the PMMA surfaces provides hydrogen-bond donors for spontaneous adsorption of the wall coating polymers.

Contact-angle measurements of the pristine and modified PMMA substrates are performed to quantify the change in wettability of the surface. We also demonstrate high-efficiency electrophoretic separations of ssDNA in the modified PMMA microchannels. Further, by performing a comparative study among different methods, we highlight the importance of surface preparation and coating polymer on separation efficiency. In addition, we show that by optimizing the wall coating, we are able to achieve significant increases in theoretical plate counts. The methods presented in this report are rapid, facile, and can be performed in an air or aqueous environments at room temperature. Therefore, these methods are amenable to automation, mass production and conducive to implementation in integrated microfluidic platforms.

2 Materials and methods

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 identified are necessarily the best available for the purpose.

2.1 Materials and reagents

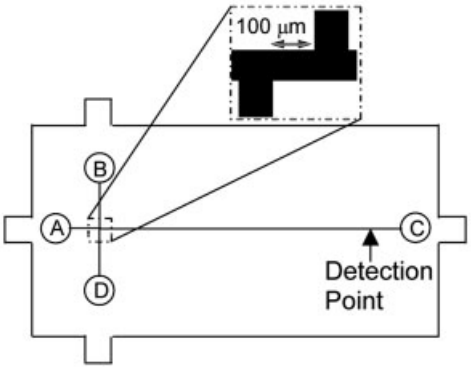
Silicon wafers were obtained from Virginia Semiconductor (Fredericksburg, VA). PMMA (Vistacryl CQ™, 1.7 mm thick) was obtained from Vista Optics (Widnes

Cheshire, UK). PC (125 μm thick) was obtained from McMaster-Carr (Dayton, NJ). A 70-base to 400-base fluorescein-labeled ssDNA ladder containing 20 fragments was obtained from BioVentures Inc. (Murfreesboro, TN). HPMC (viscosity 0.004 Pa·s (4 cP)) and PVA (87–89% hydrolyzed, average M_r 31 000 g·mol^{-1} to 50 000 g·mol^{-1}) were obtained from Sigma-Aldrich Inc. (St. Louis, MO). HNO_3 and H_3PO_4 were obtained from Fisher Scientific (Pittsburgh, PA). 89 mmol·L^{-1} Tris, 89 mmol·L^{-1} TAPS, 2 mmol·L^{-1} EDTA (TTE) with 7 mol·L^{-1} urea was used as background electrolyte for the polymer and as an electrode buffer. Biaxially oriented poly(propylene), 12 μm thick, coated on one side with a 15 μm thick adhesive made of low-density poly(ethylene)/ethylene vinyl acetate (LDPE/EVA) co-polymer resin was used as the laminating film for enclosure of microchannels and was obtained from Morane Ltd. (Oxfordshire, UK).

2.2 Device fabrication

A top-down view of the device used in this study is shown in Fig. 1. Microfluidic channels were fabricated by hot embossing patterns into PMMA substrates using photo-lithographically patterned silicon masters similar to the procedures previously published [25]. Briefly, a CO_2 laser-machined PMMA blank was aligned over a silicon master template ensuring that the fluidic access ports coincided with the raised structures on the silicon template at desired locations. The PMMA/silicon template assembly was then placed between two custom-built aluminum blocks with embedded heaters connected to a temperature controller. Subsequently, the assembly was mounted in a hydraulic press (Carver Inc., Wabash, IN) and the temperature was increased to 105°C. Once the assembly reached the desired temperature, the pressure was increased to 5.5 MPa. After approximately 30 min, the temperature was reduced to 80°C before releasing the pressure. The PMMA/silicon template assembly was then removed from the aluminum heaters and allowed to cool to the room temperature.

Sealing of the microchannel was accomplished by a thermal lamination process. First, the LDPE/EVA side of the laminating foil was placed over the microchannel side of the PMMA substrate. Then, the PMMA/laminating foil assembly was placed between a sheet of 75 g/cm^2 (20 lb; 0.09 mm) paper and a sheet of PC and rolled through a lamination machine (HRL 4200, Think & Tinker Ltd., Palmer Lake, CO) at a temperature of 160°C, and a velocity of 45.7 cm/min.



Step	Potential (V)			
	A	B	C	D
Injection	Float	0	Float	400
Separation	0	160	1500	170

Figure 1. Top-down view of PMMA micro capillary electrophoresis device. The device geometry is rectangular with four rectangular tabs used for gross device alignment to high voltage electrodes and an optical detection system. The PMMA substrate is 8.6 cm × 4.7 cm × 0.17 cm in size. Channel A is 0.25 cm long, channels B and D are 0.7 cm long, the separation channel is 7.7 cm long, and the fluidic wells are each 0.3 cm in diameter. The rectangular alignment tabs are 0.5 cm × 0.4 cm in size. The 100 μm offset "double-T" injector region is shown in the figure inset. The injection and separation voltages are shown in the Table.

2.3 Surface treatment

Figure 2 shows various methods investigated in this study for the application of coating polymers to the surface of the microchannel. Specifically, in Method 1 (■), an aqueous solution of 0.5% w/v 40DEA-60DMA was flushed through a pristine PMMA microchannel. In Method 2 (▼), the PMMA substrate imprinted with the microchannel was exposed to UV irradiation (12 to 20 mW/cm^2; UVCOS Inc., Montgomeryville, PA) for 15 min. Then, an aqueous solution of 0.5% w/v HPMC was flushed through the microchannel. It should be noted that UV irradiation was carried out prior to enclosing the microchannels and that the laminating foil was not treated with UV/ozone. In Method 3 (▲), the PMMA substrate with the microchannel was irradiated with UV light for 10 min followed by flushing with an aqueous solution of 0.5% w/v HPMC. In Method 3 (●), the microchannel was first flushed with a 3 mol·L^{-1} aqueous solution of HNO_3 and the residence time of HNO_3 in the microchannel was merely long enough to aspirate a plug of solution through the entire

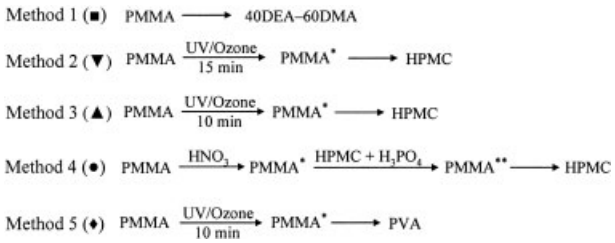


Figure 2. Schematic representation of the surface treatments investigated in the PMMA microchannel. Asterisks indicate modified surface.

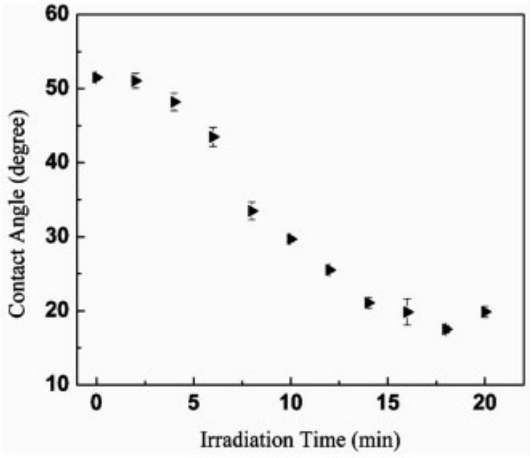
length of the microchannel. This was followed by rinsing with a 0.5% w/v solution HPMC dissolved in 20 mM H_3PO_4, and finally with an aqueous solution of 0.5% w/v HPMC. It should be noted that the HNO_3 treatment was performed in a sealed microchannel. In Method 5 (♦), the PMMA substrate with the microchannel was first irradiated with UV light for 10 min. Then, a 2% w/v aqueous solution of PVA was applied to the microchannel according to the following procedures partially adopted from the literature [6, 26]. The microchannel and reservoirs were first filled with PVA solution and incubated at room temperature for 10 min. Then, the reservoirs and channel were emptied by a vacuum pump. Thereafter, the adsorbed PVA coating was dried by heating at 110°C for 15 min. The above procedure was repeated for a total of two treatments resulting in a multilayer PVA coating. All of the microchannels were rinsed with deionized water before performing separations.

2.4 Contact angle measurements

Sessile drop-contact angle measurements utilizing approximately 6 μL drop of deionized water were performed with a contact angle analyzer (First Ten Ångstroms, Portsmouth, VA). Each of the contact angles shown in Fig. 3 were determined by averaging the values measured at three randomly chosen points on each sample surface. The samples were sonicated in ethanol for 10 min and dried using a N_2 gun prior to any treatment and the contact angle measurements were performed immediately after sample treatment.

2.5 DNA electrophoresis

All electrophoretic separations were conducted on the stage of a confocal laser-induced fluorescence microscope (Carl Zeiss MicroImaging, Inc., Thornwood, NY). Briefly, excitation of the fluorescein-labeled ssDNA was achieved by using the 488 nm line of a 30 mW argon ion laser. The laser beam was focused approximately 4 mm



Surface Treatment	Contact Angle (degree)	Std Dev (n = 3)
Method 1 (■)	64.0	3.98
Method 2 (▼)	49.4	0.40
Method 3 (▲)	46.9	1.14
Method 4 (●)	31.6	1.44
Method 5 (♦)	18.9	0.83

Figure 3. Water-contact angle measurements: (▶) UV-irradiated PMMA surface as a function of time. Error bars indicate the SD of the contact angle measurement. (■) PMMA surface coated with an aqueous solution of 0.5% w/v 40DEA-60DMA, (▼) PMMA surface irradiated with UV light for 15 min and coated with an aqueous solution of 0.5% w/v HPMC, (▲) PMMA surface irradiated with UV light for 10 min and coated with an aqueous solution of 0.5% w/v HPMC, (●) PMMA surface rinsed with 3 mol·L^{-1} solution of HNO_3 acid and coated with an aqueous solution of 0.5% w/v HPMC, (♦) PMMA surface irradiated with UV light for 10 min and coated with 2% w/v solution of PVA.

from the end of the separation channel using a 20 × 0.4 NA microscope objective. Fluorescence as a function of time at the end of the channel was monitored through a 505 nm long-pass filter by a photomultiplier tube. A high-voltage power supply (HV488, LabSmith, Livermore, CA) was used to provide voltages from 0 to 1500 V.

A solution consisting of 3% w/v 5× Nanogel (a polyacrylamide architecture) [27] dissolved in Tris-TAPS-EDTA (TTE) buffer with 7 mol·L^{-1} urea was used as a sieving matrix for the microchannel coated with 40DEA-60DMA. A solution consisting of 2.75% w/v 5× Nanogel (M_w ~ 8.8 MDa) and 0.25% w/v LPA (M_w ~ 638 kDa)

dissolved in TTE with 7 mol·L^{-1} urea was used as a sieving matrix for all other experiments. The sieving matrix buffer solution was pumped into the microchannel from reservoir C (Fig. 1) using a hydraulic laboratory press with custom built gaskets and jig with an applied pressure differential of 345 kPa. Reservoir B was filled with the sample, and reservoirs A and D were filled with buffer solution prior to analysis. Sample injection was accomplished by applying +400 V at reservoir D while keeping reservoir B at 0 V for 60 s (reservoirs A and C were left floating during the injection), for an effective electric field strength of 285 V/cm. Sample separation was performed by applying +1500 V to reservoir C, +160 V to reservoir B, and +170 V to reservoir D, while applying 0 V to reservoir A, for an effective electric field strength during separation of 190 V/cm. The "pull-back voltages" applied to prevent extraneous sample leakage from wells B and D into the separation channel were different due to the presence of the injection offset region shown in Fig. 1. Resolution was obtained by fitting Gaussian peaks to raw ssDNA separation data using PeakFit v4.06 (SPSS Inc., Chicago, IL). The separation efficiency, η, (plates m^{-1}) was calculated according to Eq. (1):

$$\eta = \frac{N}{L} = \frac{t^2}{L \cdot (\mathrm{FWHM}/2.35)^2} \quad (1)$$

where N is the number of theoretical plates, L is the migration distance in meters, t is the migration time in seconds, and FWHM is the full temporal width of the peak at half maximum [28]. The selectivity, which is defined as the peak spacing in a separation, is a measure of the sieving power of the network and was calculated using $\Delta\mu_{1,2}/\overline{\mu}_{1,2}$ with $\Delta\mu_{1,2}$ being the difference in mobility between fragments 1 and 2, and $\overline{\mu}_{1,2}$ being the average value of the mobility of fragments 1 and 2.

3 Results and discussion

Rapid, efficient electrophoretic separations of biomolecules are important for many applications including DNA sequencing, forensic DNA typing, and proteomics. The required electrophoretic separation resolution depends on the application, and as shown in Eq (1), it is affected by selectivity and peak width. In microfluidic separations, the efficiency is mostly limited by analyte-wall interactions because of the small injection plug width of the analyte band. These parameters, in general, are governed by the channel geometry, electrophoretic conditions, sieving matrix, and polymeric wall coatings, to name a few. Therefore, peak broadening can often be minimized by a robust and effective wall coating protocol, and careful characterization enables optimization of the coating protocol for high-efficiency separations. Below, we quantitatively measure wall hydrophilicity by performing contact angle measurements for various coating protocols and correlate it to electrophoretic separation efficiency.

3.1 Contact angle measurements

Changes in the water contact angles of pristine and UV-irradiated PMMA substrates as well as PMMA substrates treated with polymeric wall coatings are plotted in Fig. 3. The surface wettability of the UV-irradiated PMMA substrates varied significantly with the exposure time. The contact angle of the UV-exposed PMMA surface decreased from approximately 52 to 30° upon 10 min exposure and plateaued at approximately 20° after 14 min of exposure to UV light. The slight increase in contact angle at 20 min exposure may indicate overtreatment of the surface [29], and may be caused by a change in surface roughness or to a partial removal of the oxidized region from the PMMA surface [13]. UV irradiation of PMMA rendered it slightly yellow. However, this did not appear to have an adverse effect on the laser-induced fluorescence detection of ssDNA. The PMMA surface coated with 40DEA-60DMA (■) with a contact angle of 64° exhibited the highest degree of hydrophobicity. On the other hand, the PMMA surface coated with PVA (◆) resulted in a contact angle of 19° and exhibited the highest degree of wettability. Water contact angles of PMMA surfaces exposed to UV irradiation for 10 min or 15 min and subsequently coated with HPMC were found to be 47 (▲) and 49° (▼), respectively. The degree of hydrophilicity for both surfaces was approximately the same, which suggests that the wettability of the final surface depends on the coating polymer and not on further UV irradiation of the PMMA surface. The PMMA surface pretreated with HNO_3 and coated with HPMC (●) resulted in a contact angle of 32°. We postulate that H_3PO_4 trapped in HPMC pores is likely a factor contributing to the difference in contact angle from that obtained for PMMA surfaces exposed to UV and coated with HPMC. The trend in contact angles obtained for polymer coated surfaces was in agreement with theory as it is known that PVA is more hydrophilic than HPMC, which in turn is more hydrophilic than 40DEA-60DMA.

Oxidation, with UV/ozone or HNO_3, of PMMA renders the surface hydrophilic, and yields charged groups at the surface. Although hydrophilic surfaces are less prone to hydrophobic analyte-wall interactions, charged species would engender EOF and cause electrostatic analyte-wall interactions. Therefore, such surfaces are not directly suitable for high performance electrophoretic separation of ssDNA, which necessitates the application of charge-neutral, hydrolytically stable coating polymers on the

microchannel walls [30]. Additionally, it should be noted that charged groups on the PMMA surface are required for effective hydrogen bonding between the substrate and the coating polymer for enhanced physical adsorption of the coating polymer.

3.2 DNA separation performance

The effect of wall coating hydrophilicity on DNA separation performance was examined for a 20-base ssDNA ladder. Figure 4 shows representative electropherograms

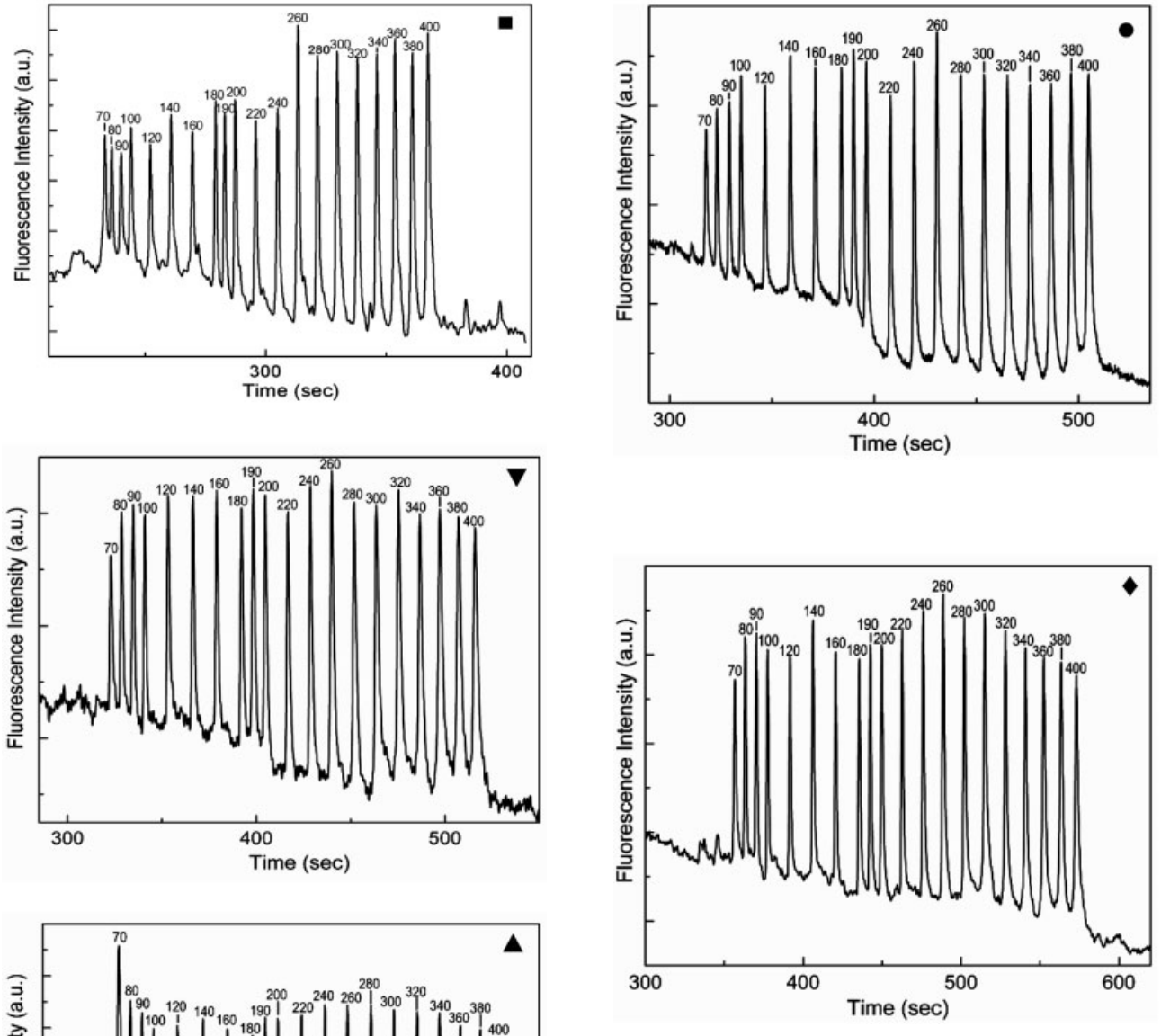


Figure 4. Typical electropherograms showing the separation of a 20-base ssDNA ladder. Electrophoretic conditions: injection field = 285 V/cm, separation field = 190 V/cm; separation buffer matrix: (A) 3% w/v 5× Nanogel in a TTE (89 mmol·L^{-1} Tris, 89 mmol·L^{-1} TAPS, 2 mmol·L^{-1} EDTA) + 7 mol·L^{-1} urea buffer; (B–E): 2.75% w/v 5× Nanogel + 0.25% LPA (molecular mass~638 kDa) in a TTE + urea buffer. (■) 40DEA-60DMA coating, (▼) 15 min UV irradiation followed by HPMC coating, (▲) 10 min UV irradiation followed by HPMC coating, (●) HNO_3 followed by HPMC coating, (◆) 10 min UV irradiation followed by PVA coating.

illustrating the CE separation for different surface treatments and polymer wall coatings. It should be noted that the coating polymers were not included in the sieving matrix solution and the surface was not recoated or reconditioned between consecutive runs. All the coating polymer protocols we investigated resulted in baseline separation of all ssDNA fragments. It can be seen from Fig. 4■ that separations in untreated PMMA surfaces coated with 40DEA-60DMA suffered from significant tailing, putatively due to hydrophobic interactions between ssDNA fragments and a relatively hydrophobic 40DEA-60DMA coating. Thus, 40DEA-60DMA was not investigated with oxidized surfaces as it is believed that it adsorbs to the microchannel surface *via* hydrophobic interactions [31]. The width of the peaks in the microchannel that was exposed to 15 min UV irradiation (Fig. 4▼) and coated with HPMC was greater than that in the microchannel exposed to 10 min UV irradiation and coated with HPMC (Fig. 4▲). As mentioned in Section 3.1, it is possible that the surface roughness increases as the UV irradiation time increases, and that the sample interactions with rougher surface tend to widen the peaks (Fig. 4▼). In comparison with Fig. 4▼ and 4▲, the PMMA microchannel oxidized with an aqueous solution of HNO_3 and subsequently coated with HPMC (Fig. 4●) showed reduced peak tailing. Similarly, PVA coated PMMA surfaces with prior exposure to UV for 10 min (Fig. 4◆) clearly suppressed analyte adsorption and also resulted in superior separation performance in agreement with the results reported for fused silica capillaries.

3.2.1 Theoretical plate count

Theoretical plates, *N*, obtained for an average of two runs for various surface treatment protocols are shown in Fig. 5. The microchannel coated with 40DEA-60DMA (■) resulted in the lowest number of theoretical plates of all surface treatments investigated with *N* ranging from 117 000 to 311 000. There was a pronounced difference in the ssDNA separation performance (theoretical plates) between the microchannels coated with 40DEA-60DMA (■) and PVA (◆). The number of theoretical plates from PVA coated microchannels (◆) varied from 266 000 (3.6×10^6 plates$\cdot$m^{-1}) to 561 000 (7.7×10^6 plates$\cdot$m^{-1}) indicating as much as a twofold increase from that obtained with the application of 40DEA-60DMA coating for most of the fragments analyzed. The number of plates from the microchannels irradiated with UV for 10 min and coated with HPMC (▲) were found to be between 110 000 and 406 000, whereas the number of plates from 15 min UV irradiated and HPMC coated (▼) microchannels ranged from 144 000 to 347 000. Even though the range

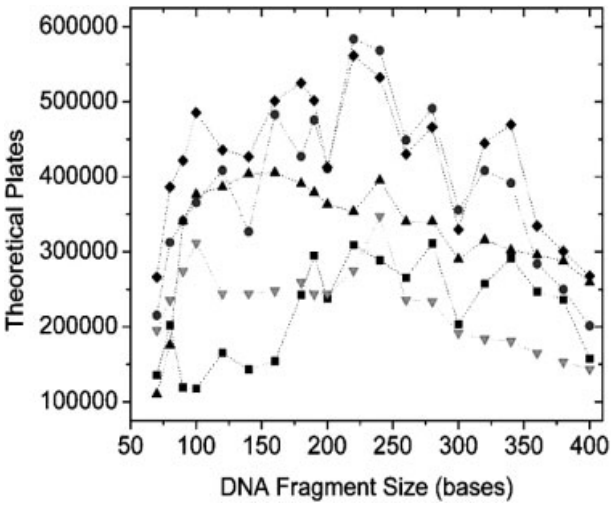


Figure 5. Theoretical plate count of ssDNA fragments, obtained for different surface treatments protocols. (■) 40DEA-60DMA coating, (▼) 15 min UV irradiation followed by HPMC coating, (▲) 10 min UV irradiation followed by HPMC coating, (●) HNO_3 followed by HPMC coating, (◆) 10 min UV irradiation followed by PVA coating. The lines are drawn to guide the eye.

of values obtained for ▼ fall within the range of values obtained for ▲, careful examination of Fig. 5 shows that ▲ produced overall greater efficiency. Specifically, the mean of the ratio of ▲ to ▼ for the number of theoretical plates was found to be 1.44 with a SD of 0.33. Significantly diminished *N* for ▼ may be attributed to increased roughness of the PMMA surface due to longer UV exposure times. The microchannels oxidized with HNO_3 and coated with HPMC (●) resulted in greater *N*, ranging from 201 000 (2.8×10^6 plates$\cdot$m^{-1}) to 583 000 (8.0×10^6 plates$\cdot$m^{-1}), compared to those treated with UV/ozone and coated with HPMC. These results are consistent with the decreasing contact angle measurements. Although the difference in contact angle between ● and ◆ is significant, it is interesting to note that *N* is comparable for both cases. This is in agreement with our speculation that the HNO_3 treatment resulted in all four of the channel walls being oxidized and coated rather than just the three PMMA walls as is the case for UV/ozone treatment of the microchannels. UV irradiation of the microchannel cover plate was not performed because contact angle measurements of the UV exposed LDPE/EVA surfaces for varying UV exposure times did not produce significant change in hydrophilicity (data not shown). More importantly, UV exposure of the LDPE/EVA surface significantly reduced bond integrity, which precluded longer exposure times. Even though the interactions of the various coating polymers with LDPE/EVA surface may contribute to improving the separation performance, they are not

believed to dominate the improvement in separation performance as LDPE/EVA only amounts to approximately 40% of the total surface area of the microchannel.

3.2.2 Selectivity

The average selectivity for various surface treatments is plotted in Fig. 6. The selectivity was found to vary from 0.001 to 0.002 for all of the wall coating protocols investigated in this study. Hence, it can be inferred that the selectivity is largely independent of the microchannel coating protocol, and that there is no incompatibility between any of the wall coatings and the sieving matrix solution.

3.2.3 Resolution

Figure 7 shows the average resolution, $\Re$, normalized to the difference in fragment size for all surface treatments investigated in this study. The average resolution obtained for 40DEA-60DMA coated microchips varied from 0.08 to 0.17 base^{-1}. As seen from Fig. 7, electrophoretic separation of ssDNA in the microchannel irradiated with UV for 15 min and coated with HPMC yielded $\Re$ from 0.08 to 0.21 base^{-1}. In contrast to 15 min UV/ozone treatment, 10 min UV/ozone treatment using the same coating resulted in higher $\Re$, 0.11 to 0.23 base^{-1}. These results are qualitatively consistent with those obtained for η. The resolution achieved using the microchannels treated with HNO_3 as oxidizing agent and coated with HPMC resulted in $\Re$ from 0.11 to 0.24 base^{-1}. Finally, 10 min UV treatment along with PVA coating yielded the highest resolution of all treatments studied with $\Re$ ranging from 0.12 to 0.27 base^{-1}. The increase in resolution from the 40DEA-60DMA coating to the PVA coating was due to a reduction in peak width and not peak spacing indicating that the reduction in analyte-wall interactions due to a more hydrophilic wall coatings is likely the reason for improved resolution.

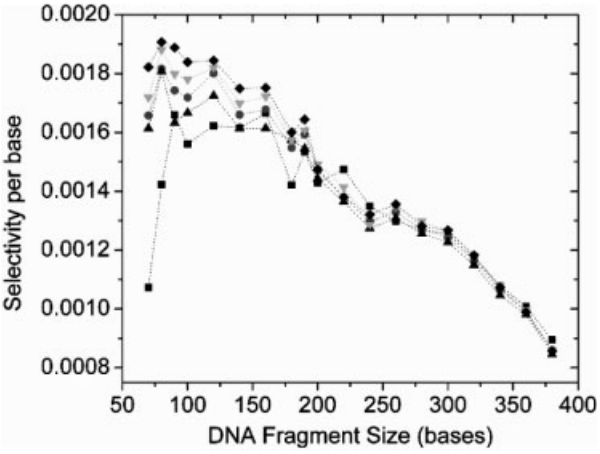


Figure 6. Selectivity of ssDNA fragments as a function of DNA fragment size, obtained for different surface treatment protocols. (■) 40DEA-60DMA coating, (▼) 15 min UV irradiation followed by HPMC coating, (▲) 10 min UV irradiation followed by HPMC coating, (●) HNO_3 followed by HPMC coating, (◆) 10 min UV irradiation followed by PVA coating. The lines are drawn to guide the eye.

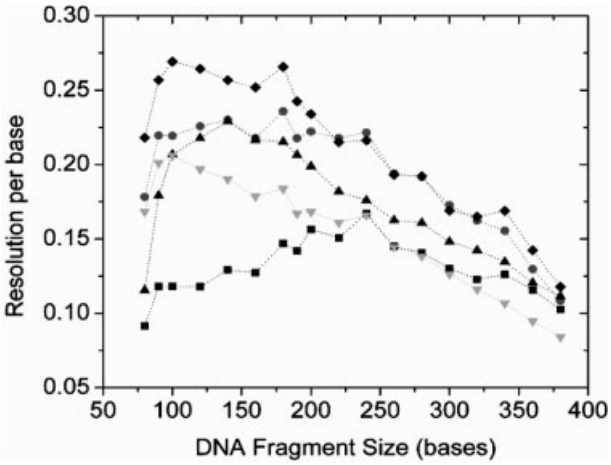


Figure 7. Resolution of ssDNA fragments as a function of DNA fragment size, obtained for different surface treatments and polymer coatings. (■) 40DEA-60DMA coating, (▼) 15 min UV irradiation followed by HPMC coating, (▲) 10 min UV irradiation followed by HPMC coating, (●) HNO_3 followed by HPMC coating, (◆) 10 min UV irradiation followed by PVA coating. The lines are drawn to guide the eye.

In summary, we have shown that the efficiency of ssDNA fragments and hence the resolution increases as the coating polymer hydrophilicity increases. HNO_3 treatment of the interior of a channel appears to activate significantly more channel surface area and does not require specialized laboratory equipment. On the other hand, UV/ozone treatment of PMMA substrates produces a significantly more hydrophilic surface than UV/ozone treatment of LDPE/EVA surfaces. Hence, the microchannels which are entirely composed of PMMA such as those fabricated with thermal bonding or solvent bonding [32] method would be expected to produce superior separation performance. The UV/ozone treatment is also simple to implement and could be easily localized to specific areas of integrated microchips with complex architecture by using photomasks allowing for spatial patterning of different surface chemistries.

4 Concluding remarks

In this work, we presented several methods for the chemical modification of PMMA surfaces for the application of wall coating polymers to suppress EOF and to

reduce analyte-wall interactions. The hydrophilicity of the PMMA surface was altered by UV irradiation and HNO_3 treatment in air at atmospheric pressure and at room temperature. Contact angle measurements were conducted to determine the change in wettability of the surface. Electrophoretic separations of ssDNA were successfully performed in microchips prepared using these methods. It was empirically observed that 10 min UV irradiated surfaces coated with HPMC yielded better separation performance than surfaces irradiated with UV for 15 min and coated with HPMC. Further, UV/ozone treated surfaces coated with PVA and HNO_3 treated surfaces coated with HPMC resulted in the highest separation performance with plate efficiency regularly exceeding 413 000 plates. Subsequently, the two coating protocols have been successfully used in our lab for ssDNA separations in longer channels to further improve resolution. We have also shown that the hydrophilicity of the wall coating polymer can increase the separation efficiency and resolution. Additionally, this approach to wall coating should be versatile and transferable to other polymeric substrates. Finally, the methods presented in this paper are less time-consuming and less laborious than those published previously and are conducive to automation for mass production of disposable microfluidic devices.

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