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Cationic polymer coatings for design of electroosmotic flow and control of DNA adsorption

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Abstract

A difficulty with the design and operation of an electrokinetically operated DNA hybridization microfluidic chip is the opposite direction of the electroosmotic flow and electrophoretic mobility of the oligonucleotides. This makes it difficult to simultaneously deliver targets and an appropriate hybridization buffer simultaneously to the probe sites. In this work we investigate the possibility of coating the inner walls of the microfluidic system with hexadimentrine bromide (polybrene, PB) and other cationic polymers in order to reverse the direction of electroosmotic flow so that it acts in the same direction as the electrophoretic transport of the oligonucleotides. The results indicated that the electroosmotic flow (EOF) in channels that were coated with the polymer could be reversed in 1× TBE buffer or 1× SSC buffer. Under these conditions, the DNA and EOF move in the same direction, and the flow can be used to deliver DNA to an area for selective hybridization within the channel. The effects of coating the surface of a nucleic acid microarray with polybrene were also studied to assess non-selective adsorption and stability. The polybrene coating significantly reduced the extent of non-selective adsorption of oligonucleotides in comparison to adsorption onto a glass surface, and the coating did not alter the extent of hybridization. The results suggest that use of the coating makes it possible to achieve semi-quantitative manipulation of nucleic acid oligomers for delivery to an integrated microarray or biosensor.

Keywords: Microfluidics; DNA microarray; Electroosmotic flow; Hybridization

1. Introduction

DNA detection technology plays a very important role in almost every field of modern life science. DNA microarray techniques, based on selective hybridization between the immobilized single-stranded DNA (ssDNA) probes and complementary targets, have been used for clinical diagnosis, forensic science, detection of genetically modified foods, and investigations into drug resistance. The most important feature of commercial microarray technology is that hundreds of thousands of DNA fragments can be immobilized onto one single slide and that highly parallel analysis can be done. Although the fundamental principles required for parallel processing have been achieved in practice, there are still numerous shortcomings that must be overcome. Diffusion limited transport of biomolecules to surface mounted materials is typically very slow, particularly at low concentrations, [1] resulting in overnight hybridization times. The environmental conditions of the procedure, such as temperature and salt concentration, must be strictly controlled, a washing procedure is needed for removal of non-selectively adsorbed target on the glass surface, resulting in a large amount of time consuming manual steps. Additionally large quantity of DNA sample (at least 20–30 μl) is often required.

A substantial step in modern analytical science was proposed when researchers tried to integrate all the usual manipulative and analytical functions into small fluidic chip using modern lithography techniques, blossoming a new field commonly reffered to as μTAS [2] or Lab on a chip. Examples of some early applications include: PCR performed in a small silicon chamber [3], in a small chamber on a glass microchip in combination with a separation channel [4], or a continuous-flow channel [5]. Various functions such as DNA sequencing have been reported using glass chips

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[6]and plastic chips [7]. Other researchers have attempted to use microfluidic techniques for DNA hybridization to overcome some of the shortcomings of microarray technology. Microfluidic devices that are suitable for fluidic circulation and mixing can improve hybridization signal intensity on DNA arrays. Wang et al. [8] made a polymethylmethacrylate (PMMA)-based microfluidic device for LDR detection. SPR imaging was used for determination of hybridization on 1-D and 2-D microarrays [9]. By applying microfluidics and microfabrication techniques, the best features of microarrays such as the capability of large-scale parallel analysis, can be combined with the handling of very small volumes of solution to achieve rapid analytical results at relatively low cost.

While pressure-driven flow has commonly been used in DNA hybridization microfluidic devices for delivery of oligonucleotides, it is well known that electroosmotic flow (EOF) offers many advantageous features. Voltage manipulation is simple and easy to control, and the technique is suitable for precisely delivering buffer solutions at nano- to pico-liter volumes. Additional device structures such as pumps and valves are unnecessary, making miniaturization simpler. Most importantly however, DNA samples may be delivered to the hybridization zone of a sensing platform in small volumes, allowing the possibility of quantitative analysis of the DNA samples.

When designing a high-voltage-driven microfluidic device for DNA hybridization detection, it becomes crucial to select an appropriate substrate. The surface properties of the material must provide for the possibility of immobilization of ssDNA, and must support a stable surface charge to enable significant EOF. Glass is a good choice for development of microfluidic devices because structures can be fabricated by lithography and etching. Also the glass material offers a reactive surface for the support of ssDNA. Commonly, hydroxyl groups on the glass surface are be modified using spacers that are terminated with functional groups, onto which ssDNA can be immobilized [10].

DNA molecules are polyelectrolytes with substantial negative charge. Such molecules move in a reversed electrophoretic direction to the EOF in a glass channel. This makes it more difficult to sequentially deliver target DNA and buffer wash solution. Furthermore, DNA also adsorbs to glass, interfering with the hybridization signal. Surface modifiers such as Denhardt's solution were often added in the hybridization buffer for reduction of non-selective adsorption [11].

Solutions that are used for reduction of non-selective adsorption in DNA microarray techniques usually contain some components that are not compatible with EOF control. For example, one of the main components in the Denhardt's solution, polyvinylpyrrolidone (PVP) was used in capillary electrophoresis [12]and on a microchip [13] for suppressing EOF. Positively charged polymers such as polybrene (hexadimentrine bromide) have been used to reverse the EOF direction in capillary electrophoresis [14–18]and electrophoresis on microchips, and it is possible to add sandwich layers of polymers for EOF control [19].

While modifiers such as Denhardt's solution can effectively block active sites on glass to curtail non-selective adsorption, it is not at all clear that cationic polymers can serve this purpose. Clearly electrostatic interactions between the negatively charged DNA and cationic the polymer are expected. A second issue that must be considered is that the polymer coatings alter the hydrophilicity of the glass surface. There are no detailed reports investigating the effectiveness of cationic polymers in their dual role as coatings to control the direction of EOF transport, and surface modifiers to reduce adsorption. In this paper, we investigate a number of cationic polymers as coatings on microfluidic chips for application in flow delivery of target ssDNA to a low resolution microarray. The effects of the polymer coating on EOF transport of DNA, and on DNA hybridization and adsorption were studied using glass chips, and microscope slides that supported covalently immobilized probe DNA. It was found that a simple coating procedure to deposit cationic polymer could result in the reversal of the direction of EOF in a glass channel. An "electrostatic interaction network" model was used to describe the polymer coating using experimental data. Real-time DNA transport was observed using a fluorescence microscope and dye-labeled ssDNA, and demonstrated controllable DNA delivery by EOF in the glass channel.

2. Experimental

2.1. Reagents

Autoclaved double-distilled water was used in the DNA immobilization/hybridization experiments. Water prepared using a cartridge purification system (Milli-Q, Millipore Corp.), was used to prepare solutions for EOF experiments. All chemicals used were analytical grade or better. Hundred times Denhardt's solution (2% (w/v) Ficoll, 2% (w/v) polyvinylpyrrolidone, 2% (w/v) acetylated BSA, in Molecular Biology Grade Water) was from Brinkmann Instruments Inc. (Mississauga, Ont., Canada). Polybrene (hexadimentrine bromide, abbreviated as PB in this paper), and a 20% solution of poly(diallyldimethylammonium) chloride (PDADMA) with low molecular weight (M_W 100,000–200,000) and medium molecular weight (M_W 200,000–350,000) were from Sigma Canada (Oakville, Ont., Canada). The reagents for glass surface silanization, such as 3-glycidoxypropyltrimethoxysilane (GOPS) and *N*,*N*-diisopropylethyamine (Hünig's base), and the reaction solvent and the washing solvents, were all from Sigma Canada.

All oligonucleotides were from Sigma GENOsys (Oakville, Ont., Canada), except for the Cy5-labeled oligonucleotides, which were prepared using a 392 DNA synthesizer (Applied Biosystems Inc., Foster City, CA).

2.2. Instruments

A high voltage (HV) supply model CZE1000R from Spellman (Hauppauge, NY, USA) was used in EOF investigations. A 10 kΩ resistor was serially connected between the grounding of the HV power supply and the microchip electrode. A chart recorder and a multimeter were used to monitor the voltage on the 10 kΩ resistor, and allowed for monitoring of the current through the microchip channel.

A Virtek Chipreader (Virtek Vision Corp, Waterloo, Canada) was used for scanning the microscope slides that were modified with immobilized/hybridized oligonucleotides. The scanned images were stored in 16-bit TIFF format and converted to numerical format using ImageQuant V5.2 software from Molecular Dynamics. Ellipsometry was used to investigate the thickness of polymer coatings on silicon wafers, glass and quartz surfaces (Rudolph Research model AutoEL II, Flanders, NJ, USA). The instrument used a wavelength of 632.8 nm, and an incident angle of 70°. Measured values of Δ and Ψ were converted to thickness and refractive index information using software that was integrated into the ellipsometer.

2.3. Coating procedure and electroosmotic flow measurements

Electroosmotic studies were done on a glass microchip from Micralyne Inc. (Edmonton, Alberta, Canada). The channel width (top position) was 50 μm and the depth of the channel was 20 μm. The length of the channel between the two reservoirs to which the high voltage was applied was 85 mm. High voltage of 1 kV was applied between the two electrodes during the EOF measurement.

The electroosmotic flow measurement was based on current monitoring during a buffer displacement experiment [20]. Briefly, the buffer in the port with positive electrode was substituted with the buffer which concentration was slightly different from that in the channel. Due to the different conductivity of the buffer, the current was supposed to change when the buffer in the positive-electrode port went through the channel, until it reached the other port. The average velocity of the buffer could be obtained by measuring the time duration. The electroosmotic mobility was calculated by the following equation.

$$\mu_{\mathrm{EOF}} = \frac{v}{E} = \frac{l/t}{V/l} = \frac{l^2}{Vt} \quad (1)$$

In Eq. (1), μ_{EOF} was the electroosmotic mobility, v was the average velocity of the buffer in the channel, E was the electric field along the channel, l was the length of the channel, V was the voltage applied to the microscope channel and t was the duration in which the substituting buffer moved from one port to the other port driven by the EOF.

The procedure for measurements in the untreated microchip channel began with sequential rinsing by 0.1 M NaOH solution for 10 min and deionized water for 10 min. 0.5× TBE buffer was filled into the channel using 1 ml syringe. High voltage was applied for 10 min to achieve a steady current. The 0.5× TBE buffer in the positive electrode port was withdrawn by a 25 μl syringe and 1× TBE buffer was filled into the port. Then the HV was applied to the channel, and the time measurement was initiated. After the current reached a plateau, the measurement channel was thoroughly rinsed with deionized water and 0.1 M NaOH solution. The measurement was repeated three times for every data point. The same procedure was used for the measurements of the EOF mobility in 1× SSC, pH 7.0 and 0.1× SSC buffer, using 0.5× SSC buffer and 0.05× SSC buffer as the substituting buffers, respectively.

The polymer coating procedure began with flushing the microchip channel with 0.1 M NaOH and deionized water for 15 min, sequentially. Then the polymer solution (either 2.5% PB solution or 5% PDADMA solution), was injected into the microchip channel. The polymer solution was kept in the channel for 15 min and then was flushed out with deionized water. The EOF measurements followed that same procedure as for the untreated microchip except that the substituting buffer was filled into the port with the negative electrode since the direction of EOF had been reversed by the coated polymer.

2.4. Oligo hybridization procedure

Oligonucleotide immobilization on glass slides was done using published protocols [21]. Briefly, a glass slide (Fisher Finest, 75 mm × 25 mm, 1.1 mm thick) was cleaned with soap and put into water for a 30 min sonication. The slide surface was further treated with a mixture of 30% hydrogen peroxide, ammonia and water (v/v, 1:1:5) at 80 °C for 20 min, and 30% hydrogen peroxide, hydrochloric acid and water (v/v, 1:1:5) at 80 °C for 20 min, sequentially. After cooling, the slide was sequentially washed with distilled water, methanol, dichloromethane, and anhydrous ethyl ether. The slide was kept in an oven of 120 °C before reaction. The above procedure was used for removing contamination, creating surface attachment sites such as hydroxyl groups and controlling the surface roughness [22]. The treated slide was put into a 500 ml round bottom flask with 30 ml of GOPS and 1 ml Hünig's base in 100 ml toluene, and was left to reflux overnight. The slide was then washed with methanol, dicholormathane and anhydrous ethyl ether, and was kept in a vacuum desiccator before use. Ten micrometers of the ssDNA probe 5′-NH_2-C_{12}-dA_{20} in water was manually applied to the glass slide using a pipette tip after the glass surface had underwent GOPS modification. The slide was kept in a humid environment overnight at room temperature. Before hybridization, the slide was thoroughly rinsed with 1× PBS buffer and was dried in air.

Hybridization on the slide was allowed to proceed by adding 1 μM the dye-labeled oligonucleotide, 5′-Cy5-dT_{20} or 5′-Cy5-$dT_8dA_3dT_9$, in 1× SSC buffer. Twenty microliters of target oligonucleotide solution was spotted onto the slide

and formed a thin layer on the hybridization zone. The slide was kept in a humid environment for 3 h, at room temperature. After hybridization, the slide was washed with 1× SSC buffer containing 0.01% SDS, and was then rinsed in 0.1× SSC buffer containing 0.1% SDS. After drying, the slide was scanned using the Virtek Chipreader.

For investigations of surface modification, a 5× Denhardt's solution, 2.5% PB solution or 5% PDADMA solution was respectively applied to the slides with the immobilized probes for 10 min, followed by washing using 1× SSC buffer before hybridization.

2.5. Ellipsometry

Silicon wafers, and microscope slides composed of glass and quartz, were cleaned and sonicated before treatment. The treatment procedure was similar to that described in Section 2.4. Polymer solution was applied to the surface for 15 min, followed by washing with water before the measurement. After drying in air, the ellipsometric data was measured using the AutoEL instrument.

3. Results and discussion

3.1. Electroosmotic flow when using polymer coating

Fig. 1 shows the results of the current monitoring based EOF mobility measurements in 1× TBE and 1× SSC buffer both before and after coating with PB, low molecular weight PDADMA and medium molecular weight PDADMA. As can be seen the polymer coatings were successful in reversing the direction of the EOF. In all cases, the EOF in the polymer coated channels were stable for at least 1 day. The reproducibility of EOF mobility measurements was good in a day with a standard deviation of 6%. In high salt concentration 1× SSC buffer, Joule heating problems had a severe effect on the current, and the maximum salt concentration associated with 0.5× SSC buffer was used to quantitatively monitor the current change. The coated polymer could be removed by flushing 1N NaOH solution through the channel for 15 min. After NaOH treatment and subsequent rinsing with water, the EOF was determined to be the same as that observed for an untreated uncoated glass channel.

The data of Fig. 1 indicates that there is no significant difference in EOF mobility for the three different types of cationic polymer coatings that were examined. From a practical perspective, the medium molecular weight PDADMA had a much higher viscosity than the two solutions, and it was difficult to introduce the medium molecular weight PDADMA into a channel by syringe.

For DNA transport in the free solution, the apparent mobility of DNA, μ_{app}, is approximated by the summation EOF mobility of the bulk solution, μ_{EOF}, and the electrophoretic mobility of the negatively charged molecules, $\mu_{electrophoretic}$, as described by Eq. (2).

$$\mu_{app} = \mu_{electrophoretic} + \mu_{EOF} \quad (2)$$

By coating the channel with cationic polymer, the direction of μ_{EOF} was changed to be in the same direction as that of the electrophoretic mobility. The apparent mobility of DNA was increased as shown in Fig. 2, and the transport time to move the DNA through the channel was shortened. Concurrently, it was determined that buffer solution could easily be delivered for washing without contamination of the wash solution, given that the oligonucleotide movement and the bulk solution movement were in the same direction.

3.2. Surface modification of slides used for hybridization

3.2.1. Effects of cationic polymer coating on the adsorption of DNA

It is necessary to ameliorate or eliminate non-selective adsorption of DNA on the glass substrate in order to

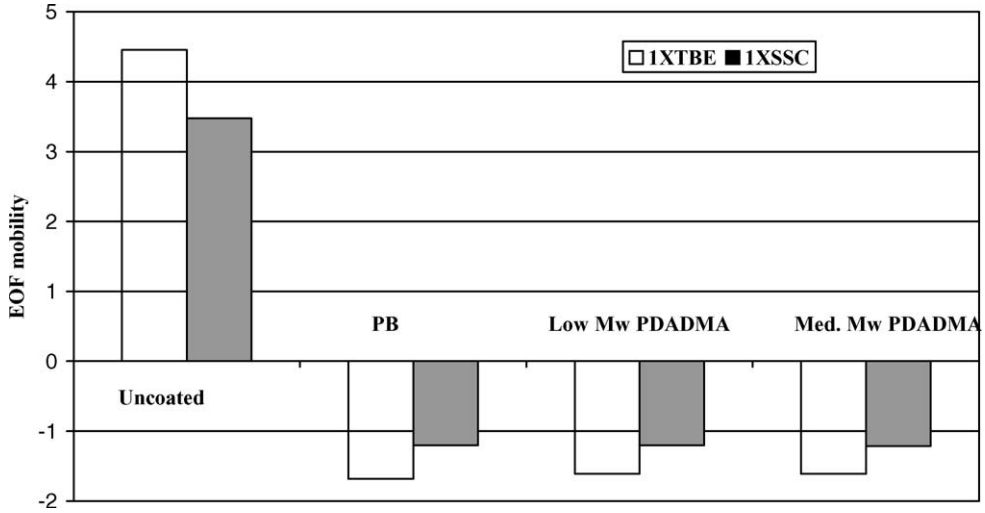

Fig. 1. Electroosmotic mobility measurements made using a Micralyne microchip. *Y*-axis, EOF mobility ($\times 10^4$, $cm^2\,V^{-1}s^{-1}$). Channel size 50 μm × 20 μm × 85 mm, applied voltage 1000 V.

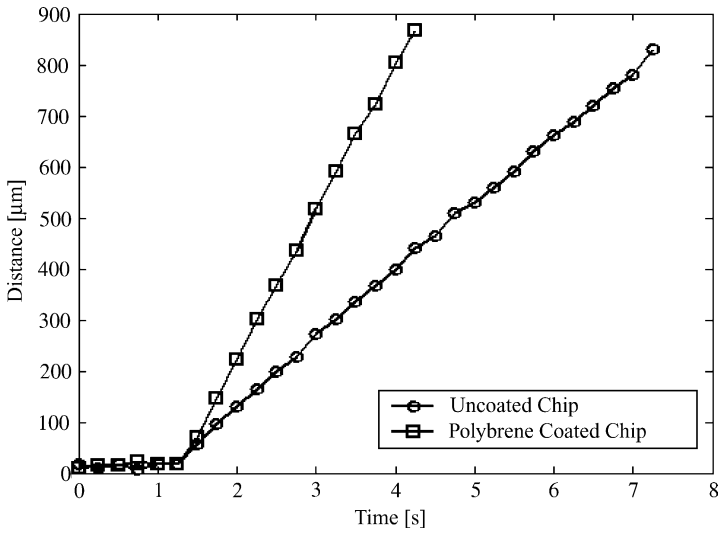

Fig. 2. Progression of a fluorescein tagged 20 mer ssDNA sample plug with time for a PB coated and an uncoated glass microchip. 1× TBE buffer was used. Instantaneous sample location based on point of maximum concentration. Applied voltage during dispensing phase was approximately 90 V cm^{-1}. The direction of DNA motion was from the negative to the positive port.

reduce background signal. Commonly a blocking procedure would be implemented, and would rely on Denhardt's solution or some other surface modifier for surface passivation [23,24]. Cationic polymer has not been used for this purpose because electrostatic interaction between the negatively charged oligonucleotides and the positively charge polymer surface would be expected to enhance non-selective adsorption. This work investigated the effects of the various cationic polymer coatings on DNA adsorption. Glass microscope slides were cleaned and the upper parts of the slides were coated with either 5% PB solution, 5% low molecular weight PDAMDA or 5% medium molecular weight PDADMA. The slides were washed with water and dried in air, and then 10 μM 5′-Cy5-dT_{20} oligo was manually deposited to the slides on both the untreated and polymer treated areas as was left standing overnight. After washing, the slides were scanned with the Virtek Chipreader and representative fluorescence intensity images are shown in Fig. 3.

The dye-labeled oligonucleotide demonstrated adsorption on both the untreated and polymer treated areas. It was difficult to wash the adsorbed oligonucleotides from the glass surface, even when using long wash times at 30–40 °C. Adsorbed dye-labeled oligonucleotide were observed to spread across the surface during washing, suggesting removal from polymer coated areas, but even more tenacious re-adsorption at uncoated glass areas. A significant difference between the background of these two areas is shown in Fig. 3(d). With the cationic polymer coating, the contamination during the washing steps was almost eliminated, while the spread on the untreated glass was relatively severe. These results clearly showed that the cationic polymer coatings of PB and PDADMA could be used to reduce DNA adsorption in comparison to unmodified glass.

Further study of the adsorption behavior on polymer coated surfaces involved reduction of the contact time so that it was consistent with microfluidics manipulations. A period of 15 min was used for 1.0 μM dye-labeled DNA adsorption to simulate the target DNA in the microfluidic channel. Before applying dye-labeled dT_{20}, slides were treated with PB (Fig. 4a), low molecular weight PDADMA (Fig. 4b) and medium molecular weight PDADMA (Fig. 4c) on the upper parts of the slides for 10 min, respectively. The lower parts of the slides were concurrently treated with 5× Denhardt's solution. The center sections of the slides were left untreated. After surface treatment, the slides were washed, and were dried in air. Then 10 μM 5′-NH_2-C_{12}-dA_{20}in water was dropped on the three different zones and the slides were kept in a high humidity environment for 3 h. The slides were then thoroughly washed with 1× PBS buffer and water. Finally, complementary Cy5-labeled dT_{20} in 1× SSC buffer was spread onto the whole slide and was allowed to react for 15 min before washing with 1× SSC buffer with 0.1% SDS for 10 min, and rinsing with 0.1× SSC buffer with 0.1% SDS. The fluorescence images of these slides are shown in Fig. 4(a)–(c). Fig. 4(d) shows two digitized curves of Cy5 intensity along three zones representing PB coating, untreated glass, and coating based on Denhardt's solution. Line 1 shows the background fluorescence intensity level and Line 2 passes through the spots where 5′-NH_2-C_{12}-$dA_{20}$was applied. As can be seen the PB coating was effective in suppressing non-specific DNA adsorption. The background fluorescence level of the areas coated with PB was somewhat higher than that observed in the areas that had been treated with Denhardt's solution, but were much lower than that observed for uncoated glass.

The intensities of the spots within the polymer-coated zones suggested that hybridization had occurred between the adsorbed dA_{20} and dT_{20}Cy5 oligonucleotides. PB and low molecular weight PDADMA coatings did not fully block adsorption of DNA, and some evidence of hybridization could

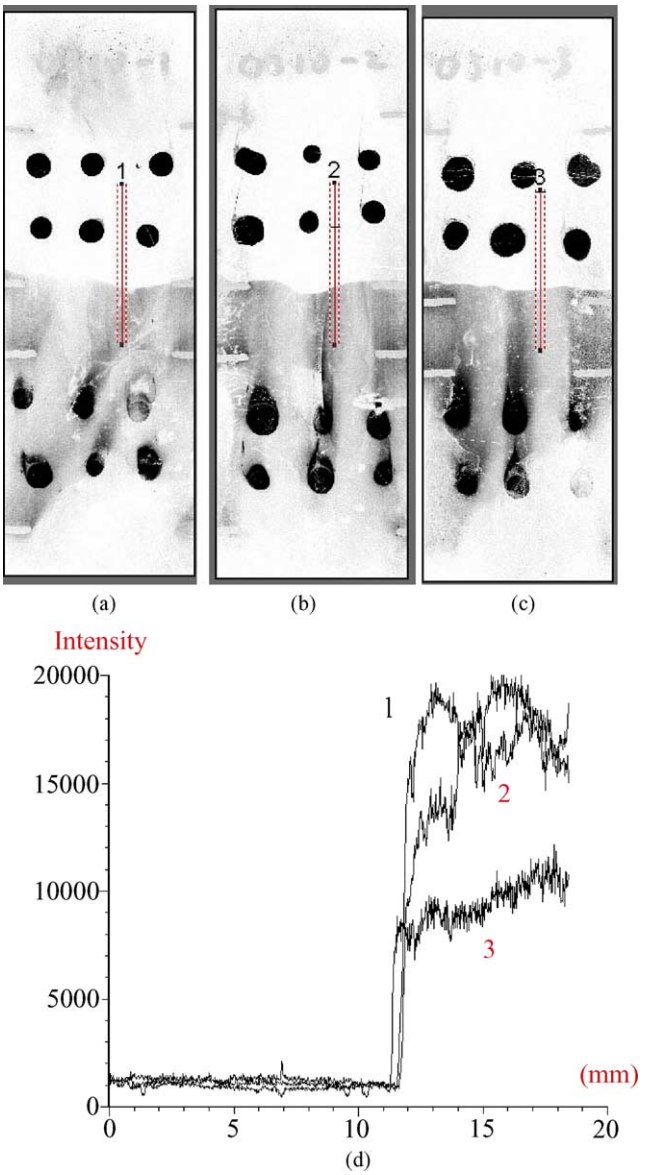

Fig. 3. Comparison of the background fluorescence from uncoated and coated glass slides. The slides were scanned by Virtek chipreader under Cy5 channel using 10% of the full laser power and 800 (out of 1000) sensitivity. In slides (a), (b) and (c), the upper areas were coated with PB, low molecular weight PDAMDA, and medium molecular weight PDADMA, respectively. The lower parts were uncoated. Three lines (1, 2, and 3) with 20 pixel width were chosen, and the background values were digitized and shown in (d).

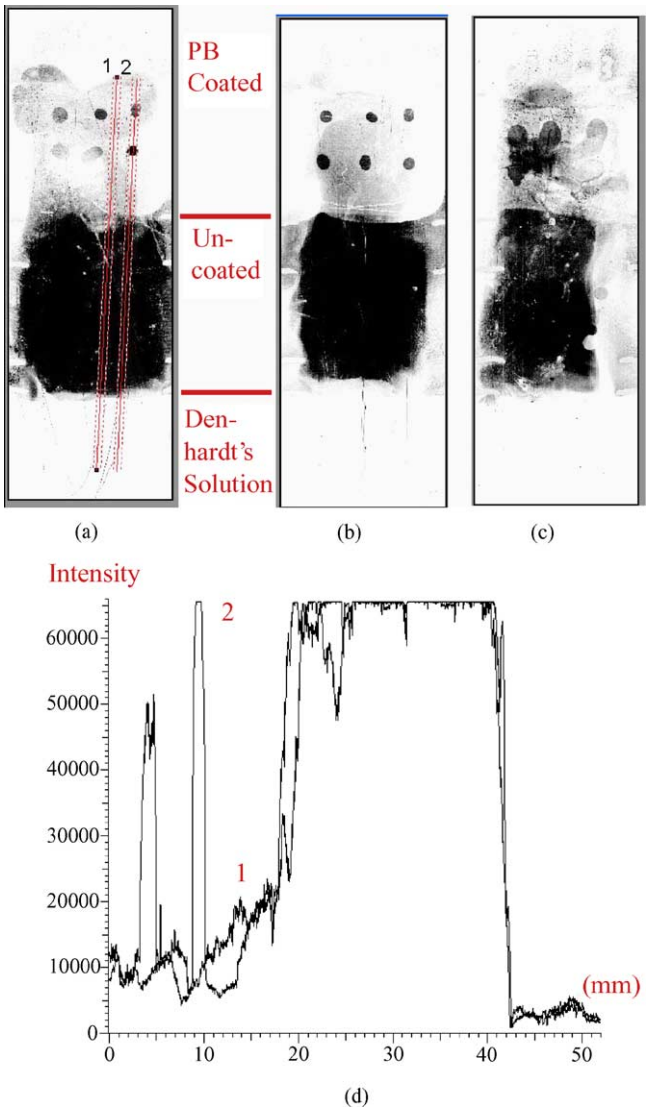

Fig. 4. Comparison of adsorption of dye-labeled oligonucleotides on PB coated glass and on uncoated glass. Laser power and sensitivity settings were the same as those in Fig. 3. (a) The upper part of the slide was coated with PB, the lower part was coated with Denhardt's solution, and the center section was untreated. Two lines of background (curve 1) and the line across the hybridization spots (curve 2) on the three different zones were digitized in (d). Ten-pixel width was used for averaging the intensity along the two lines. For (b) and (c), the upper areas were coated with low molecular weight PDADMA and medium molecular weight PDADMA, respectively. The other conditions were the same as those in (a). (d) The digitized curves of the intensity in (a).

be seen. Interestingly, the dA_{20} spots in the zones that were treated with Denhardt's solution did not brighten after application of dye-labeled complementary DNA, indicating that Denhardt's solution could fully block the glass surface and the dA_{20} oligo could not be adsorbed even after 3 h of incubation. It was not possible to determine whether there may have been any selective hybridization on the untreated glass as the background fluorescence was too high to distinguish any further change in intensity. The fluorescence data indicates that a PB coating could greatly reduce adsorption of ssDNA on a time scale relevant to microfluidic applications. However, ssDNA could still be adsorbed onto the polymer-coated surface if it was allowed to react for longer periods of time (hours).

The slides that were coated with PDADMA showed similar results to those that were modified with PB. It was observed that ssDNA would readily spread on surfaces that were treated with the medium molecular weight PDADMA (Fig. 4c). This aspect, along with the viscosity problem discussed in Section 3.1, suggests that medium molecular

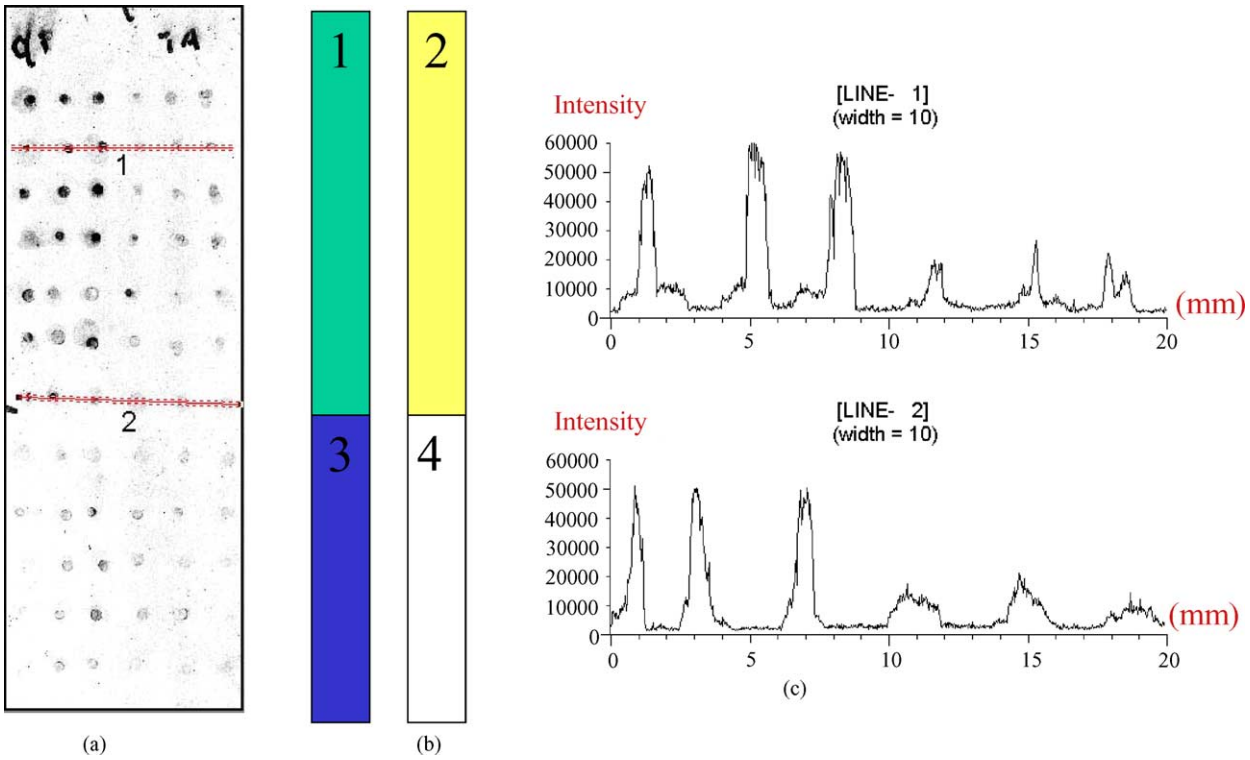

Fig. 5. (a) Fluorescence image of the result of hybridization on a slide containing fully matched and partially mismatched target. The intensity of the two rows of hybridization spots for matched and mismatched target on the PB coated zone appear in (c), respectively. Laser power and sensitivity settings were the same as those in Fig. 3. (b) The layout of the four different zones on the slide. Zones 1 and 2, with PB treatment after immobilization; Zones 3 and 4, without PB treatment; Zones 1 and 3, fully matched target 5′-Cy5dT$_{20}$ was applied; and Zones 2 and 4, 5′-Cy5-dT$_8$dA$_3$dT9 with three-base mismatch was applied. (c) Digitalized intensity of two rows of hybridization spots in (a). Ten-pixel width was chosen.

weight PDADMA is not a preferred material for surface modification.

3.2.2. *Effects of cationic polymer coating on hybridization*

Coating with PB and the low molecular weight PDADMA was effective in reducing the non-selective adsorption of the oligonucleotides on glass surfaces. The other issue to be considered was whether the cationic polymer coating impeded the hybridization reaction. To examine the effects of the cationic polymer coating on hybridization, slides with immobilized 5′-NH$_2$-C$_{12}$H$_{24}$-dA$_{20}$ were made using the procedure described in Section 2.4. 2.5% PB solution was applied to the upper part of the slide for 10 min, and the slide was then washed with water and then dried. 1 μM of fully complementary 5′-Cy5dT$_{20}$ and 1 μM of 5′-Cy5-dT$_8$dA$_3$dT9 (three-base pair mismatch) were applied to various areas of the slide. After a reaction period of 3 h, the slide was washed following the same procedure in Section 3.3.2 and scanned using the Chipreader. The scanned slide image is shown in Fig. 5(a). There were four zones on the slide as shown in Fig. 5(b). It is apparent from these images that there is significant differences in signal intensity between the fully matched target and the mismatched target. The background intensity was low, and a high signal to background ratio was achieved by use of the PB coating. At higher washing temperatures (30–40 °C), the signal to background ratio was improved, and the ratio between the signal of fully matched spots and mismatched spots was significantly increased from 2:1 to 5:1 when compared to the slide washed at the room temperature.

3.3. *Ellipsometry applied to polymer coatings*

In order to understand the polymer coating properties, it was necessary to examine the physical structure of the polymer membrane on the glass surface. An ellipsometer was used to confirm the deposition of polymer, and to estimate the range of thickness of the coatings on silicon, glass and quartz surfaces. The polymer coating procedure was similar to that described in Section 3.2. Table 1 provides ellipsometric data where 10 different points were measured for each surface.

According to the ellipsometry theory [25], the Δ value of glass and quartz without coating is 0°. The Δ value of silicon wafer is 180°. On the glass and quartz surfaces, Δ and Ψ values had minute changes before and after polymer coating. The samples were not highly reflective, and precision was poor. Highly reflective silicon wafers with a thin layer of SiO_2were also used, as these samples provided strong signals and were similar in surface chemistry to fused silica and glass. Before the polymer coating was deposited, a thickness of 20 Å of SiO_2 layer was determined by ellipsometry. A

Table 1
Ellipsometric data for coatings of cationic polymer PB onto silicon wafer surfaces

Surface	Before polymer coating			After polymer coating		
	Δ	Ψ	Calculated thickness of SiO_2 layer (Å)	Δ	Ψ	Calculated thickness of polymer layer (Å)
Silicon wafer without treatment	173.2 ± 0.2	10.50 ± 0.56	21.0 ± 0.7	171.5 ± 0.5	10.64 ± 0.02	6.1 ± 1.7
Silicon wafer with treatment	163.9 ± 1.6	9.65 ± 0.11	50.0 ± 4.7	161.7 ± 1.0	9.67 ± 0.15	6.7 ± 5.7

thickness of 6 Å was calculated according to the changes of Δ and Ψ, using a refractive index estimate of 1.5. The same thickness of polymer was also observed on the silicon wafer after treatment with NH_3/H_2O_2 and HCl/H_2O_2. On the treated silicon wafer, the thickness of the SiO_2 layer was about 50 Å. The measured Δ and Ψ values and calculated thickness are shown in Table 1. Compared with the actual molecular size of the polymers, the thickness of the coatings as determined by ellipsometry was very thin.

The PB or PDADMA polymer were likely adsorbed onto the glass and silicon dioxide surfaces by electrostatic interaction. This is consistent with the observation that the polymer coating could be readily washed away using 1 M NaOH with complete restoration of the original EOF mobility. The layer that was formed likely had monolayer dimensions, and may have incompletely covered the surface. Incomplete coverage would be consistent with adsorption over longer incubation periods, which suggests that oligonucleotides could still reach the glass surface. The mechanism of function of the polymer coatings in EOF was a direct consequence of surface charge. The effectiveness of the coatings in reduction of adsorption of DNA was likely due to the hydrophobic backbone of the polymer.

4. Conclusions

Cationic polymers such as polybrene and poly(diallyldimethylammonium) chloride can be used as coatings in microfluidic channels for reversing the electroosmotic flow direction. This is crucial for DNA sample delivery and buffer washing procedures in electroosmotically operated DNA hybridization microchips. These polymer coatings are not only compatible with the hybridization process, they also significantly reduce the adsorption of non-specific DNA onto the glass surface. There was no significant difference in the effects of coatings of polybrene or low molecular weight PDADMA for the purpose of EOF control and hybridization. A single layer of incomplete coverage of the polymer on the glass is consistent with the experimental results.

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