Surface Modification of Polydimethyl Siloxane Using Polyethylene Oxide Copolymers

by

Shikha Manchanda

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Graduate Supervisory Committee:

Alexandra Ros, Chair
Mark Hayes
Yan Liu

ARIZONA STATE UNIVERSITY

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ABSTRACT

Polydimethyl siloxane is a commonly used fabrication material for microfluidic devices. However, its hydrophobic nature and protein adsorption on the surface restricts its use for microfluidic applications. Also, it is critical to control the electroosmotic flow for electrophoretic and dielectrophoretic manipulations. Therefore, surface modification of PDMS is essential to make it well suited for bioanalytical applications. In this project, the role of polyethylene oxide copolymers F108 and PLL-PEG has been investigated to modify the surface properties of PDMS using physisorption method. Measuring electroosmotic flow and adsorption studies tested the quality and the long-term stability of the modified PDMS surface. Static and dynamic coating strategies were used to modify the PDMS surface. In static coating, the PDMS surface was incubated with the coating agent prior to the measurements. For dynamic coating, the coating agent was always present in the solution throughout the experiment. F108 and PLL-PEG were equally effective to prevent the protein adsorption under both strategies. However, dynamic coating was more time saving. Furthermore, effective reduction of EOF was observed with F108 coating agent under dynamic conditions and with PLL-PEG coating agent under static conditions. Moreover, PLL-PEG dynamic coatings exhibited reversal of EOF. These important findings could be used to manipulate EOF and suggest optimal coating agent and strategies for PDMS surface treatment by the physisorption method.

DEDICATION

To my family and my loving daughter Saina.

ACKNOWLEDGMENTS

I want to thank my advisor Dr Alexandra Ros for her guidance and support.

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Chapter 1

ABSTRACT

Polydimethyl siloxane is a commonly used fabrication material for microfluidic devices. However, its hydrophobic nature and protein adsorption on the surface restricts its use for microfluidic applications. Also, it is critical to control the electroosmotic flow for electrophoretic and dielectrophoretic manipulations. Therefore, surface modification of PDMS is essential to make it well suited for bioanalytical applications. In this project, the role of polyethylene oxide copolymers F108 and PLL-PEG has been investigated to modify the surface properties of PDMS using physisorption method. Measuring electroosmotic flow and adsorption studies tested the quality and the long-term stability of the modified PDMS surface. Static and dynamic coating strategies were used to modify the PDMS surface. In static coating, the PDMS surface was incubated with the coating agent prior to the measurements. For dynamic coating, the coating agent was always present in the solution throughout the experiment. F108 and PLL-PEG were equally effective to prevent the protein adsorption under both strategies. However, dynamic coating was more time saving. Furthermore, effective reduction of EOF was observed with F108 coating agent under dynamic conditions and with PLL-PEG coating agent under static conditions. Moreover, PLL-PEG dynamic coatings exhibited reversal of EOF. These important findings could be used to manipulate EOF and suggest optimal coating agent and strategies for PDMS surface treatment by the physisorption method.

Chapter 2

INTRODUCTION

Microfluidic devices have gained much importance in recent years[1-6].These devices have the potential to provide fast analysis using only small sample volume. Microdevices deal with the high surface to the volume ratio. The fabrication material also has a profound effect on the performance of the analysis made in these micro devices. There is a wide variety of materials used for microfluidic devices such as silicon, quartz, glass or elastomers, e.g. polydimethyl siloxane (PDMS)[7]. The latter has been extensively used as a fabrication material for microfluidic devices[8]. Low fabrication cost, minimum clean room requirements and compatibility with biological samples makes PDMS suitable for microfluidic applications[9]. But, PDMS is hydrophobic in nature, which leads to unstable EOF flow[10], the tendency to adsorb protein[11] and consequently its low reusability.

Therefore, surface modification of PDMS is very important to carry out reliable and reproducible analysis. Several methods have been reported to modify the PDMS surface. Modification of PDMS using surfactants[12-15], chemical modification[12, 16-19] using photoinduced grafting[20, 21] or UV initiated grafting[17, 18], and bulk modification[22] by adding prepolymer additives. Modification of PDMS using surfactants is based on the weak interactions between the surface and the surfactant, called physisorption method. We have employed this method using polyethylene glycol (PEG) based copolymers for the modification of PDMS.

PEG derivatives have been demonstrated to significantly control EOF and reduce protein adsorption[12, 16, 23, 24].The coating materials based on their structure and the interaction with PDMS have been categorized as non-ionic surfactants and charged polymers[25]. The physisorption method is typically based on the hydrophobic and electrostatic interactions with the PDMS surface. This project deals with two coating agents, the copolymers F108 and PLL-PEG (polylysine – polyethylene). F108 is a triblock copolymer, which has two polyethylene glycol units and one polypropylene oxide unit (PEG_x-PPO_y-PEG_x, x = 132 and y = 52). On native PDMS, the hydrophobic PPO unit interacts with the PDMS and hydrophilic PEG chains extrude out on the surface. However, on hydrophilic PDMS surface F108 has a flat conformation[12] related to the interaction of PEO units with PDMS. Figure 1 shows the interaction of F108 on both hydrophobic and hydrophilic PDMS surfaces.

PLL-PEG adsorption is based on electrostatic interactions on the PDMS surface. The PLL backbone is positively charged at neutral pH, which interacts with the negatively charged PDMS surface. Lee and Vörös[15] reported PLL-PEG as an excellent coating material to reduce protein adsorption. Another group[12] has tested F108 to control EOF, but its effectiveness to reduce protein adsorption still needs to be studied. This group has tested EOF under the static conditions, but not the dynamic. In this project we characterized these two coating agents to reduce protein adsorption and to control electroosmotic flow using both the strategies static and dynamic. The quality of the surface-modified PDMS using these two

coating agents was tested by electroosmotic flow measurements and by performing adsorption studies. The long-term stability of the modified PDMS was also examined by the EOF measurements. We have also tested the dependence of the concentration of coating agents to modify the quality of the PDMS surface.

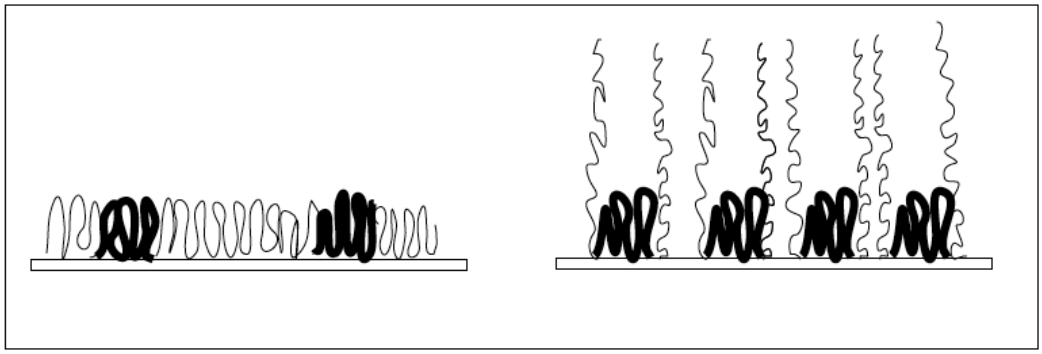


Figure 1. a) Molecular conformation of F108 on a hydrophilic PDMS surface. Both PEG (thin lines) and PPO (thick lines) chains are interacting with the PDMS surface and b) on a hydrophobic PDMS surface. The hydrophobic PPO chains are attached to the PDMS surface via hydrophobic interactions and hydrophilic PEG chains are extruding out on the surface.

Chapter 3

BACKGROUND AND THEORY

3.1 PDMS and its characteristics

PDMS is a silicon based organic polymer with a repeating unit of [-Si $(CH_3)_2O$-]. It is an inexpensive biocompatible material and micro fabrication with PDMS can be carried out with minimum clean room requirements. With a suitable master, several molds of PDMS can be made using soft lithography[8]. It is optically transparent down to 280nm, meaning it passes the light through it and therefore is useful for the detection schemes[26]. PDMS is hydrophobic in nature with a contact angle of ~108º [27]. With the exposure to the plasma treatment the PDMS surface can be made hydrophilic. The hydrophilic PDMS micro channels can be easily filled with aqueous solutions. All these advantageous characteristics of PDMS make it a very useful fabrication material for microfluidic devices.

3.2 Plasma treatment

Plasma treatment is used to achieve irreversible seal of PDMS with other suitable material like glass to form closed microchannel. This irreversible seal is formed by covalent siloxane, Si-O-Si bonds. The oxygen plasma pretreats the non-modified native PDMS, which makes PDMS surface hydrophilic due to the oxidation of siloxane to silanol groups. However, PDMS recovers hydrophobicity in a short period of time[27]. The possible reason for this hydrophobic recovery is reorientation of silanol groups and the diffusion of low molecular species from the bulk of PDMS to the surface[28]. The unstable surface charges due to hydrophobic

recovery on the PDMS, lead to the poor repeatable migration times[29] in electrophoretic applications and reduce separation efficiency. Therefore, an effective and stable surface modification is necessary for good separation efficiency and reproducible measurements.

3.3 Role of PEG derivatives

PEG derivatives have been demonstrated to modify the PDMS surface effectively. The PEG chains are brush like polymers, which prevents the adsorption of proteins[12, 15, 30] and control EOF[29]. It has been reported that the PEG architecture influences the adsorption of protein on the PDMS surface. High PEG chain density leads to low protein adsorption[23]. PDMS surface modified with PEG derivatives are more stable and helps in preventing protein adsorption and to control electroosmotic flow. Lee and Vörös group[15] demonstrated a simple modification of PDMS surfaces through the adsorption of a graft copolymer (PLL-g-PEG) from aqueous solution. They reported a reduction in fibrinogen adsorption onto the oxidized PDMS surface. This group has also characterized the resistance to protein on metal oxide surfaces layered with PLL-PEG[31]. Ros et al.[12] have tested the influence of PEG chains to control EOF. They varied the length of the PEG chains and found that longer PEG chains resulted in higher EOF reduction. The PEG chain density has a direct impact on EOF mobility. Textor et al.[16] have used PLL-PEG copolymers to covalently attach on aldheyde plasma modified substrates. This modification was reported to be stable at extreme pH values or high ionic strength buffer (2400mM NaCl). In another study, photo

induced grafting polymerization was used to treat the PDMS surface. Polyethylene glycol monoacrylate (PEGMA) and polyethylene glycol diacrylate (PEGDA) have been used to modify the PDMS surface[20]. The PEGDA modified PDMS surface has been reported to be stable for over two months. PEG based modifier like PLA-PEG can be used as a prepolymer derivative[22]. It is reported that the bulk modification of PDMS using PLA -PEG reduced the adsorption of protein and also controls EOF. This PEG based copolymer is mainly used for tissue engineering applications[32]. The PEG based copolymers like F108 are also suitable for cell patterning[33]. Most of the PEG based copolymers are highly protein resistant, and therefore suitable for many microfluidic applications. With such advantages of PEG based polymer, Yoon et al.[34] has reported the whole chip made of PEG. This PEG structured microfluidic channel can be repeatedly used.

PEG based polymers are highly capable to modify the PDMS surface. The PEG chains in these polymers are able to resist protein[12, 19] and to control EOF[35]. Long-term stability and reusability have been observed on the PDMS modified with PEG copolymers[36]. Both the chemical and physical modifications are possible with PEG copolymers depending on the need.

3.4 Surface modification methods and strategies

There are two methods to do the surface modification of PDMS, the first one is physisorption method and the second one is chemisorption. The chemical modification is based on the covalent attachment of the coating agent with the surface. This modification method requires a specific functional group on the

microchannel to induce the chemical reaction. Various examples include chemical grafting of alkyne – PEG[37], photoinduced grafting using polyethylene glycol monoacrylate and polyethylene glycol diacrylate[20] and UV initiated grafting using benzophenone[18] and self assembled monolayer's (SAM) [38]. The SAM layers covalently attached on the PDMS surface through the gold layer deposited on the PDMS. The physisorption method is based on weak Van der Waals forces, electrostatic interactions or hydrophobic interactions with the PDMS surface. In this method, the coating agent could detach from the PDMS surface because of the weak interactions. But, the physisorption method is very simple and easy to apply. There are two strategies to physically adsorb coating materials on PDMS surfaces, one is static coating and the other one is dynamic coating[39]. In static coating, the PDMS surface is incubated prior to the experiment and then rinsed with pure buffer to remove unbound residues. In dynamic coating, the coating agent is always present on the PDMS throughout the experiment. Figure 2 shows the schematic of the strategy to apply coating agents.

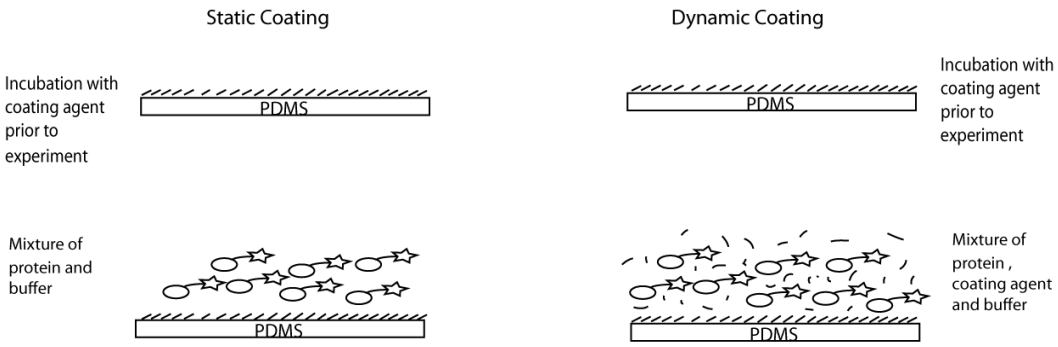


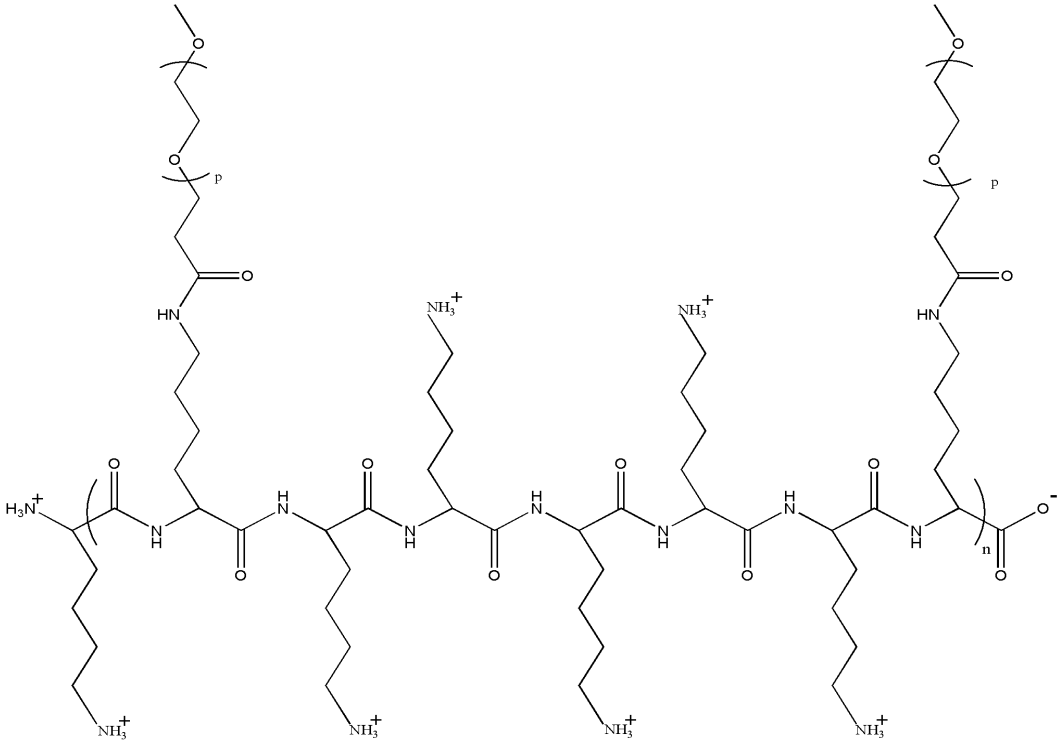
Figure 2. Schematics of static vs. dynamic coating. The figure shows the coating agent (short black lines) adsorbed on the surface. The other symbol (circles with stars) depicts the proteins tagged with fluorophores. Under static conditions the PDMS surface was incubated with the coating agent prior to the experiment and then after washing with the fluorescently tagged protein. In dynamic coating, the coating agent was always present both during pre-incubation and during incubation with protein.

3.5 Coating agents

F108 and PLL-PEG are the two coating agents that have been used to modify the PDMS surface in this work. Both these coating agents have long PEG chains. These PEG chains have been demonstrated to be effective in reducing the adsorption of proteins on the PDMS surface and to control EOF[29, 40]. F108 is a triblock copolymer with two PEG (polyethylene glycol) units or otherwise known as PEO units (polyethylene oxide) and one PPO unit, PEG_x-PPO_y-PEG_x, x = 132 and y = 52. On a hydrophilic PDMS surface, F108 has flat conformation resulting in an interaction of PEG chains with the PDMS[12]. PLL-PEG[41] (polylysine - polyethylene glycol) is a polycation-PEG grafted co-block polymer. The PLL backbone exhibits positively charged amino groups, which attract towards the negatively charged PDMS surface, thus leading to electrostatic interactions[16, 31, 41].The PEG chains stick out on the PDMS surface. Figure 3 a) shows the

molecular structure of PLL-PEG and b) F108 respectively.

a)



b)

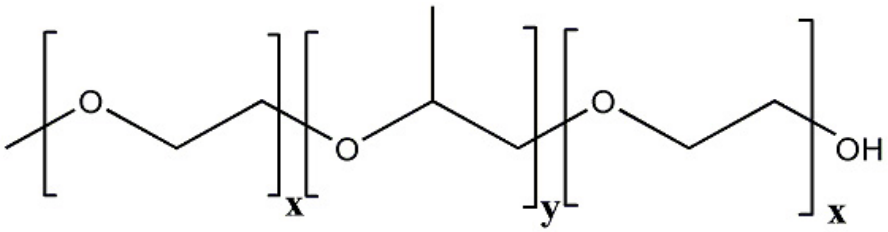


Figure 3. Molecular structure of the surface coating agents: a) PLL-PEG (n=19, p = 113) b) F108 (with x = 132 and y = 52)

3.6 Electrical double layer and electroosmotic flow

After the plasma treatment, PDMS has negative charges[42] on its surface because of the deprotonation of the silanol groups in aqueous solution.

$$SiOH \rightleftharpoons SiO^- + H^+$$

These charges are the basis of electroosmotic flow (EOF) in PDMS channels[10]. These negative charges on the PDMS surface attract the counter ion charges from the buffer apparent in the channel. The ions, which are directly attracted on the wall of the PDMS channel, form an immobile layer, called Stern layer and the other mobile layer, is called Diffusive layer. The Diffusive layer contains ions of both charges, but one is enriched depending on surface charge. These two layers together are called as electrical double layer or Debye layer. The thickness of the Debye layer is usually in the nanometer range. Figure 4 shows the stern model of the electrical double layer. When an external electric field is applied to a microchannel filled with buffer, the charges in the Debye layer get accelerated across a channel. These ions migrate towards the cathode moving the bulk solution by viscous drag. This bulk flow of ions is called electroosmotic flow. The ions move with the uniform velocity on the application of electric field and exhibits flat velocity as shown in Figure 5.

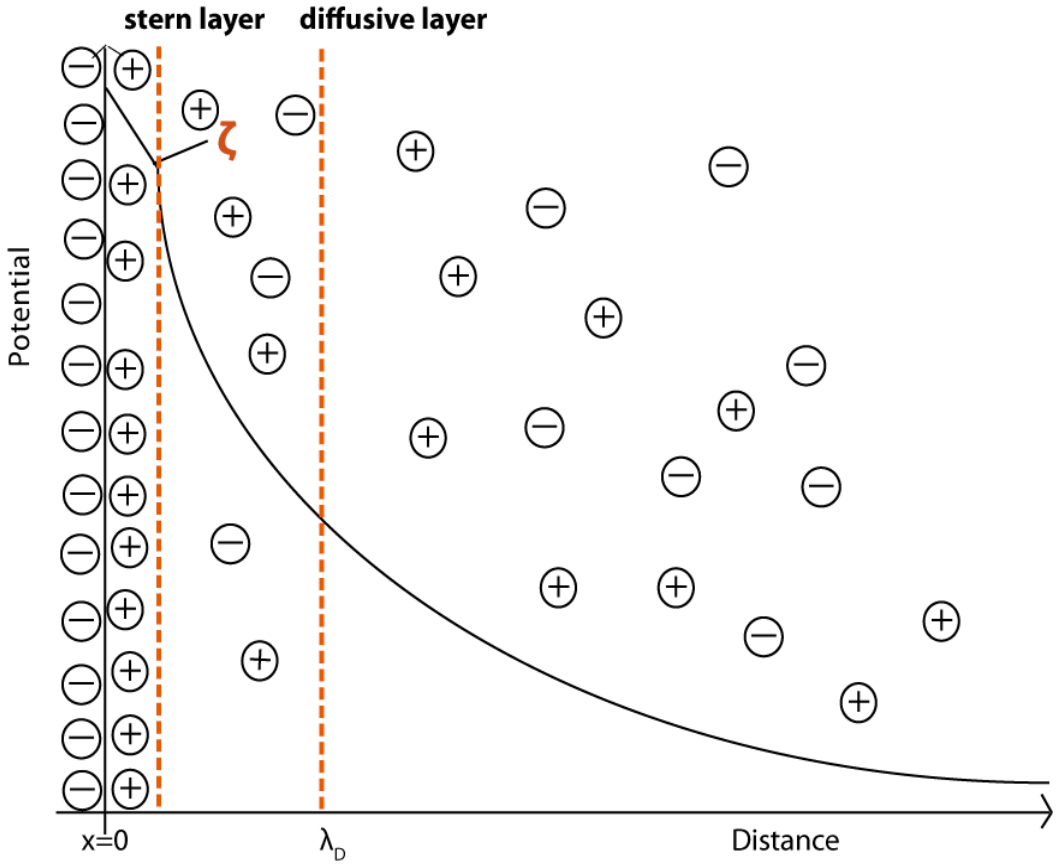


Figure 4. Scheme showing the Stern model of the electrical double layer. The immobile layer formed at the immediate vicinity of the PDMS wall is the Stern layer. The other layer is mobile and denoted the Diffusive layer. The Zeta potential is the potential at the Stern layer. The electrostatic potential decreases linearly through the stern layer and then exponentially.

The shear forces in the liquid lead to a unique flow in the channel with the electroosmotic velocity denoted by, $\vec{v}_{eo}$ given by the Smoluchowski equation,

$$\vec{v}_{eo} = \frac{\vec{\mathrm{E}}\varepsilon\zeta}{\eta} \qquad \text{Eqn. 1}$$

Where $\vec{v}_{eo}$ is the velocity, $\vec{\mathrm{E}}$ is the electric field strength applied, ε is the permittivity of the solution, ζ is the zeta potential and η is the viscosity of the solution.

and, the electroosmotic mobility μ_{eo} is given by

$$\vec{v}_{eo} = \mu_{eo}\vec{E} \qquad \text{Eqn. 2}$$

Rearranging and substituting, $\vec{E}$ = V/L, where V is the voltage applied, L is the length of the channel and $\vec{v}_{eo}$ = L/t, *t* is time, the electroosmotic mobility results in:

$$\mu_{eo} = L^2/Vt \qquad \text{Eqn. 3}$$

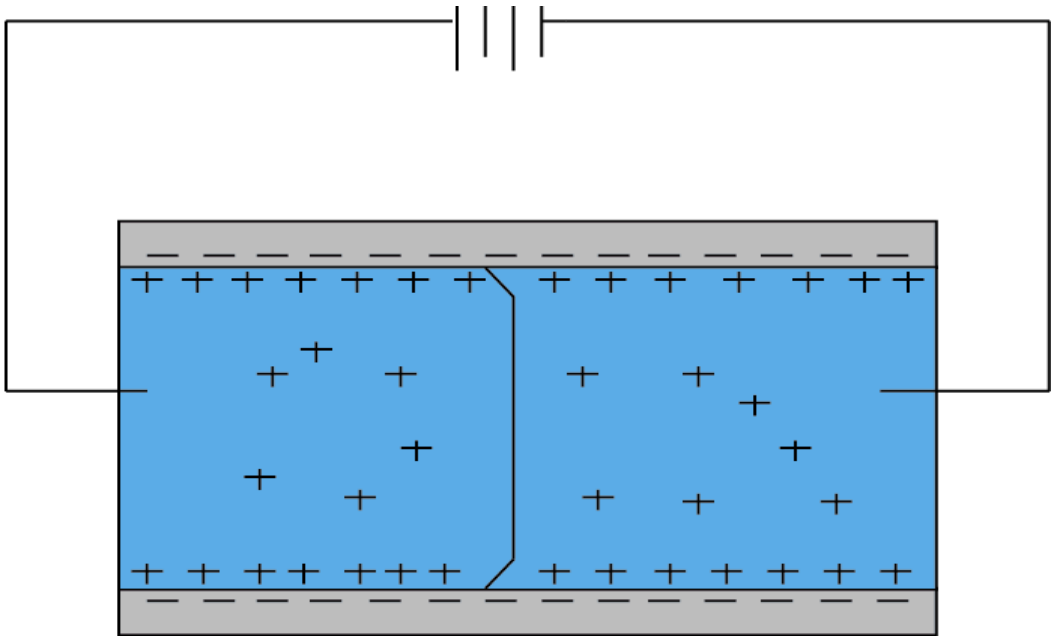


Figure 5. Scheme showing electroosmotic velocity profile. PDMS exhibits negative surface charges on the microchannel wall and positive ions are enriched in close vicinity to the PDMS surface in accordance with the Stern model. Upon application of an electric field a bulk flow arises, exhibiting a flat velocity profile.

3.7 Zeta Potential

In an EDL, the electrostatic potential near the surface drops linearly through the Stern layer and then it drops exponentially (figure 4). The Zeta potential is the potential at the Stern layer and denoted by ζ. It is an important characteristic to determine the EOF on charged surfaces. It is a function of the double

layer and the surface charge density on the PDMS surface. There are different methods to calculate ζ. Li et al.[43] has reported a simple method to calculate the zeta potential on PDMS coated surface using the Smoluchowski equation and the slope of the current time relationship observed from the current monitoring method. According to this group, the zeta potential values on PDMS coated glass surface are -87 and -68mV for 10^{-3} M KCl and 10^{-3} M $LaCl_3$ respectively.

3.8 Current Monitoring Method

This method is based on recording the time history of the current during CZE (capillary zone electrophoresis)[44.] The microchannel and both reservoirs are filled with an electrolyte at a high concentration and as the microchannel gets conditioned with the high concentration electrolyte the buffer in the anode reservoir gets exchanged with the lower concentration buffer. When a voltage is applied, the lower concentration electrolyte migrates through the microchannel and it displaces an equal volume of higher concentration electrolyte in the microchannel. The time at which the complete buffer exchange occurs is called as exchange time, *t*. The '*t'* value can be calculated by the interception of the two linear fits. Fig 6a. shows the schematic of the current monitoring method and 6b. shows an example of a recorded current vs. time trace.

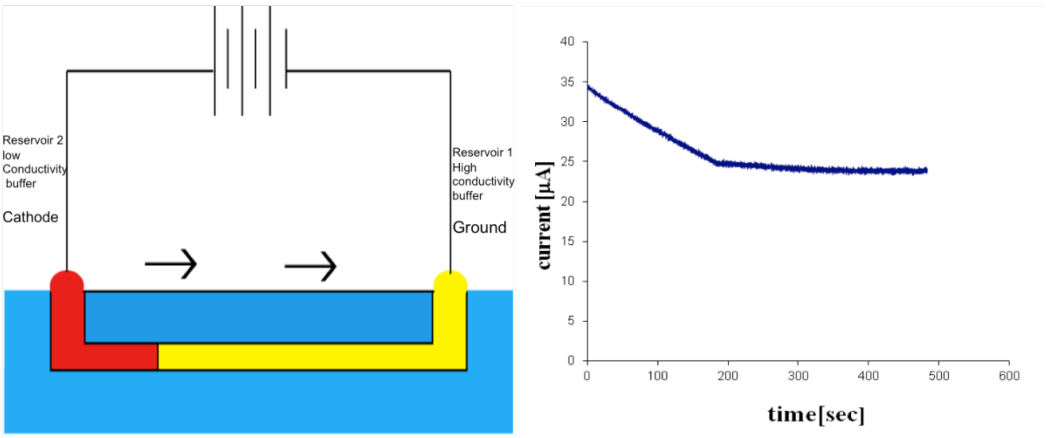


Figure 6. a) Schematic of current monitoring method. The channel was conditioned with the high concentration buffer and then replaced with the lower concentration buffer. b) Example of resulting trace of current vs. time curve.

3.9 Fluorescence microscopy imaging

Fluorescence is a phenomenon in which a molecule called a fluorophore absorbs light at a particular wavelength and subsequently emits light of longer wavelength. Fluorescence measurements are very useful for adsorption studies of protein to surfaces because of their sensitivity. Fluorescence microscopy allows to image the fluorescence intensities on a PDMS surface. In fluorescence microscopy imaging a sample is illuminated with light of a specific wavelength range. The fluorophores in the sample are excited and then emit light at a different longer wavelength. Two types of filters are used in this technique, an excitation filter and an emission filter as well as a dichroic mirror. The excitation filter passes the light of the desired wavelength similar to the excitation wavelength of the fluorescence material. Dichroic mirror directs this light on the immobilized fluorophore. Upon excitation of the fluorophore the emitted light passes through the emission filter.

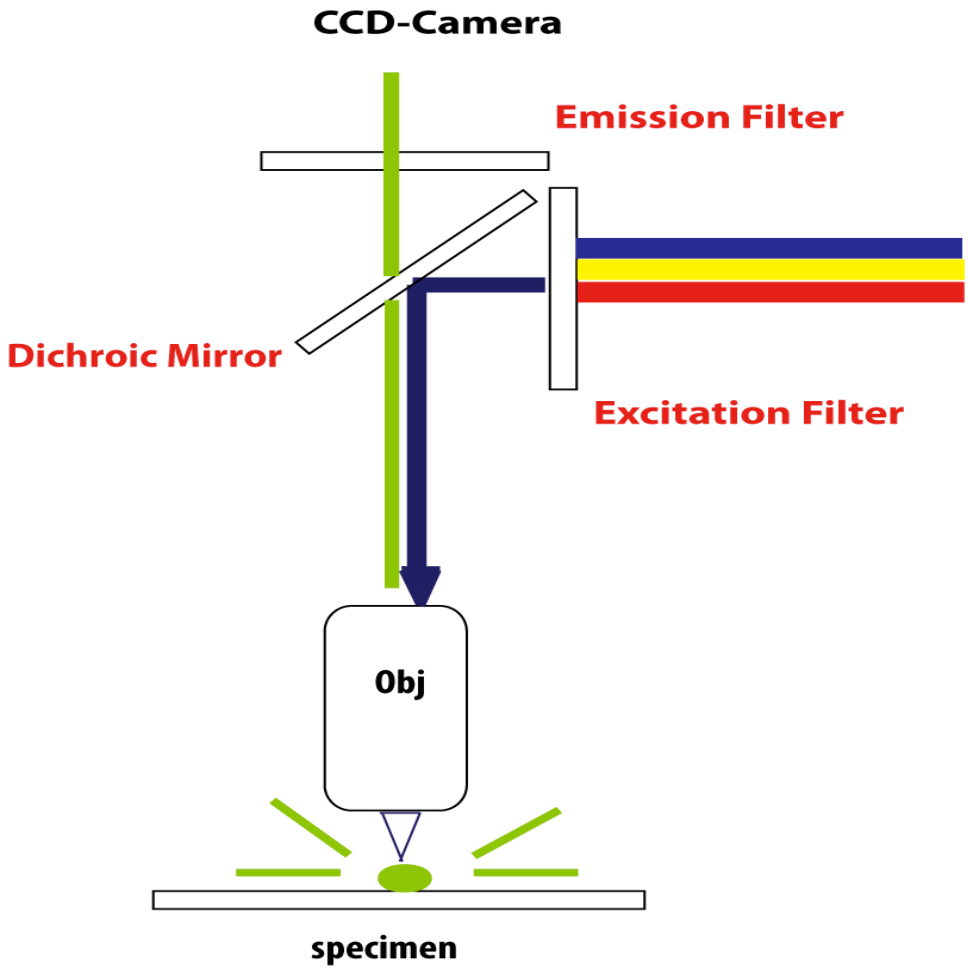


Figure 7. *Fluorescence microscopy imaging technique uses two types of filters: an excitation filter and an emission filter as well as a dichroic mirror. The sample is tagged with the fluorophores, which absorbs light at a particular wavelength, gets excited and emits light of longer wavelength.*

Chapter 4

MATERIALS AND METHODS

4.1 Chemicals and reagents

Silicon wafers were purchased from University Wafer. Negative photoresist SU-8 was purchased from Microchem (Newton, USA). Polydimethyl siloxane (Sylgard 184) was from Dow Corning (USA). Potassium phosphate monobasic, potassium phosphate dibasic and F108 a triblock polymer (PEG-PPO-PEG) was obtained from Sigma-Aldrich (USA). Alexa488-labelled BSA and Alexa-488 labelled fibrinogen were from Invitrogen (USA). Polylysine polyethylene glycol [(20)-[3.5]-(20)], PLL-PEG was obtained from SurfaceSolutions (Switzerland). Glass slides were obtained from Fischer Scientific and Pt wire was from Alfa Aesar (USA). Millipore water was from a Synergy purification system (Millipore, USA).

4.2 Chip fabrication

In this work, PDMS microfluidic devices were fabricated using soft lithography[26, 45]. The straight channels required for measuring EOF were created on a silicon wafer using negative photoresist SU-8 by photolithography. This master wafer was silanized using tridecafluoro-1, 1,2,2-tetra-hydro-octyl-1-trichlorsilane (TDTS) to make it hydrophobic.

PDMS (25g) was mixed with the curing agent (2.5g) and was poured on the master wafer and cured at 85º C for 4 h. Then PDMS was peeled off from the wafer and the required channel was cut. Reservoirs were punched to enable access

to the channel. To form closed micro channels, the PDMS slab and the PDMS coated glass substrate were treated with oxygen plasma in plasma cleaner (Harrick, USA) for 60 seconds at 500-millitorr. The dimension of the linear micro channel was 25 x 55 μm^2 in height and width and 3.1 cm in length for PDMS chip. For adsorption studies, thin glass slides (48x60 mm^2) were spin coated with PDMS diluted with hexane (50:50) using a spin coater.

4.3 Surface derivatization

Coating materials were dissolved in 20mM phosphate buffer (potassium phosphate monobasic and potassium phosphate dibasic anhydrous) at pH 7. The assembled PDMS chip was incubated overnight with the coating agent for overnight incubation and then rinsed with phosphate buffer for EOF measurements under static condition. For dynamic coating, the PDMS surface was incubated with coating agent for 5 min before the experiments and the coating agent was present throughout the measurements. For, adsorption studies, under static conditions the PDMS surface was incubated overnight with the coating agent. It was further rinsed with phosphate buffer and was incubated with protein for 2 hrs in the dark. For dynamic coating, the PDMS surface was pre-incubated with the coating agent for 10 min and then with protein solution containing coating agent.

4.4 EOF measurements

The current monitoring method[44] was used to determine the electroosmotic flow mobility. Platinum wires were inserted in the reservoirs to apply potential to the

channel. Labsmith (HV 446, Labsmith, USA) was used as a high voltage power source via electrodes. The polarity of the power supply is chosen so that the electroosmotic flow is cathodic i.e. from the anode reservoir to the cathode reservoir. 30mM and 15mM phosphate buffer were used to determine the buffer exchange time. The channel was conditioned with the 30mM phosphate buffer and then replaced by 15mM phosphate buffer. The decrease in current was recorded at 300V/cm, applied via electrodes dipped in the reservoirs. Voltage was applied and current recorded using Labsmith (HV 446, Labsmith, USA). The electroosmotic mobility was calculated using eq. 3. The time t was calculated by the interception of the two linear fits of the current trace. For PLL-PEG dynamic coating, the polarity was reversed. The same method was used for the EOF measurements for dynamic coating with PLL-PEG but the buffer was exchanged from the cathode reservoir.

4.5 Protein adsorption measurements

Fluorescence microscopy imaging technique was used for adsorption studies. The fluorescence intensity of fluorophorescently tagged protein adsorbed on treated PDMS surface was measured to study the effectiveness of the coating agent. The surfaces on the PDMS coated glass slide were sectioned using PDMS slabs to prevent the mixing of coating agent with variable concentrations. Under static conditions, each sectioned PDMS surface was incubated overnight with the different concentration of the coating agent and then with a droplet of protein prepared in buffer for 2hrs. Under dynamic condition, the surface was incubated

for 10 min with the coating agent, which was further incubated for 30 min with labeled protein solution containing phosphate buffer and coating agent. After incubation, the surfaces were washed with pure buffer. For washing, PDMS surface was rinsed with pure buffer for 1min and this step is repeated three times to remove any unbound residues. After washing the PDMS surface was dried and the fluorescence intensities were measured. F108 and PLL-PEG were used as the coating agents and BSA-Alexa 488 (Invitrogen, USA) and Fibrinogen - Alexa 488 were used as proteins for concentration dependent experiments. The concentration range tested for F108 and PLL-PEG was from 5μM to 10mM and 0.04μM to 20μM. Fluorescence intensity measurements of adsorbed protein were carried out on a IX74 inverted microscope (Olympus, USA) using a QuantEM camera (Photometrics, USA). The objective magnification was 40X for all the adsorption experiments.

Chapter 5

RESULTS AND DISCUSSIONS

Here, we studied the properties of PDMS surface modified with the PEG based coating agents under varying conditions. We tested the effectiveness of the modified PDMS surface by EOF measurements and adsorption studies. Dynamic and static coating strategies were used to investigate the influence of the coating agent on the PDMS surface.

5.1 Effect of coatings on EOF

The effect of F108 and PLL-PEG coatings on electroosmotic flow has been characterized by the current monitoring method[44] and electroosmotic flow mobilities have been obtained from this method. In this project we used 20 KDa of PLL to which 5KDa PEG was grafted in a ratio of 3.6. With PLL-PEG, the PEG chain density can also alter the EOF. We compared the mobility after applying the coating agent with the mobility on the native PDMS. The electroosmotic flow was always from the anode to the cathode except[9, 46] for the PLL-PEG dynamic coating. Table 1 summarizes the mobility observed and the % reduction in the mobilities after the surface modification with coating agent with respect to the plasma treated PDMS. It was observed that F108 dynamic coating is more effective to control the electroosmotic flow as compared to the static. With F108 static coating, 43% reduction in the mobility was observed. However with F108 dynamic coating, 93 % reduction was observed[47]. It has been argued that the PEG chains coated on the charged PDMS surface increase the local viscosity in

the electrical double layer region referring to equation 1. The coating agent adsorbed on the PDMS surface has a certain thickness. PLL-PEG is a charged polymer. This adsorbed layer of PLL-PEG polymer creates a viscous layer that masks the charges on the wall, reducing the surface charge density and hence the EOF. F108 has a flat conformation[12] with a thickness of ~ 3nm on oxidized PDMS surface. Under static conditions these coatings agents altered the viscosity in the electrical double layer region but not in the bulk solution. But when applied dynamically, the running buffer has PEG chains, which increases the viscosity of the bulk solution. Under both the strategies due to the increase in the viscosity, the EOF mobility should decrease.

Further, to compare F108 and PLL-PEG under static conditions, ~ 80 % reduction in EOF mobility was observed with PLL-PEG, which is larger than was observed with F108 static coating (Table 1). The long PEG chains in PLL-PEG can alter the EOF by increasing the viscosity of the solution in the channel. The brush like chains extruding out in the double layer on the charged surface of PDMS (Fig 3a). PLL-PEG has strong electrostatic interactions with the PDMS surface as compared to the hydrophobic interaction of F108. This strong electrostatic interaction of PLL-PEG with the PDMS surface accounts for the stable PDMS surface covering. However, with PLL-PEG dynamic coating, the direction of the EOF flow was reversed which was not the case for F108 dynamic coating. The reasons accounted for this behavior are (1) dynamic coating replenishes the PDMS surface and (2) masking of surface charges with PLL-PEG leading to the

positive charges on PDMS surface, thus the negative mobility. With PLL-PEG dynamic coating the flow remains reversed even after several measurements as shown in Table 1.

The long-term stability with PLL-PEG static coating was also tested. The PDMS surface modified with PLL-PEG under static condition was stable for several days. In contrast to F108 static coating as reported in the literature[12] no significant change in μ_{eo} with repetitive measurements after several days was observed.

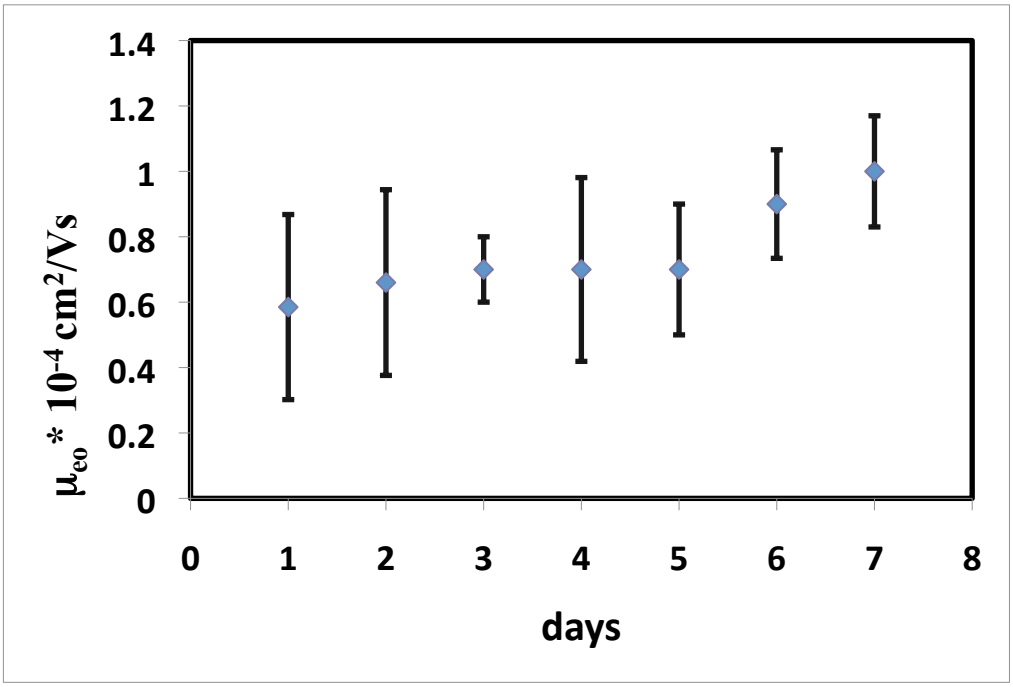


Figure 8. Long-term data of repetitive EOF measurements with PLL-PEG coating agent under static conditions.

In summary, dynamic coating strategy exhibits stronger reduction in EOF in comparison to the static coating. The surface coatings had an appreciable effect on the quality of EOF. The reproducibility and the stability were improved with the coatings. All the measurements are the average values of at least three

repetitions from each channel, with three channels in total. Table 1 below shows the summarized EOF mobility observed after the treatment of PDMS surface. The reduction in the mobility with respect to the untreated PDMS surface is also listed in Table 1.

Table 1. *EOF mobility of treated PDMS surface and % reduction with respect to the untreated PDMS surface (n.d : not determined)*

	$\mu_{eo}*10^{-4}$[cm^2/Vs]	% Reduction	EOF direction	Zeta potential
F108 static[12]	1.75[0.3]	43	Cathodic	24.7mV
F108 dynamic	0.53[0.07]	88	Cathodic	25mV
PLL-PEG static	1.00[0.15]	80	Cathodic	14.12mV
PLL-PEG dynamic	- 0.85[0.21]	N/a	Anodic	-11.3mV
PLL-PEG dynamic (after 17 days)	- 1.1[0.84]	N/a	Anodic	n.d.

5.2 Concentration dependence of F108 on EOF

We also studied the effect of coating agent concentration on EOF measurements to find the saturation concentration at which the maximum reduction in electroosmotic mobility occurs. The range of F108 concentration tested was from 1μM to 2mM. The maximum reduction in electroosmotic mobility was ~ 80 % with respect to the untreated surface at around 100μM. After that there was no further reduction in the mobility with further increase in the F108 concentration. The percentage reduction in EOF mobility increases dramatically at

lower concentrations of F108 and then it reaches a saturation point, where the isotherm becomes flat. At lower concentration of F108, PDMS surface is not covered completely with the agent; therefore the standard deviations are high. But when the coating agent on the PDMS surface covered properly and reached the saturation, the standard deviations are much less and the maximum reduction in the EOF mobility observed. Figure 9 shows the isotherm where the % reduction in EOF mobility is plotted vs. concentration of F108. For dielectrophoretic manipulations, the EOF can be altered as desired by changing the concentration of the coating agent on PDMS surface. This gives the flexibility to control EOF.

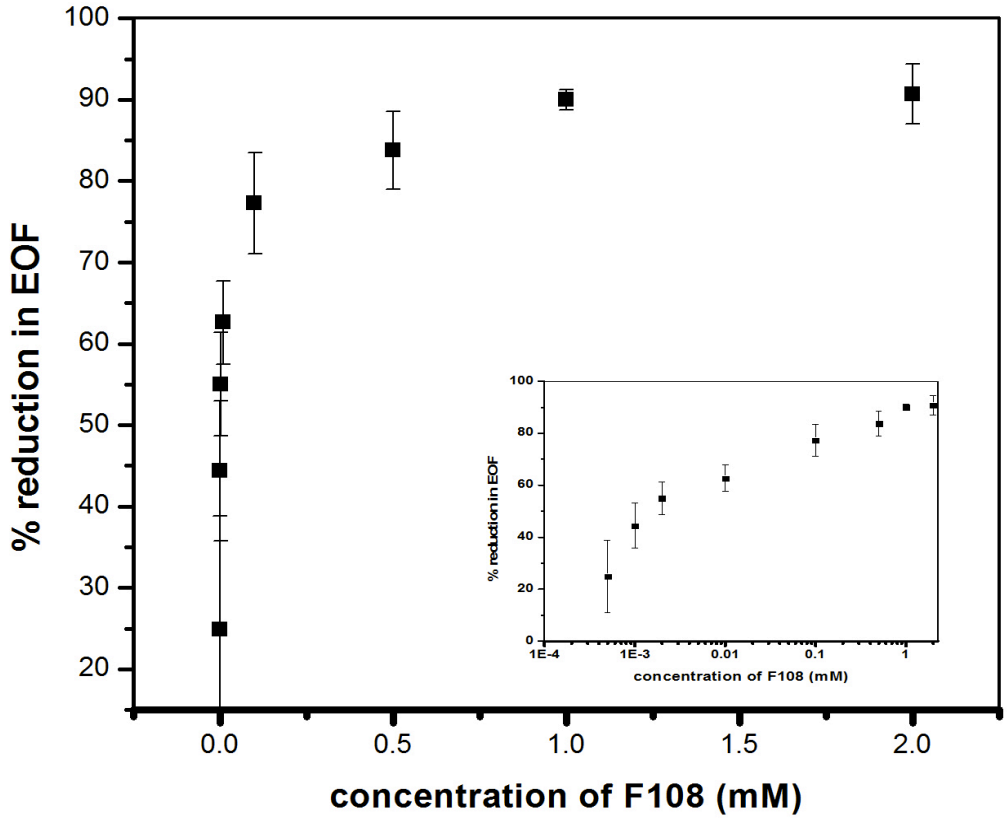


Figure 9. Concentration dependence of F108 on EOF under dynamic conditions. All values are the average values obtained from at least two repetitions in three channels.

5.3 Adsorption studies of protein

The PDMS surface was modified with coating agent, which was further incubated with fluorescently labeled protein. Relative intensity of fluorescence was measured for coating agent modified and plasma oxidized PDMS surfaces. The fluorescence microscopy imaging technique was used to probe the surface and count the intensity from the fluorophores. Bovine serum albumin (BSA) known to adsorb readily to surfaces was used for the adsorption studies. The concentration of protein chosen was 100nM. Fibrinogen, another protein present abundant in blood plasma was also tested for the adsorption studies. The concentration of fibrinogen used was 5mg/mL. We applied both dynamic and static coating strategies for adsorption studies using F108 and PLL-PEG as coating agents. The following Figure 10 shows the schematic procedure to conduct adsorption studies.

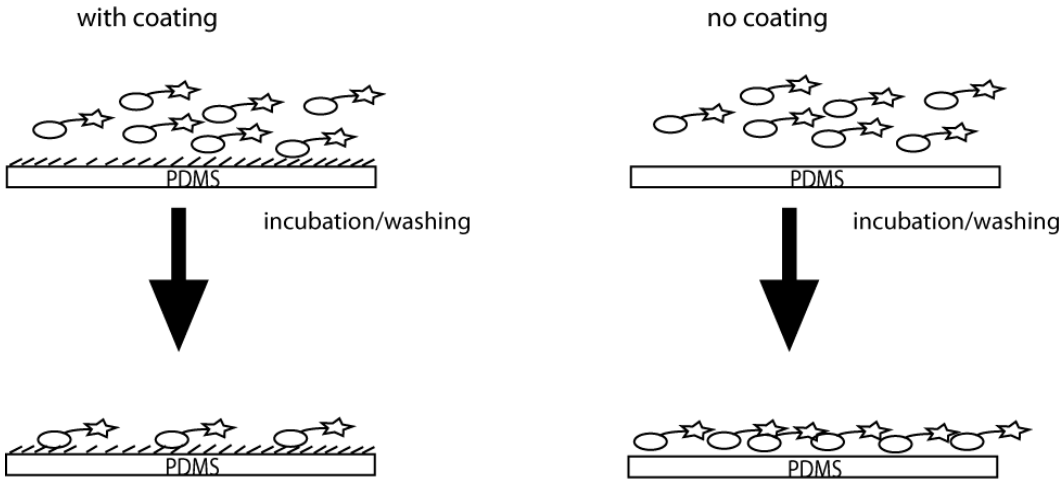


Figure 10. Schematics of protein adsorption studies. The figure shows the coating agent (short black lines) adsorbed on the surface. The other symbol (circle with star) depicts the proteins tagged with fluorophores. The left figure shows less adsorption of proteins on the oxidized PDMS surface after it was incubated with the coating agent. On the right side the proteins are readily adsorbed on the non-modified oxidized PDMS surface.

5.3.1 Static coating of F108 with BSA and fibrinogen

To test the effectiveness of F108 and its behavior on the PDMS surface, we incubated the PDMS surface with the coating material, by varying its concentration from 5μM to the maximum of 10mM. Here, we are interested to know the saturation point where the maximum reduction in the adsorption of protein occurs on the modified PDMS. After incubation and the washing steps as mentioned above, PDMS surface was probed by fluorescence microscopy imaging. As shown in Figure 11, the isotherm observed with F108 and BSA, F108 copolymer readily helped in reducing the protein adsorption. The standard deviations are little higher at the lower concentration of F108 but when the concentration of the coating agent increases and the PDMS surface gets covered with it properly, the standard deviations are much less. This is due to less protein adsorbed on the PDMS surface, when the PDMS surface covered with the coating agent. At lower concentrations, the PDMS surface was not fully covered with the agent and the proteins are interacting with the PDMS surface. The BSA protein (pKa ~ 4.7) [48] is negatively charged at pH 7 and the PDMS surface is also negatively charged. But when the concentration of F108 increases, it forms more stable covering on the PDMS. PEG molecules are believed to form a protective hydration shell around it in aqueous solution[16]. The PEG molecules bind water through hydrogen bonding. These hydration shells around PEG molecules prevent protein to interact with the PEG molecule and also to interact with the PDMS surface, thus preventing protein adsorption. For BSA, the saturation point was

observed at ~ 1mM, where the maximum reduction in the protein adsorption has been observed.

For fibrinogen, we performed the same studies, and the saturation point was observed at around same concentration of F108 as it was with BSA. Around 80% reduction in the protein adsorption has been observed. Swart et al.[49] has reported the effectiveness of F108 to reduce protein adsorption on non-porous membranes. Our results with F108 are in accordance to reported literature data. In our case F108 efficiently helped to reduce protein adsorption on PDMS surface. Figure 12 shows the isotherm observed with fibrinogen. All the values shown in the figure below are the average of three independent measurements and the error bars indicate the standard deviation. Thus, we can conclude that F108 works effectively in reducing the adsorption of proteins on the PDMS surface.

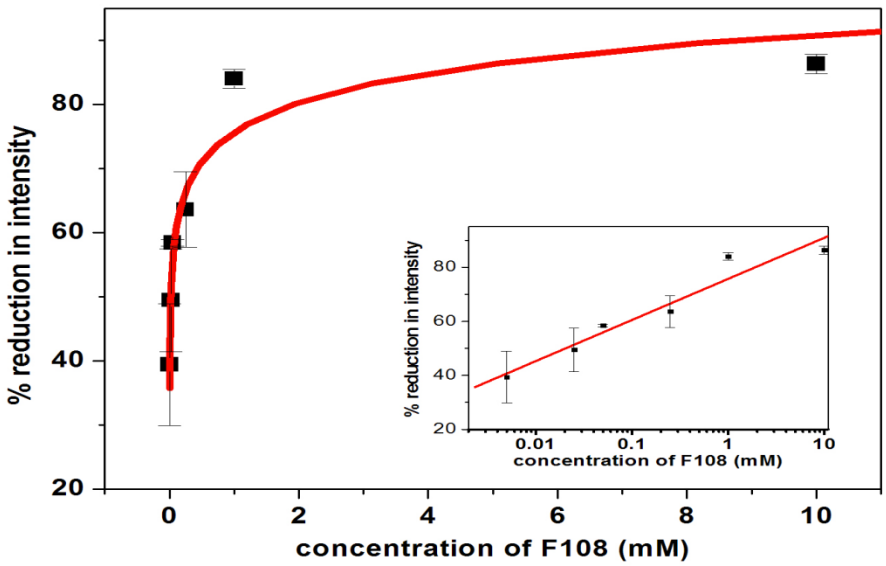


Figure 11. Protein adsorption for varying concentration of coating agent F108 under static condition: all values are the average of three independent measurements.

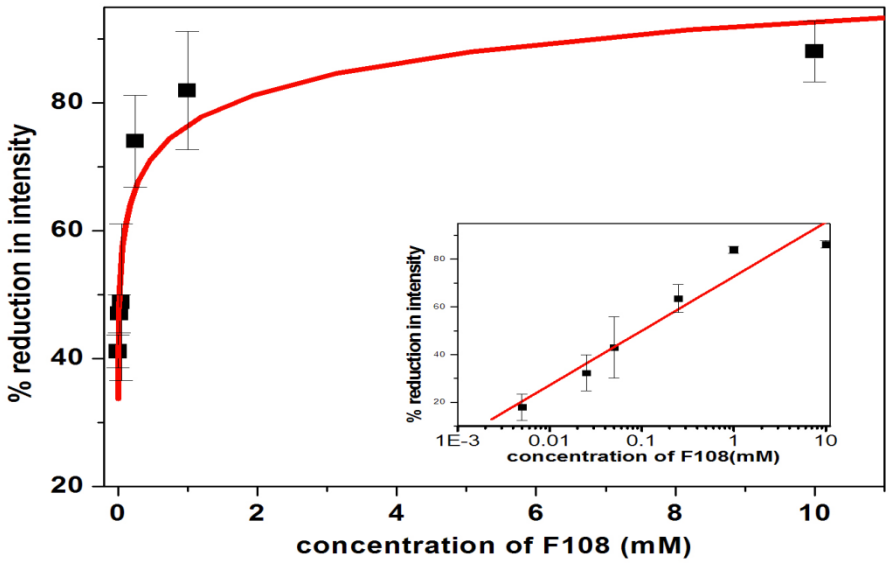


Figure 12. Protein adsorption for varying concentration of coating agent F108 under static condition: all values are the average of three independent measurements.

5.3.2 Static coating PLL-PEG (BSA)

Here, we tested PLL-PEG for the adsorption studies. This polymer worked equally effective as F108 to reduce the adsorption of protein. However, the saturation point with PLL-PEG was observed at 100 fold less concentration as was observed with F108. For PLL-PEG, the concentration varied from 0.04µM to the maximum 20µM to incubate the PDMS surface. Similar percentage ~80% in the reduction of protein adsorption was observed as compared with F108. Figure 13 shows the isotherm observed with PLL-PEG. At ~ 10µM the PDMS surface was almost covered with PLL-PEG. The PLL group attached to the PDMS surface via electrostatic interactions and the PEG chains are extruding out on the surface preventing the protein to adsorb on the surface. Our data using PLL-PEG

as a coating agent is in accordance with other reported literature values[16, 23, 30, 31]

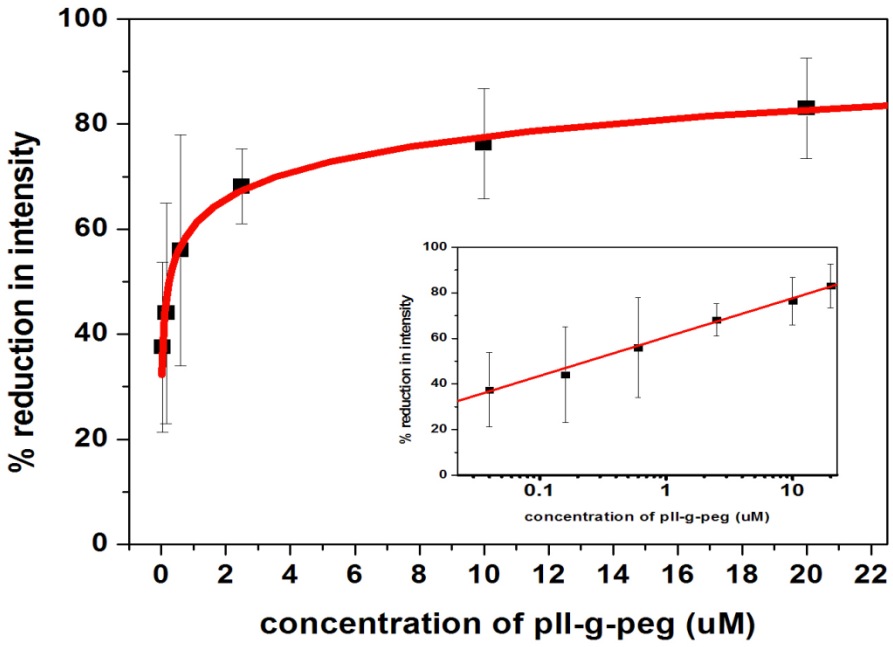


Figure 13. Protein adsorption for varying concentration of coating agent F108 under static condition: all values are the average of three independent measurements.

5.3.3 Time Dependence

For static coating, the PDMS surface was coated overnight, which is more time consuming. Therefore, we tested the amount of time sufficiently required to coat the PDMS surface properly by the coating agent to reduce protein adsorption. Here, we incubated the PDMS surface with the coating agents for different intervals of time. After treating the PDMS surface with the coating agent, it was treated with the mixture (100nM BSA and 1mM F108) of protein and the coating agent for 2 hrs. It was observed that time is not a factor for treating PDMS surface. Around same percentage of reduction in the adsorption of protein

was observed for the minimum (2 min) and the maximum time (1 hr) of incubation. So, we choose 10 min incubation time for our experiments with dynamic coating. Figure 14 shows the percentage reduction in intensity at different interval of incubation time.

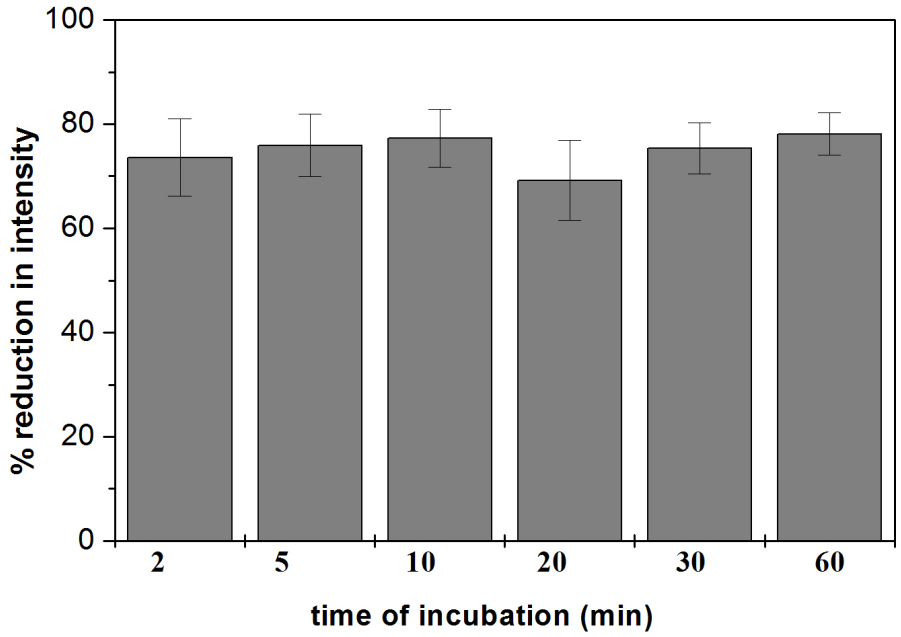


Figure 14. Time dependence of incubation under dynamic conditions with F108 coating agent.

5.3.4 Dynamic coating of F108 with BSA

Under dynamic condition, the PDMS surface was incubated with F108 for 10 min and was further incubated with the mixture of protein and F108 for 2 hr. Dynamic coating showed similar results as was concluded for static coating with F108.We applied same concentration of coating agent (lowest 5μM and highest 10mM) as used for static coating. At around 1mM the saturation point was observed with

the maximum of ~89% reduction in the adsorption of protein. Dynamic coating is more time saving and if the coating does not interfere with the analytes, then it would be a good strategy. Figure 15 shows the isotherm observed after applying the coating dynamically.

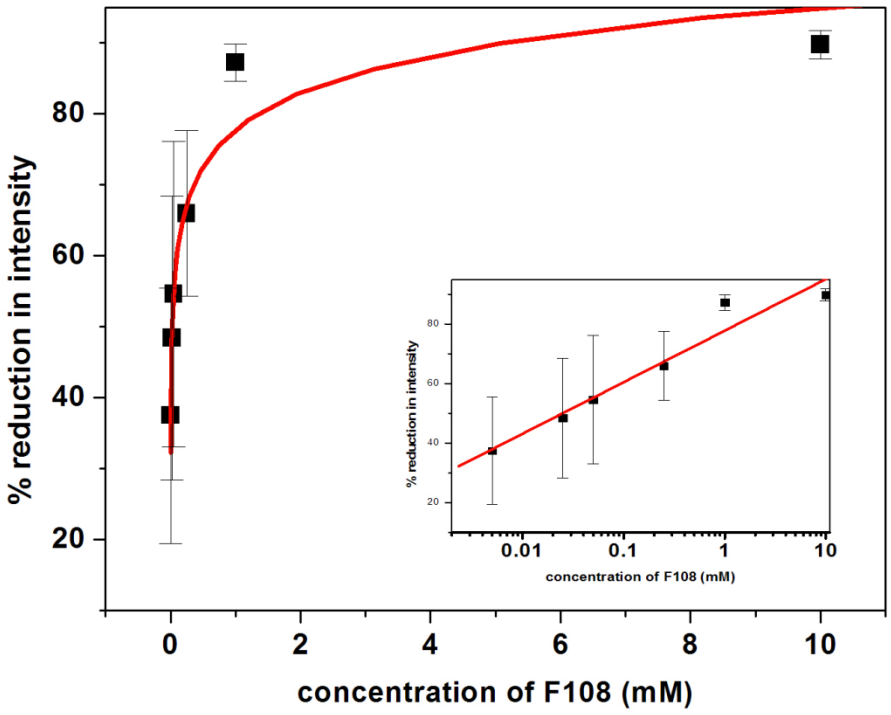


Figure 15. Protein adsorption for varying concentration of coating agent F108 under dynamic condition: all values are the average of three independent measurements

Chapter 6

CONCLUSIONS

We tested the effect of F108 and PLL-PEG coating agents on EOF and protein adsorption on PDMS surface. Coating agents were tested under both static and dynamic conditions. These coating agents could significantly reduce the electroosmotic mobility. The dynamic strategy is found to be more effective as compared to the static method to control electroosmotic flow. With F108 dynamic coating more than 90 % reduction was observed[12]. However, with F108 static coating only 43% reduction has been observed. The possible reason accounted for the effectiveness of dynamic coating is the replenishment of the PDMS surface with the coating agent. Interestingly, PLL-PEG static coating resulted in ~ 80% reduction in EOF mobility. This is attributed to the strong electrostatic interactions of PLL-PEG as compared to the weak hydrophobic interactions of F108 with the PDMS surface. With PLL-PEG dynamic coating, however, the flow was reversed. The charges on the PDMS surface get positive with the dynamic application of PLL-PEG. This interesting finding could be used to manipulate the EOF in a specific direction relative to electrophoretic transport. With the change in the concentration of PLL-PEG coating agent, the EOF mobility can be controlled and can be eliminated to null. This is advantageous for DC analytical application in which EOF is not desired.

The adsorptions were tested via fluorescence microscopy. The proteins were tagged with the fluorophore Alexa - 488. Both F108 and PLL-PEG copolymers

were equally effective in reducing the adsorption of proteins on the PDMS. Further, static and dynamic coating both were equally effective to reduce the adsorption. However, the dynamic coating strategy is more simple and time saving. If the interference of coating agent with the sample is not an issue, the dynamic coating is the best option for physisorbed modification. We were able to find the saturation point where the maximum reduction in the protein adsorption was observed. To determine the maximum coverage of the PDMS surface, the concentration of the coating agent varied. At lower concentrations of the coating agent the adsorption of protein was still high. This is due to the direct interaction of PDMS with the proteins. But, when the PDMS get covered with the coating agent, the protein interacts less with the charged PDMS surface. The protective hydration shell around the PEG molecules prevents the proteins to interact with the PDMS surface and reduces protein adsorption. These PEG molecules not only reduced the adsorption of proteins but also reduce the degree of protein unfolding and denaturing[17].

In summary, the surface modification of PDMS using polyethylene oxide copolymer has the potential to reduce adsorption of proteins and to control EOF, which is useful for various microfluidic applications. These modified PDMS surfaces are stable for days and can produce reproducible results. The PEG based modified PDMS are highly protein resistant and able to control EOF.

REFERENCES

1. Arora, A.; Simone, G.; Salieb-Beugelaar, G. B.; Kim, J. T.; Manz, A., *Analytical Chemistry 82* (12), 4830-4847.

2. Reyes, D. R.; Iossifidis, D.; Auroux, P. A.; Manz, A., *Analytical Chemistry* **2002,** *74* (12), 2623-2636.

3. Auroux, P. A.; Iossifidis, D.; Reyes, D. R.; Manz, A., *Analytical Chemistry* **2002,** *74* (12), 2637-2652.

4. Dittrich, P. S.; Tachikawa, K.; Manz, A., *Analytical Chemistry* **2006,** *78* (12), 3887-3907.

5. Whitesides, G. M., *Nature* **2006,** *442* (7101), 368-373.

6. West, J.; Becker, M.; Tombrink, S.; Manz, A., *Analytical Chemistry* **2008,** *80* (12), 4403-4419.

7. Zhang, X. L.; Haswell, S. J., *Mrs Bulletin* **2006,** *31* (2), 95-99.

8. McDonald, J. C.; Whitesides, G. M., *Accounts of Chemical Research* **2002,** *35* (7), 491-499.

9. Mata, A.; Fleischman, A. J.; Roy, S., *Biomedical Microdevices* **2005,** *7* (4), 281-293.

10. Ren, X. Q.; Bachman, M.; Sims, C.; Li, G. P.; Allbritton, N., *Journal of Chromatography B* **2001,** *762* (2), 117-125.

11. Wu, D. P.; Zhao, B. X.; Dai, Z. P.; Qin, J. H.; Lin, B. C., *Lab on a Chip* **2006,** *6* (7), 942-947.

12. Hellmich, W.; Regtmeier, J.; Duong, T. T.; Ros, R.; Anselmetti, D.; Ros, A., *Langmuir* **2005,** *21* (16), 7551-7557.

13. Garcia, C. D.; Dressen, B. M.; Henderson, A.; Henry, C. S., *Electrophoresis* **2005,** *26* (3), 703-709.

14. Huang, B.; Wu, H. K.; Kim, S.; Zare, R. N., *Lab on a Chip* **2005,** *5* (10), 1005-1007.

15. Lee, S.; Voros, J., *Langmuir* **2005,** *21* (25), 11957-11962.

16. Blattler, T. M.; Pasche, S.; Textor, M.; Griesser, H. J., *Langmuir* **2006,** *22* (13), 5760-5769.

17. Hu, S. W.; Ren, X. Q.; Bachman, M.; Sims, C. E.; Li, G. P.; Allbritton, N., *Analytical Chemistry* **2002,** *74* (16), 4117-4123.

18. De Smet, N.; Rymarczyk-Machal, M.; Schacht, E., *Journal of Biomaterials Science-Polymer Edition* **2009,** *20* (14), 2039-2053.

19. Ionov, L.; Synytska, A.; Kaul, E.; Diez, S., *Biomacromolecules 11* (1), 233-237.

20. Sugiura, S.; Edahiro, J. I.; Sumaru, K.; Kanamori, T., *Colloids and Surfaces B-Biointerfaces* **2008,** *63* (2), 301-305.

21. Yang, L. Y.; Li, L.; Tu, Q.; Ren, L.; Zhang, Y. R.; Wang, X. Q.; Zhang, Z. Y.; Liu, W. M.; Xin, L. L.; Wang, J. Y., *Analytical Chemistry 82* (15), 6430-6439.

22. Xiao, Y.; Yu, X. D.; Xu, J. J.; Chen, H. Y., *Electrophoresis* **2007,** *28* (18), 3302-3307.

23. Michel, R.; Pasche, S.; Textor, M.; Castner, D. G., *Langmuir* **2005,** *21* (26), 12327-12332.

24. Choi, H. -G.; Zhang, Z.; Boccazzi, P.; Laibinis, P.E.; Sinskey, A.J.; Jensen, K.F**.**, *Proceedings of the 7th lnternational Conference on Miniaturized Chemical and Biochemical Analysis Systems* **2003,**1105-1108.

25. Wong, I.; Ho, C.-M., *Microfluidics and Nanofluidics* **2009,** *7* (3), 291-306.

26. McDonald, J. C.; Duffy, D. C.; Anderson, J. R.; Chiu, D. T.; Wu, H. K.; Schueller, O. J. A.; Whitesides, G. M., *Electrophoresis* **2000,** *21* (1), 27-40.

27. Morra, M.; Occhiello, E.; Marola, R.; Garbassi, F.; Humphrey, P.; Johnson, D., *Journal of Colloid and Interface Science* **1990,** *137* (1), 11-24.

28. Kim, J.; Chaudhury, M. K.; Owen, M. J.; Orbeck, T., *Journal of Colloid and Interface Science* **2001,** *244* (1), 200-207.

29. Luo, Y. Q.; Huang, B.; Wu, H.; Zare, R. N., *Analytical Chemistry* **2006,** *78* (13), 4588-4592.

30. Marie, R.; Beech, J. P.; Voeroes, J.; Tegenfeldt, J. O.; Hook, F., *Langmuir* **2006,** *22* (24), 10103-10108.

31. Huang, N. P.; Michel, R.; Voros, J.; Textor, M.; Hofer, R.; Rossi, A.; Elbert, D. L.; Hubbell, J. A.; Spencer, N. D., *Langmuir* **2001,** *17* (2), 489-498.

32. Lucke, A.; Tessmar, J.; Schnell, E.; Schmeer, G.; Gopferich, A., *Biomaterials* **2000,** *21* (23), 2361-2370.

33. Liu, V. A.; Jastromb, W. E.; Bhatia, S. N., *Journal of Biomedical Materials Research* **2002,** *60* (1), 126-134.

34. Yoon, J.-H. H. a. J.-Y., *Journal of Biological Engineering* **2009,**3:6,1-6.

35. Chen, W.; Yuan, J. H.; Xia, X. H., *Analytical Chemistry* **2005,** *77* (24), 8102-8108.

36. Feng, J.-J.; Wang, A.-J.; Fan, J.; Xu, J.-J.; Chen, H.-Y., *Analytica Chimica Acta 658* (1), 75-80.

37. Zhang, Z.; Feng, X.; Xu, F.; Liu, X.; Liu, B.-F., *Electrophoresis 31* (18), 3129-3136.

38. Prime, K. L.; Whitesides, G. M., *Science* **1991,** *252* (5009), 1164-1167.

39. Liu, Y.; Fanguy, J. C.; Bledsoe, J. M.; Henry, C. S., *Analytical Chemistry* **2000,** *72* (24), 5939-5944.

40. Gaudioso, J.; Craighead, H. G., *Journal of Chromatography A* **2002,** *971* (1-2), 249-253.

41. Morgenthaler, S.; Zink, C.; Staedler, B.; Voeroes, J.; Lee, S.; Spencer, N. D.; Tosatti, S. G. P., *Biointerphases* **2006,** *1* (4), 156-165.

42. Tandon, V.; Bhagavatula, S. K.; Nelson, W. C.; Kirby, B. J., *Electrophoresis* **2008,** *29* (5), 1092-1101.

43. Sze, A.; Erickson, D.; Ren, L. Q.; Li, D. Q., *Journal of Colloid and Interface Science* **2003,** *261* (2), 402-410.

44. Huang, X. H.; Gordon, M. J.; Zare, R. N., *Analytical Chemistry* **1988,** *60* (17), 1837-1838.

45. Duffy, D. C.; McDonald, J. C.; Schueller, O. J. A.; Whitesides, G. M., *Analytical Chemistry* **1998,** *70* (23), 4974-4984.

46. Lucy, C. A.; Underhill, R. S., *Analytical Chemistry* **1996,** *68* (2), 300-305.

47. Viefhues, M.; Manchanda, S.; Chao, T. C.; Anselmetti, D.; Regtmeier, J.; Ros, A., *Analytical and Bioanalytical Chemistry 401* (7), 2113-2122.

48. Tang, S.-W.; Chang, C.-H.; Wei, H.-H., *Applied Physics Letters 97* (4).

49. Govender, S.; Jacobs, E. P.; Bredenkamp, M. W.; Swart, P., *Journal of Colloid and Interface Science* **2005,** *282* (2), 306-313.