

Site-specific Anti-adsorptive Passivation in Microchannels

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Abstract

A novel methodology for generating site-specific anti-adsorptive coatings on microchannel walls was reported. The coating process consists of two steps. The first step is to coat the glass channel wall with linker molecules of TMSMA. And then the anti-adsorptive polymer, PEGMA, is photo-grafted onto the surface via a UV induced radical reaction using a patterned photo mask. We evaluated the proposed anti-adsorptive coating method by investigating the adsorption behavior of BSA-FITC and amine-modified polystyrene beads. This method offers valuable advantages because the coating process is carried out after the entire chip fabrication steps have been completed. The functional materials of interest could be grafted onto the microchannel wall which is difficult to access mechanically. Moreover, this method enables site-specific multiple materials coating preserving the organic layers undamaged during the chip fabrication processes.

Keywords: Antifouling, PEG, Surface coating, Photo grafting, Protein, Microfluidics

Introduction

Recently, the demand for site-specific and safe functional coating of microchannel walls that could be performed after chip fabrication has undergone a rapid increase¹. As the number of functions of microfluidic systems becomes greater, integrated microfluidic channel networks will require different components to play different roles. Every component needs its own surface that can be achieved only by using organic functional layers. Protein anti-adsorptive coating is one of the functions essential for an intelligent

microfluidic system. Such functional surfaces, however, are readily damaged by such harsh microfabrication processes as found with bonding. As a consequence, microchannel coating should be carried out after the chip fabrication. Unfortunately, it is difficult to coat certain molecules or polymers precisely where needed in a specific microfluidic channel network because of the limitations of existing mechanical approaches.

For most biological microanalyses incorporating proteins, surface adsorption of the protein is a significant problem. This is especially the case when the analytic system includes microchannels. The situation actually worsens as the surface to volume ratio increases. As a result, various methods have been developed over the last several decades to prevent or minimize nonspecific adsorption of protein. Anti-adsorptive surface coating methods are classified into dynamic and static coating procedures. Static anti-adsorptive surface coating has long been addressed in the fields of chromatography and capillary electrophoresis. In the present study, we focused on static coating because dynamic coating is not appropriate for site-specific anti-adsorption. Bi-functional reagents of silane derivatives were employed for static anti-adsorptive coating², however; polyacrylamide has replaced these reagents due to hydrolysis at elevated pH³. Poly(ethylene glycol) (PEG) is another material that has been used for a long time^{4,5} and many processes for pegylation reportedly were very reproducible and easy to implement⁵.

Recent methods for static anti-adsorptive coating include treatment with plasma^{6,7}, thermal energy induction⁸, and vapor deposition⁹. Plasma treatment and vapor deposition processes can be site-specific; however, they can be patterned only on flat and open surfaces, and not for ready-made microchannels. Also, heat transfer coating poses the fundamental problem of thermal conduction for the site-specific coating. Responding to these design and development challenges, some research groups have utilized a photo-grafting method to create porous polymer monoliths in predefined patterns¹⁰⁻¹¹. Stachowiak¹² coated PEG onto a polymer chip monolith following single-step and two-step photo grafting methods.

In this study, we report on a method to coat open glass microchannels with PEG based on a two-step photo-grafting protocol. Glass is a robust and commonly used chip material for the manipulation and

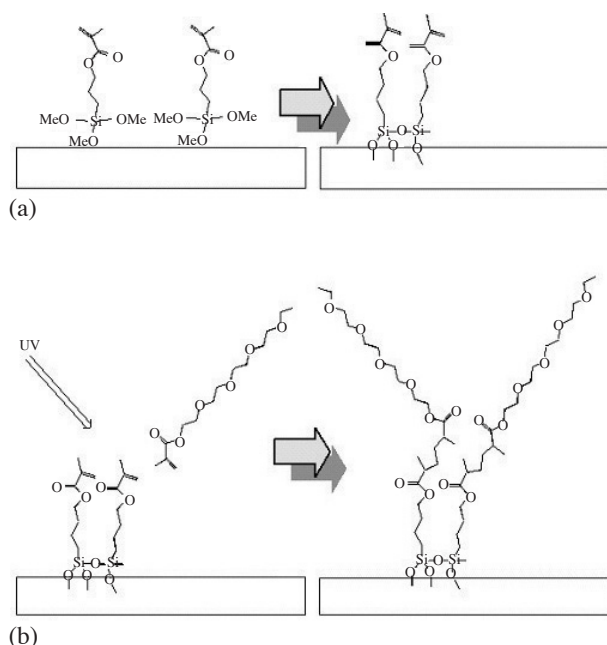


Figure 1. Schematic for the site-specific anti-adsorptive coating process. The linker, TMSMA is coated to the glass microchannel walls (a) and then the PEGMA is photo-grafted to the linker by UV irradiation initiating the radical reaction (b).

separation of proteins. Moreover, mass transport of samples and solutions in the miniaturized microanalytical system requires specific functional parts in the channel network which is a mostly open channel structure. Thus, site-specific functional coating is a powerful addition to chip-based microanalytical systems where a protein anti-adsorptive surface is the essential component.

Trimethylsilylmethacrylate (TMSMA) was used as the linker molecule (Figure 1a), which was bound to poly(ethylene glycol) methacrylate (PEGMA) as a result of patterned UV-irradiation (Figure 1b).

Results and Discussion

A solution of bovine serum albumin-fluorescein isothiocyanate (BSA-FITC) in phosphate buffered saline (PBS) (pH 7.4) was adsorbed to the surface wall of the microchannel. The BSA solution (1 mg/mL) was passed through the glass microchannel (30 μm wide and deep) by a syringe pump (KDS100, KD Scientific, USA). The flow rate was kept at 1 $\mu\text{L}/\text{h}$ for 7 h. After rinsing the microchannel with deionized water, a fluorescence image was captured by the CCD camera connected to a fluorescence microscope

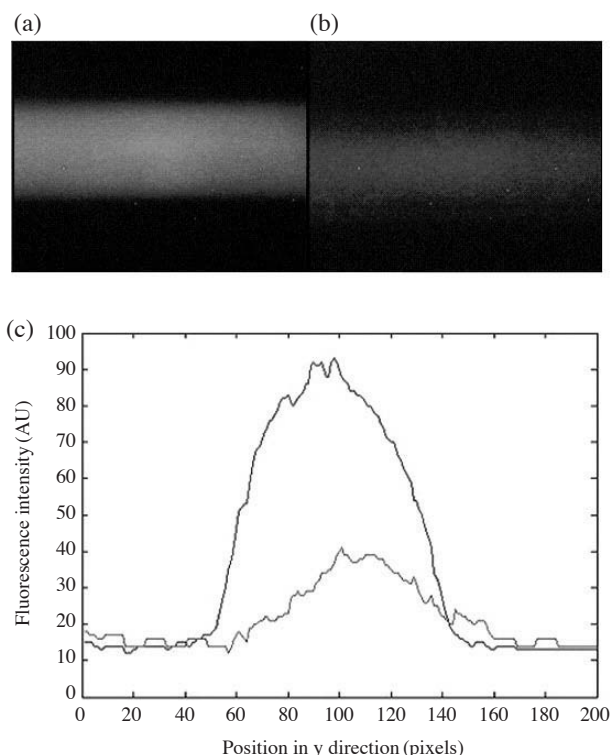


Figure 2. BSA-FITC solution passed through the naked glass microchannel and subsequent fluorescence image was captured by fluorescence microscopy (a). A surface treated microchannel was similarly contaminated by BSA-FITC and a fluorescence image captured (b). The fluorescence intensities have been plotted (c).

(Nikon TE-2000). Fluorescence from BSA-FITC on the microchannel wall surfaces was readily observed. Figures 2a and 2b show differences in BSA adsorption between the glass microchannels treated and not treated by PEG photo-grafting. The fluorescence intensity across the microchannel can be seen in Figure 2c.

The anti-adsorptive coating method for a porous polymer monolith¹² reportedly suppressed the fluorescence intensity from BSA adsorption to less than 10%. A previous report on a non-biofouling surface coating on both a glass and a polydimethylsiloxane (PDMS)⁵ product demonstrated that fluorescence intensity declines to a similar extent. In the present work, Figure 2c indicates that about 30% of protein adsorbed remains after rinsing. Compared with the preceding reports, this result does not seem impressive. It should be noted, however, that a glass microchannel structure involves the additional variable of flow rate during UV irradiation. Thus, further effort should be made to optimize the coating process compatible with improved performance. In addition, the structure and the materials of the substrate on which

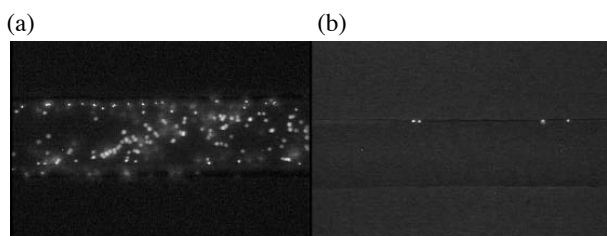


Figure 3. The amine-modified polystyrene beads passed through the naked glass microchannel and their fluorescence images captured (a). The same solution was passed through the surface-treated microchannel and the fluorescence image captured (b). The number of adsorbed beads was seen to be significantly reduced.

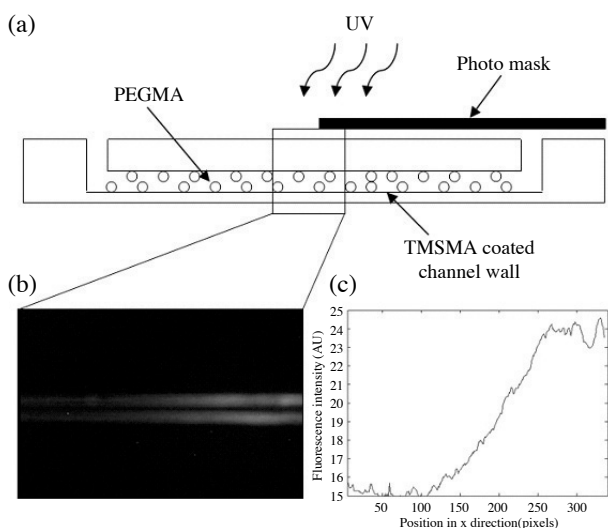


Figure 4. Schema of site-specific PEG photo-grafting (a). After the BSA-FITC solution was passed through, the fluorescence image in the box of picture (a) was taken (b). Fluorescence intensity profile across the microchannel (c).

coating occurs differ from the previous studies and hence, comparison in terms of performance would not be relevant. Nonetheless, the results shown in Figure 2 clearly indicate that protein adsorption onto the open glass microchannel can be substantially reduced by the proposed direct coating method.

We have in addition exposed microbeads to both naked and PEG coated glass microchannel walls. The amine-modified polystyrene beads in PBS (pH 7.4) was prepared and allowed to pass through the glass microchannel (30 μm wide and deep). The flow rate was controlled by a syringe pump set at 1 $\mu\text{L}/\text{h}$ being for 1 h interval. At pH 7.4, amine modified beads are positively charged whereas the glass surface remains negatively charged. Marked adsorption of beads to the glass channel walls (Figure 3a) was observed while

in pegylated microchannel (Figure 3b) only a few beads were adsorbed.

Finally, photo-grafting of PEGMA to the linker (TMSMA) onto the glass microchannel was patterned by using a photo mask (Figure 4a). TMSMA coating was followed by attaching a printed film mask to the microchip. Figure 4b indicates the fluorescence image taken after protein adsorption at the end of 7 h. The flow rate and the concentration of the BSA-FITC solution were identical as in our first experiment (Figure 1). From intensity differences, we determined that the protein adsorption of the right side of the channel was higher than the left side. Fluorescence intensity along the microchannel (width of 30 μm) is shown in Figure 4c. The intensity profile indicates that the photo mask selectively inhibited and allowed the photo-grafting reaction between PEGMA in the solution and TMSMA onto the surface.

Conclusion

To achieve a site-specific anti-absorptive micro-channel wall coating, we proposed the use of photo-grafting PEG onto TMSMA tethered to open glass microchannel via UV irradiation. Most important of the several valuable advantages offered by the proposed method of fabrication is that all coating processes can be performed after completion of microchip manufacture. Both adsorption experiments with FITC-BSA and the amine-modified beads clearly demonstrated that PEG coating on a specific part of the microfluidic network substantially reduced protein adsorption without obstructing the channel. This site-specific anti-adsorptive coating method can be applied to many glass micro fluidic chips with open micro-channel networks, especially for microanalytical systems that are integrated with multiple functional coating components.

Materials and Methods

Materials

Corning 2947 glass slides were used to fabricate the glass microchannel. TMSMA, PEGMA and 2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropionophenone, the photo initiator, were purchased from Sigma-Aldrich, USA. Acetone, sulfuric acid, hydrogen peroxide and the buffered oxide etching solution used in the microchip fabrication processes and methanol were purchased from Mallinckrodt Baker, USA. The HMDS, Photo resist AZ 4620 and developer solution 400K was obtained from Clariant, Switzerland.

A UV-cabinet (Ultra LUM, model: UVA-515) was

used to implement photo-grafting. FITC-BSA solution was purchased from Biomed, USA. Amine-modified bead (polystyrene, 1.0 μm , yellow green fluorescent 2% in water) was used as received from Bangs Laboratory, USA.

Glass Micro Chip Fabrication

Corning 2947 glass was cleaned using a sulfuric acid and hydrogen peroxide solution (3 : 1). Photolithography was used to pattern microchannel on the slide glass. Glass to glass fusion bonding was performed at approximately 600°C in an oven. The width and depth of the microchannel for all experiments was kept at 30 μm .

Linker Coating

The glass microchannel were filled with acetic acid 0.4% (v/v) and TMSMA 0.4% (v/v) in methanol for 2 h and dried for 15 min on a hot plate (90°C, Figure 1a). During contact time, the TMSMA solution was prevented from drying by sealing the microchip reservoir using a paraffin film. Because the linker is very sensitive to the light and thermal energy, all linker coating processes were conducted in a dark room.

PEG Photo Grafting

50% (v/v) of PEGMA and 2% (wt/wt) of the photo initiator in water solution was filled in the microchannel and exposed to ultra violet (UV) light for 10 min in a UV-cabinet. The methacrylate groups in TMSMA and PEGMA were bound (Figure 1b) via the radical reaction initiated by UV light.

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