Patternable Protein Resistant Surfaces for Multifunctional Microfluidic Devices via Surface Hydrophilization of Porous Polymer Monoliths Using Photografting

Timothy B. Stachowiak,[†] Frantisek Svec,[‡] and Jean M. J. Fréchet*[,†,‡]

College of Chemistry, University of California, Berkeley, California 94720-1460, and The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720-8139

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Surface-modified macroporous polymer monoliths that resist the adsorption of proteins have been prepared using both single- and two-step photografting of hydrophilic monomers. The adsorption of protein was measured using a fluorescence assay based on bovine serum albumin labeled with fluorescein. Acrylamide, 2-hydroxyethyl methacrylate, vinyl pyrrolidinone, and poly(ethylene glycol) methacrylate (PEGMA) monomers were grafted and evaluated for their ability to prevent protein adsorption. Photografted layers of PEGMA reduced protein adsorption to less than 2% relative to unmodified surfaces. The sequential two-step photografting process consisted in (i) the formation of covalently bound surface photoinitiator sites followed by (ii) surface-localized graft polymerization. Monomer concentration and irradiation time during photografting were found to be the most important parameters for optimization of the two-step process. For simultaneous single-step photografting, the solvent and the presence of photoinitiator were the key variables. Initiator-free single-step photografting was less efficient than the two-step technique, yet resulted in similar prevention of protein adsorption after grafting for an extended period of time. The utility of photografting as a simple, patternable modification technique was demonstrated by first creating a hydrophilic surface within the monolith that was subsequently patterned with a layer of reactive 2-vinyl-4,4-dimethylazlactone polymer chains followed by the immobilization of green fluorescent protein.

Introduction

A challenge to the implementation of increasingly complex and integrated microanalytical systems is the need for a multiplicity of different surface chemistries to be located precisely within the different sections of a microfluidic device. Applications such as microscale chromatographic separation and solid-phase extraction rely on specific interactions between analyte molecules and the surfaces of the device. In contrast, other applications may require that surface interactions be eliminated. For example, adsorption of compounds onto microfluidic mixers, filters, supports for enzyme immobilization, or the walls of an electrophoresis channel should be minimized. The need to reduce surface interactions in some regions of a device while maximizing the interactions in other parts requires access to techniques for the well-controlled patterning of chemical functionalities within the device.

Porous polymer monoliths have attracted significant attention as versatile, high-surface-area matrices useful for the creation of a variety of functional modules within microfluidic devices.[1] Polymer monoliths with desired porous properties are easily and rapidly prepared using photoinitiated free radical polymerization at specific locations directly within the microfluidic channels.[2,3] The surface chemistry and porous properties of the monoliths can be tailored to suit a variety of applications by adjusting the composition of the initial monomer solution and the polymerization conditions.[4] For example, the porous structure of monoliths can be utilized to improve mixing in microfluidic channels,[2] whereas thermally responsive polymer monoliths can operate as temperature-actuated microvalves.[5] Hydrophobic monoliths are suitable as supports for solid-phase extraction and can also be used for electrochromatographic separations in microchips.[3,6−10] In addition, enzymatic microreactors capable of effecting the rapid digestion of proteins can be created in microchannels using monoliths with immobilized trypsin.[11]

* To whom correspondence should be addressed. Fax: (510) 643-3079. E-mail: frechet@berkeley.edu.
[†] University of California, Berkeley.
[‡] The Molecular Foundry.

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These individual functional monolithic modules now need to be combined and accurately positioned within a device to create integrated analytical systems. Preparing such modules by consecutive in situ polymerizations of mixtures of monomers containing the desired functionalities would be quite challenging because of the complexity of performing several polymerization steps in close succession, the necessity of individually optimizing each polymerization mixture, and the difficulty of avoiding overlap of chemistries intended for adjacent areas.

Thus, using a single, optimized monolith as a generic scaffold for subsequent surface modification via photografting of monomers that possess the required chemical functionalities is a very attractive approach for creating integrated, multifunctional devices based on porous polymer monoliths.[12,13] For example, ionizable monomers have been photografted onto polymer monoliths to create the surface chemistry needed for electrochromatographic separations.[13,14] Because the grafting reaction is initiated by UV light, the location of grafts can be precisely controlled using photomasks, allowing different sections of a single monolith to be functionalized with different surface chemistries. Therefore, we used the spatial resolution of photografting to fabricate monolithic devices with dual functionality that combined solid-phase extraction and enzymatic digestion.[15] However, because the original monolithic matrix is hydrophobic adsorption of proteins and peptides cannot be restricted to the desired solid-phase extraction portion of the device.

In this work, surface modification of polymer monoliths via photoinitiated photografting with hydrophilic monomers in order to limit protein adsorption has been investigated using both single-step and two-step techniques. In our initial work, we explored a single-step photografting technique originally developed for flat surfaces by Rånby,[16] in which grafting is achieved by irradiation of a solution of functional monomer containing a photoinitiator such as benzophenone.[12−15] Upon photoexcitation, the benzophenone molecules abstract hydrogen atoms from the polymer surface and the resulting surface radicals initiate surface graft polymerization. Although very convenient, this procedure also creates significant amounts of nongrafted polymer within the solution; removal of the viscous liquid from the small pores is difficult and, in some cases, permanent clogging of the pores is observed.

To avoid solution polymerization, researchers have developed a number of sequential photografting techniques that address some of the limitations of the conventional single-step photografting.[17−22] In general, these approaches are based on localizing the photoinitiator at the polymer surface so that graft polymerization is favored and formation of polymer in solution is reduced. Most of these techniques rely on the simple adsorption of the photoinitiator onto the polymer. However, physically adsorbed initiator can be released from the surface when the polymer is exposed to the monomer solution, resulting in solution polymerization and reduced surface grafting. Therefore, Ogiwara et al. used poly(vinyl acetate) to entrap photoinitiator at the polyethylene surface.[17] Ulbricht at al. used initiator-saturated monomer solutions to inhibit the desorption of preadsorbed photoinitiator from the polymer.[18] In addition, photoinitiator has also been entrapped within the polymer itself by swelling the polymer with an appropriate solvent-containing photoinitiator.[21] A superior alternative sequential technique, developed by Ma et al. for polypropylene microfiltration membranes, involves the covalent attachment of photoinitiator to the polymer before monomer is introduced.[22] This technique avoids the use of additional species on the surface or in the monomer solution and ensures that the initiator is located at the internal surface of the porous polymer. Using this sequential photografting technique, as well as other single-step techniques, we have prepared patterned hydrophilic regions on polymer monoliths and evaluated their ability to effectively resist protein adsorption.

Experimental Section

Materials. Acrylamide (99+%, AAm), butyl methacrylate (99%, BuMA), ethylene dimethacrylate (98%, EDMA), 2-hydroxyethyl methacrylate (97%, HEMA), 1-vinyl-2-pyrrolidinone (99+%, VP), 1-decanol (99+%), benzophenone (99+%, BP), 3-(trimethoxysilyl)-propyl methacrylate (98%), and *tert*-butyl alcohol (99.5%) were purchased from Sigma-Aldrich. Cyclohexanol (99+%) and 2,2-dimethoxy-2-phenylacetophenone (99+%, DMPA) were purchased from Fluka. Undecaethyleneoxide methacrylate (PEGMA,11) and triethyleneoxide methacrylate (PEGMA,3) were purchased from Sartomer. BuMA and EDMA were purified by distillation under a reduced pressure. The HEMA and PEGMA monomers were passed through a column containing inhibitor remover beads of basic alumina (Aldrich). All other reagents were of the highest available purity and used as received. Fluorescein-labeled bovine serum albumin (BSA) was obtained from Invitrogen. Green fluorescent protein (GFP) was a gift from the research group of Professor Douglas S. Clark (University of California, Berkeley); the His-tagged GFP had been expressed in *E. coli* and purified using nickel affinity chromatography. 2-Vinyl-4,4-dimethylazlactone (VAL) was a gift from the 3M Company (St. Paul, MN). UV transparent Teflon-coated fused silica capillary (100 μm ID) was obtained from Polymicro Technologies (Phoenix, AZ).

Instrumentation. An OAI Model 30 deep UV collimated light source (San Jose, CA) fitted with a 500 W HgXe lamp was used for UV exposures. The irradiation power was calibrated to 15.0 mW/cm^2 using an OAI Model 306 UV power meter with a 260 nm probe head. Solutions were pumped through capillaries using a KDS 210 syringe pump from KD Scientific Inc. (Holliston, MA).

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Microscopic evaluations were performed using a Nikon TE200 inverted fluorescence microscope (Scientific Instrument Company, Sunnyvale, CA). Images were acquired with a Micropublisher 5.0 RTV CCD camera from QImaging (Burnaby, BC, Canada).

Activation of Capillaries. The walls of the fused silica capillaries were first activated with 3-(trimethoxysilyl)propyl methacrylate to enable covalent attachment of the monolith to the walls through the resulting pendent vinyl groups. Capillaries were first rinsed with acetone and water using a syringe pump and then activated with 0.2 mol/L sodium hydroxide for 30 min at a flow rate of 0.5 μL/min; afterward, they were rinsed with water. Next, the capillaries were flushed with 0.2 mol/L hydrochloric acid for 30 min at a flow rate of 0.5 μL/min, rinsed with water again, and finally flushed with ethanol. A 20 wt % solution of 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol with pH adjusted to 5 using acetic acid was pumped through the capillaries at a flow rate of 0.5 μL/min for 60 min. The capillary was then washed with acetone, dried in a stream of nitrogen, and left at room temperature for 24 h.

Preparation of Porous Polymer Monoliths. A polymerization mixture consisting of 24 wt % BuMA, 16 wt % EDMA, 50 wt % 1-decanol, 10 wt % cyclohexanol, and 1 wt % DMPA (with respect to monomers) was purged with nitrogen for 10 min. The 10 cm long activated capillary was filled with this mixture, placed under the light source, and irradiated with UV light for 10 min at a distance of 30 cm from the UV source. After photopolymerization, the porogenic solvents were flushed from the polymer monolith by pumping methanol through the column at a flow rate of 0.5 μL/min for 8 h. The pore size of the monolith was $\sim$1.6 μm, as measured by mercury intrusion porosimetry.[13]

Sequential Two-Step Photografting. The monolith in capillary was flushed with a deaerated 5 wt % BP solution in methanol at a flow rate of 0.5 μL/min for 30 min. The ends of the capillary were sealed with rubber septa to prevent flow and evaporation, and the capillary was exposed to UV irradiation through a photomask. The photomask had 6 openings of 5 mm width spaced equally 5 mm apart. The exposure times were varied in the different exposed regions by sequentially covering the different openings of the mask. After photografting of the initiator alone, the monolith was rinsed with methanol at a flow rate of 0.5 μL/min for 20 min to remove unbound initiator. Next, a deaerated 0.1 mol/L solution of monomer in water was pumped at a flow rate of 0.5 μL/min through the activated monolith for 30 min. The column was then sealed and exposed to UV irradiation through the realigned photomask in the same regions previously exposed in the first step. The monolith was then rinsed with water at a flow rate of 0.5 μL/min for 60 min to remove the unreacted hydrophilic monomers.

Single-Step Photografting. Two different approaches were tested. For the first, initiator-free photografting, the porous monolith was flushed with an aqueous 0.1 mol/L solution of monomer at a flow rate of 0.5 μL/min for 30 min. In the second, the photografting mixture consisted of 15 wt % monomer and 0.25 wt % BP dissolved in a 75:25 wt % water:*tert*-butanol mixture. After filling the pores of the monolith with any of these monomer solutions, we sealed the capillary with rubber septa at both ends and exposed it to UV irradiation through a photomask. Next, the monolith was rinsed with water at a flow rate of 0.5 μL/min for 60 min to remove excess reagents.

Fluorescence Assay of Protein Adsorption. This assay was designed to mimic the conditions used for immobilizing proteins onto a monolith to obtain an enzymatic microreactor.[11] For protein immobilization, the column is exposed to a relatively high concentration solution of protein for the duration of the immobilization reaction time. However, afterward, only covalently immobilized protein should remain and there should not be any nonspecific adsorption of protein. A 0.5 mg/mL solution of fluorescein-labeled BSA was passed through the monolith for 1 h at a flow rate of 0.25 μL/min. The pores were then rinsed with water for 1 h at 1.0 μL/min to remove excess BSA. After rinsing with water, we took micrographs of the capillary and analyzed the fluorescence intensity across the width of the monolith using ImageJ software (NIH); the fluorescence intensities were quantified as pixel intensities from the 8-bit images. The reported intensities were taken from the maximum (center) of the intensity profiles. In all experiments, each grafted region was adjacent to non-grafted ones on the same polymer monolith. The non-grafted regions served as controls for measuring the relative extent of protein adsorption.

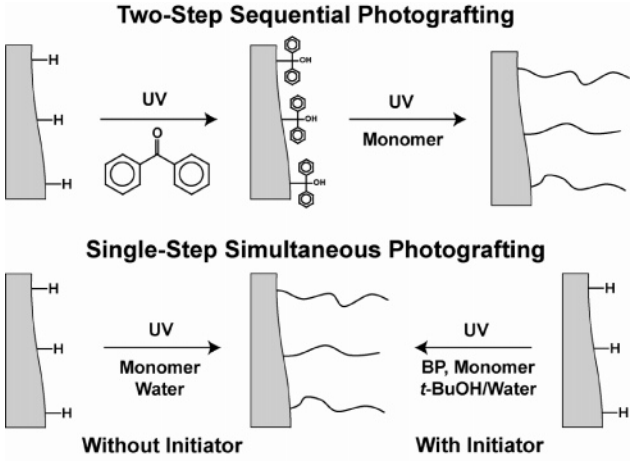

Figure 1. Scheme of the single- and two-step photografting procedures.

Immobilization of Green Fluorescent Protein. First, a polymer monolith completely photografted with PEGMA was prepared using the sequential two-step method. An exposure time of 2 min was used in the first step (initiator immobilization) and a 0.1 mol/L PEGMA,11 solution was photografted for 2 min in the second step. To enable the specific covalent immobilization of protein onto this polymer monolith, patterned photografting of the reactive monomer 4,4-dimethyl-2-vinylazlactone was then performed on the PEGMA-grafted monolith through a photomask (4 min exposure time). The photografting solution consisted of 15 wt % vinylazlactone and 0.22 wt % BP dissolved in a solution of 75:25 wt % *tert*-butanol:water. After grafting vinylazlactone, we rinsed the monolith with acetone for 1 h at a flow rate of 0.5 μL/min to remove excess reagents. Next, to covalently immobilize GFP onto the monolith, we pumped a 1 mg/mL solution of GFP through the monolith at a flow rate of 0.25 μL/min for 1 h. Finally, the capillary was rinsed with water at 1.0 μL/min for 1 h to remove excess GFP.

Results and Discussion

As indicated in the introduction, our approach consists of the preparation of a "generic" poly(butyl methacrylate-*co*-ethylene dimethacrylate) monolith with optimized porous properties followed by the introduction of the desired hydrophilic surface chemistry via photografting. Separation of the monolith synthesis and surface modification processes allows each process to be optimized independently. In this study, we investigated a two-step photografting approach that involves covalent attachment of photoinitiator to the pore surface of the monolith. Specifically, the sequential photografting technique consists of the two simple steps shown in Figure 1. In the first step, initiator moieties are formed at the pore surface by UV irradiation of a polymer monolith that is in contact with the benzophenone solution. This

compound abstracts hydrogen from the polymer surface and creates a free radical. However, in the absence of monomers, the surface radical and the newly formed semipinacol radical combine to form surface-bound initiator. The second step of graft polymerization is then carried out with a solution containing only monomer. UV irradiation liberates the immobilized latent free radicals that initiate graft polymerization from the surface. Besides reducing the formation of nongrafted polymer in solution (in comparison to the single-step techniques, where both initiator and monomer are present in solution), the sequential approach provides additional control over grafting because the overall process, including hydrogen abstraction and grafting polymerization, is decoupled into two separate steps. Thus, this two-step sequential photografting technique allows for a greater degree of control over the grafting process.

Although benzene was used as the solvent for benzophenone in the original study by Ma et al. concerning the photografting of acrylic acid,[22] other reports have shown that the choice of solvent has only a small effect on the formation of immobilized initiator.[23,24] Indeed, we observed no difference in grafting performance when methanol was used as the solvent instead of benzene (data not shown). Therefore, methanol was chosen in place of benzene for our experiments because it poses fewer health and environmental hazards and is more compatible with the future use of this technique in microfluidic devices fabricated from plastics that may swell or dissolve in hydrocarbon solvents.

Screening of Monomers for Prevention of Protein Adsorption. The literature is quite rich with descriptions of synthetic polymers that decrease the nonspecific adsorption of proteins on surfaces.[25−29] In particular, neutral hydrophilic polymers have been shown to be very effective in reducing protein adsorption.[25] Therefore, we selected acrylamide, 2-hydroxyethyl methacrylate, vinyl pyrrolidinone, and poly(ethylene glycol) methacrylate monomers to prepare patterned protein-resistant surfaces on polymer monoliths via photografting.

Many measurements of surface hydrophobicity or protein adsorption are not readily adaptable to porous materials such as our polymer monoliths. Contact angles of water on grafted planar surfaces are often measured; however, although this measurement may indicate the hydrophilicity of a surface, contact angles do not correspond directly with actual ability to resist protein adsorption.[25] Quartz crystal microbalance,[30] surface plasmon resonance,[31,32] ellipsometry,[33] and optical waveguide lightmode spectroscopy (OWLS)[34] techniques have been used to quantitatively measure protein adsorption on a variety of flat surfaces but, to the best of our knowledge, have not been used within macroporous materials. It should also be noted that results obtained with a flat surface analog may not be truly indicative of the actual properties of the grafted surface within the tortuous porous structure of a polymer monolith because of the effect on the grafting process of the very high surface-to-volume ratio of the polymer monolith compared to a flat surface. Therefore, we developed a fluorescence assay that can be performed "on-monolith" and does not require the use of a flat surface analog.

Photografted regions of the polymer monoliths were tested for their ability to resist protein adsorption by subjecting the monolith to a solution of fluorescein-labeled bovine serum albumin (BSA), which served as the model protein for this study. Human serum albumin is the most abundant protein in blood serum and is well-known as an adhesive protein that binds to a wide variety of surfaces; its analog, BSA, is therefore often used as a test protein for adsorption studies.[35−38] After BSA exposure, the columns were washed extensively with water and protein adsorption was then observed using a fluorescence microscope. The extent of adsorption of protein was characterized using the value of the maximum intensity in the fluorescence intensity profile. It is worth noting that the surface concentration of the adsorbed protein is not directly correlated to fluorescence intensity, and thus, these results are not rigorously quantitative. However, they are certainly useful for comparing different grafting conditions using a variety of monomers and provide a good estimate of relative protein adsorption. Successful prevention of protein adsorption is indicated by a low intensity of fluorescence. In contrast, high fluorescence intensity indicates substantial adsorption of the fluorescent protein and poor performance of the surface. Whether the degree of adsorption prevention achieved by the techniques described here is adequate for a specific application must be evaluated for that specific application and is the subject of our ongoing work.

Polymer monoliths were photografted with the different hydrophilic monomers using the two-step sequential photografting technique and evaluated for protein adsorption using the fluorescence assay. The results of these in situ tests are shown in Figure 2. Under our photografting conditions, both grafted AAm and VP exhibited little, if any, prevention of protein adsorption. Fluorescence of the photografted regions had the same intensity as the nongrafted control regions of the monolith. Therefore, these results may also serve as a baseline for a comparison of the performance of other grafted

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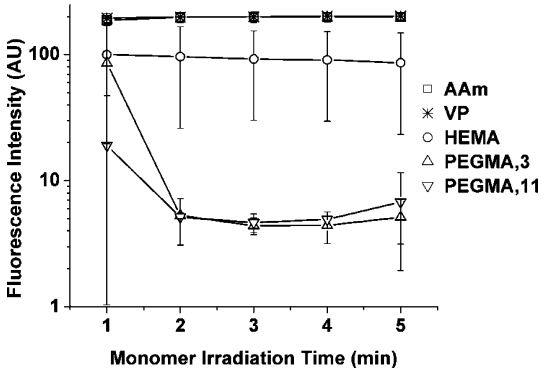

Figure 2. Fluorescence intensity of adsorbed fluorescein-labeled BSA on regions of a porous polymer monolith photografted with five neutral hydrophilic monomers using the two-step sequential method. Average fluorescence intensity ($n = 3-4$) is plotted as a function of monomer grafting time for monomers. Conditions: First step, irradiation for 4 min in the presence of a 5 wt % solution of benzophenone in methanol; second step, monomer concentrations of 0.1 mol/L in water, irradiation time 1−5 min.

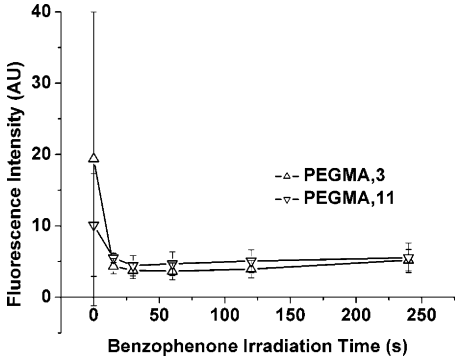

Figure 3. Effect of irradiation time in the first step of the two-step photografting process on average fluorescence intensity ($n = 5$) of adsorbed fluorescein-labeled BSA. Conditions: First step, irradiation for 0−240 s in the presence of a 5 wt % solution of benzophenone in methanol; second step, irradiation time of 3 min, PEGMA monomer concentrations of 0.1 mol/L in water.

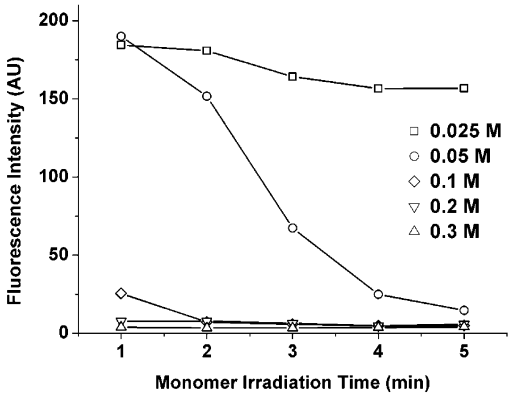

Figure 4. Effect of PEGMA,3 monomer concentration, and grafting time in the second step on the adsorption of fluorescein-labeled BSA on regions of a photografted polymer monolith. Conditions: First step, 1 min irradiation in the presence of a 5 wt % solution of benzophenone in methanol; second step, irradiation time 1−5 min, PEGMA,3 monomer concentrations of 0.025−0.3 mol/L in water.

monomers in the prevention of protein adsorption. For example, grafted HEMA exhibited only moderate reduction in the amount of adsorbed protein. In contrast, Figure 2 clearly demonstrates that both of the PEGMA monomers tested significantly outperformed the other monomers in preventing adsorption of the model protein. The effectiveness of the different monomers is in agreement with studies of protein adsorption on self-assembled monolayers of alkanethiols with a variety of different terminal functional groups, including amide, hydroxyethyl, and ethylene glycol moieties.[25] Photografted PEGMA monomers reduced the amount of adsorbed protein by 98% on the basis of the relative fluorescence intensities of grafted and nongrafted regions on the same monolith.

Effect of Irradiation Time on Surface Initiator Formation. The first step of the sequential photografting process controls the number of surface initiator sites that are formed. The final density of initiator sites is determined by both the concentration of the benzophenone solution and the irradiation time. The concentration of benzophenone affects the rate of surface initiator formation. However, selecting a single concentration and simply changing the irradiation time represents an effective means for controlling the final surface density of surface initiator sites. On the basis of previous reports, we selected 5 wt % benzophenone solution for this study.[22] This concentration represents a good compromise between achieving a high rate of surface initiator formation, which requires a high BP concentration, and maintaining the low UV absorbance needed for transmission of UV light through the entire thickness of the sample.

The effect of varying the density of surface initiator moieties on the prevention of protein adsorption was investigated again by performing protein adsorption assays on photografted polymer monoliths. The BP irradiation time was varied in the first step, whereas the conditions used for monomer grafting were fixed in the second step. Figure 3 shows that the amount of protein adsorption varied little with BP irradiation time, even though the density of surface initiator sites was expected to increase with increasing irradiation time.[22] However, our assay does not measure the surface density of benzophenone, but rather the prevention of protein adsorption after the entire two-step photografting process. The density of grafted polymer chains may be less than the density of surface initiator sites, or the effective prevention of the protein adsorption may not be noticeably improved beyond a certain density of grafted polymer chains on the pore surface. The protein adsorption for these photografting conditions remains approximately 98% lower than that for regions that were not photografted with the PEGMA monomers.

Effect of Monomer Concentration and Grafting Time on Graft Polymerization. In the second step of the sequential photografting procedure, graft polymerization of the monomer is initiated from initiator sites immobilized on the surface of the polymer monolith. UV irradiation time and monomer concentration are the key variables that can be adjusted to control this step of the photografting process. Figure 4 clearly demonstrates that both variables have a significant effect on the adsorption performance of the grafted polymer layer. At each particular monomer concentration, increasing irradiation time resulted in a decrease in the amount of adsorbed protein. The effect of irradiation time is most obvious at the two lowest monomer concentrations. For example, Figure 4 shows that with a monomer concentration of 0.05 mol/L, the transition is made from essentially

no protein adsorption resistance to almost maximum prevention of adsorption in the range of exposure times measured.

Monomer concentration also has a dramatic influence on the effectiveness of the grafted layer to prevent nonspecific protein adsorption. As also shown in Figure 4, grafting with a very low PEGMA concentration of 0.025 mol/L was ineffective across all irradiation times, whereas at higher concentrations of monomer in the range of 0.2−0.3 mol/L, the protein resistance of the grafted layers is at its maximum for even the shortest irradiation time. In approaching the limit of high concentration and long polymerization time, there appears to be a lower bound to the amount of adsorbed protein. Studies of protein adsorption on self-assembled monolayers of alkanethiols with terminal ethylene glycol oligomers have shown that prevention of adsorption is not strongly dependent on the PEG chain length.[25,39−41] In addition, other studies indicate that PEG chain density is more important than chain length in preventing protein adsorption.[38,42,43] Thus, the lower bound observed in our results likely represents the attainment of a fully covered pore surface with a sufficient density of grafted poly(PEGMA) chains. The monomer concentration most directly affects the rate at which such a layer is formed. At this apparent lower bound, grafting PEGMA using the two-step sequential photografting technique reduces nonspecific protein adsorption to less than 2% of that found for nontreated monoliths.

Photoinitiator-Free Single-Step Photografting. Only a very limited number of reports describe photografting in the absence of any photoinitiator. For example, Uchida et al. demonstrated the photografting of acrylamide onto poly(ethylene terephthalate) film achieved with no initiator added.[44] Self-initiated photografting of styrene, maleic anhydride, and several acrylate monomers onto polyethylene films has also been reported.[45−47] We investigated this technique as an alternative to the sequential two-step photografting process. We found that PEGMA could be grafted onto the polymer monoliths from aqueous solutions that contained no photoinitiator. We even observed significant prevention of protein adsorption in regions of the polymer monolith grafted using this photoinitiator-free technique as shown in Figures 3 (for BP irradiation time = 0 s) and 5. However, this initiator-free grafting is not as efficient as the two-step sequential process, which uses benzophenone as the photoinitiator (Figure 5). Longer irradiation times are required for the photoinitiator-free process in order to achieve a level of prevention of protein adsorption similar to that obtained in the two-step process.

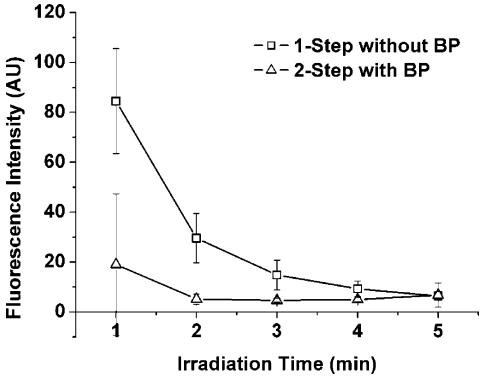

Figure 5. Comparison of the sequential two-step and the single-step photoinitiator-free photografting methods ($n = 4$) on the basis of the fluorescence intensity of adsorbed fluorescein-labeled BSA onto photografted regions of polymer monolith. Conditions: First step of the two-step method, 2 min irradiation in the presence of a 5 wt % solution of benzophenone in methanol; second step and the single-step method, monomer solution of 0.1 mol/L PEGMA,11 in water; irradiation time 1−5 min.

We hypothesize that the initiator-free photografting observed in our experiments results from photoexcitation of the carbonyl groups in the monomers and/or in the polymer monolith matrix. Many photoinitiators, such as BP or DMPA, are based on carbonyl photochemistry, in which photoexcitation of the carbonyl group results in an excited triplet state that is capable of generating free radicals by α-cleavage or by hydrogen abstraction.[48] Although no photoinitiator is present in our system, both the monomers and the methacrylate-based monolith contain numerous carbonyl groups that may generate radicals by various mechanisms. Surface radicals may be generated directly by excitation of carbonyls in the polymer monolith and subsequent cleavage of the neighboring carbon−carbon bond. Additionally, photoexcited monomers may abstract hydrogen atoms from the polymer monolith to initiate surface grafting. To test this mechanism, we carried out both two-step and initiator-free photografting while using a glass filter during UV irradiation. The glass filter uniformly blocks UV light with a wavelength less than about 300 nm. Because the carbonyl group in benzophenone is conjugated with two aromatic rings, its UV adsorption maximum is shifted to longer wavelengths (∼340 nm) compared to the carbonyls in the monomers or polymer (∼280 nm).[48] Figure 6 shows that this shift allows benzophenone to efficiently initiate graft polymerization even when the far UV light is blocked by the glass filter, whereas the self-initiating photografting process is significantly hindered. The prevention of protein adsorption is nearly identical for the two-step process with or without the presence of a glass filter during irradiation. This result is very important, because it demonstrates that the two-step process is better suited for the preparation of monolith-based microfluidic devices fabricated from glass substrates.

Single-Step Photografting in the Presence of Photoinitiator. In our initial attempts to prepare protein-resistant polymer monolith surfaces, we used a single-step photo-

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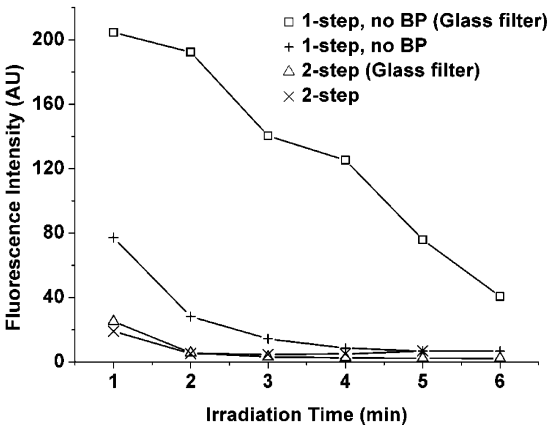

Figure 6. Effect of glass filter during irradiation steps of the sequential two-step and the single-step photoinitiator-free photografting methods ($n = 4$) based on the fluorescence intensity of adsorbed fluorescein-labeled BSA onto photografted regions of polymer monolith. Conditions: First step of the two-step method, 2 min irradiation in the presence of a 5 wt % solution of benzophenone in methanol; second step and the single-step method, monomer solution of 0.1 mol/L PEGMA,11 in water, irradiation time 1−6 min.

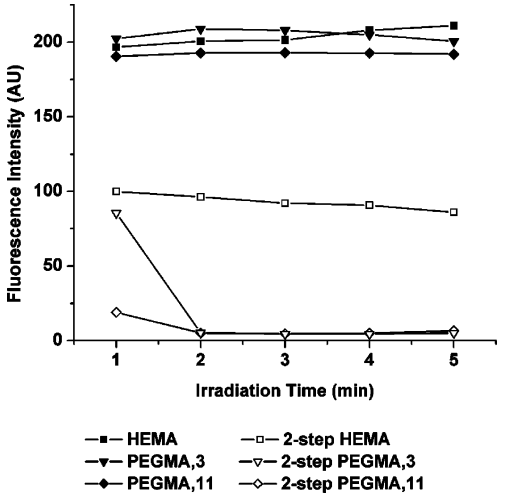

Figure 7. Fluorescence intensity of adsorbed fluorescein-labeled BSA on polymer monolith photografted with different monomers using the single-step method with photoinitiator. Conditions: 10 wt % monomer solution containing 0.25 wt % benzophenone in 1:3 *tert*-butanol-water mixture; irradiation time 1−5 min. Data from Figure 5 for photografted regions of PEGMA[11] prepared with the two-step method are included for comparison.

grafting technique with photoinitiator included in the photografting solution. We have successfully used this technique to prepare monoliths with pore surfaces grafted with ionizable, polar monomers, such as 2-acrylamido-2-methyl-1-propanesulfonic acid and [2-(methacryloyloxy)ethyl] trimethylammonium chloride.[12,14] In this more common single-step process, the photografting solution contains both monomer and photoinitiator. To prepare a homogeneous photografting mixture, we used a water/*tert*-butyl alcohol mixture to dissolve both the hydrophilic monomer and the hydrophobic photoinitiator. However, Figure 7 shows that using this single-step photografting method with a variety of neutral hydrophilic monomers did not result in the desired prevention of protein adsorption. The fluorescence intensity of the grafted regions was essentially the same as that of nongrafted areas exhibiting "full" protein adsorption.

Because initiator-free photografting with aqueous monomer solutions was effective at preventing protein adsorption

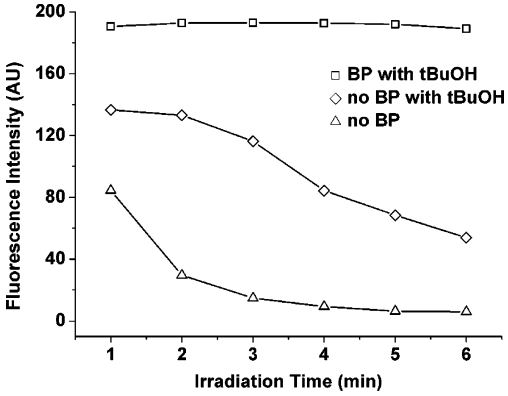

Figure 8. Effect of *tert*-butanol and benzophenone on the single-step photografting of PEGMA,11 on the polymer monolith. Conditions: 10 wt % PEGMA,11; grafting from an aqueous solution (no BP), from a 1:3 *tert*-butanol:water mixture (no BP with *t*BuOH), and from a 1:3 *tert*-butanol: water mixture containing benzophenone (BP with *t*BuOH); irradiation time 1−6 min.

(vide supra), whereas photografting using the typical single-step approach was not, we speculated that the presence of *tert*-butyl alcohol in the photografting mixture might control the extent of photografting. To test the effect of *tert*-butyl alcohol, we performed an initiator-free photografting of PEGMA using the alcohol-water solution as solvent rather than pure water. Figure 8 shows that far more protein was adsorbed on the grafted regions obtained using the mixed *tert*-butyl alcohol/water solvent compared to grafting from an aqueous solution, clearly indicating that the alcohol has a negative effect on the grafting process. The high concentration of the tertiary alcohol may result in excessive chain transfer from the growing grafted polymer chains to this solvent. Additionally, the hydrogen atoms of *tert*-butyl alcohol are located within methyl groups, which are less likely to undergo hydrogen abstraction than the hydrogens in the methylene groups of the monomers or the polymer monolith surface.[49] Yet, hydrogen abstraction from the abundant alcohol molecules may still occur and the solvent radicals that are formed can terminate the growing polymer chains. However, although the presence of *tert*-butyl alcohol in the grafting mixture reduces grafting efficiency, it does not reduce performance to the extent seen with the one-step photografting technique that includes photoinitiator. Surprisingly, the presence of photoinitiator added to the grafting mixture further reduces the effectiveness of the photografting and prevention of protein adsorption (Figure 8). We assume that the photoinitiator present in the bulk solution facilitates the formation of polymer in solution, depleting the supply of monomers and increasing solution viscosity, both factors that may reduce grafting efficiency. It is also possible that initiator is incorporated into the grafted layer in a way similar to the formation of surface initiator sites in the first step of the two-step photografting process, thus increasing the hydrophobicity of the grafted layer and increasing the amount of adsorbed protein. Fortunately, the use of the two-step process avoids both these problems. First, no cosolvents are needed to obtain a homogeneous solution, because the monomer and initiator are used in separate solutions. In

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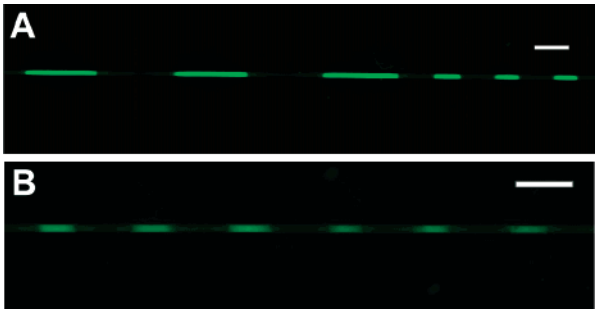

Figure 9. (A) Adsorption of fluorescein-labeled BSA on a polymer monolith with patterned regions of photografted PEGMA,11. The dark regions correspond to grafted parts of the monolith that resist protein adsorption; the bright fluorescence corresponds to nongrafted regions of the monolith that adsorb the fluorescent protein. (B) Patterned immobilization of green fluorescent protein onto a PEGMA and 1-vinyl-4,4-dimethylazlactone grafted polymer monolith. The monolith was first completely grafted with PEGMA,11 using the two-step method. Patterned regions of vinyl azlactone were photografted onto the modified monolith and subsequently reacted with the protein. Scale bars are 1 mm.

addition, polymer formation in solution is limited as the photoinitiator is immobilized at the polymer surface.

Patterned Photografting and Protein Immobilization. An important benefit of photografting is the ability to pattern the grafted functionality at precise locations within the monolith. This photolithographic-like approach allows different sections of a monolith to be functionalized with the different surface chemistries required for the fabrication of multifunctional devices. Patterned regions of PEGMA-grafted polymer monolith shown in Figure 9A are easily distinguishable from nongrafted regions after a solution of fluorescein-labeled bovine serum albumin is passed through the monolith. The regions grafted with PEGMA do not adsorb this protein, whereas the nongrafted hydrophobic monolith is highly susceptible to adsorption of the fluorescently labeled protein. This demonstration clearly shows the resolution and patterning ability of photografting and highlights the effective prevention of nonspecific protein adsorption on PEGMA-grafted regions of the polymer monolith.

Passivation of polymer monoliths with photografted PEGMA may be useful for the preparation of an inert enzyme-immobilization matrix that can be used for flow-through enzymatic microreactors. As a demonstration of this application, a monolith was first modified with a layer of photografted PEGMA. The modified monolith was then photografted in patterned regions with the reactive monomer, 2-vinyl-4,4-dimethylazlactone. Covalent immobilization of a model protein onto this monolith was then achieved by passing a solution of green fluorescent protein (GFP) through the monolith; the azlactone functionalities on the monolith surface reacted with amines on the periphery of the GFP to form a stable covalent linkage to the polymer monolith matrix. Indeed, Figure 9B shows that immobilization of GFP was achieved only in the desired areas, whereas very little fluorescence is observed for those parts of the monolith that were masked.

Conclusions

Photografting hydrophobic polymer monoliths with hydrophilic monomers is an effective means of reducing protein adsorption. Monomer concentration, grafting time, and photografting solvent are the most important parameters in obtaining a protein resistant layer of grafted hydrophilic polymer chains. Interestingly, this study demonstrated that the nontraditional photografting approaches, i.e., the initiator-free and two-step sequential photografting techniques, were more effective than the conventional photografting approach for creating grafted hydrophilic layers that effectively prevent protein adsorption. This result suggests that these approaches may also be better suited for other photografting applications, such as grafting ionizable monomers needed for electrochromatographic separations, reactive monomers for enzyme immobilization, and hydrophobic monomers for reversed-phase chromatography or concentration. We are currently pursuing these alternative photografting techniques and investigating the effects of solvent and initiator in order to improve the surface modification of polymer monoliths. The mechanism of initiator-free grafting, partly elucidated in this report, is also currently under further investigation. In addition, the photografting techniques investigated in this report should be helpful for creating multifunctional microanalytical devices. Patterned photografting can be used to create different chemical functionalities on a polymer monolith in order to build up a multifunctional system. In particular, hydrophilic-grafted monoliths seem well-suited for use as inert enzyme-immobilization matrices for flow-through enzymatic microreactors. The hydrophilic surface modification should help preserve enzyme activity, prevent adsorption of substrate and product molecules, and enhance spatial patterning by reducing nonspecific adsorption of enzyme outside of the reactor region. Hydrophilic photografting should also be useful for creating nonfouling surfaces in applications such as mixers, valves, and filters.

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