Nonfouling Polymer Brushes via Surface-Initiated, Two-Component Atom Transfer Radical Polymerization

Matthew T. Bernards, Gang Cheng, Zheng Zhang, Shengfu Chen, and Shaoyi Jiang*

Department of Chemical Engineering, University of Washington, Seattle, Washington 98195

Received January 25, 2008; Revised Manuscript Received March 12, 2008

ABSTRACT: A significant challenge in the field of biomaterials is the nonspecific adsorption of proteins. It has been suggested that overall neutral materials composed of mixed charge components present protein-resistant properties. This work describes the development of a novel nonfouling polymer brush formed from a surface-initiated, two-component atom transfer radical polymerization. The polymer brushes are composed of varying mixtures of positively and negatively charged monomers depending on the polymerization conditions. The polymer brushes were characterized by both atomic force microscopy and electron spectroscopy for chemical analysis to determine the thickness and composition, respectively. The nonspecific adsorption of fibrinogen, lysozyme, and bovine serum albumin was measured using a surface plasmon resonance biosensor. It was found that when the polymer brush surface coating was formed as a statistical copolymer from the two charged components, it had nonfouling properties for all three probe proteins.

Introduction

There is a significant need for the development of novel nonfouling materials for applications such as biosensors, biomaterials, drug delivery vehicles, and marine coatings.[1,2] One of the most widely studied nonfouling materials is poly(ethylene glycol) (PEG) or oligo ethylene glycol (OEG).[3–5] However, these materials have been shown to be subject to oxidation in biochemically relevant solutions.[4–6] Another class of nonfouling materials is the phosphorylcholine (PC) family of materials, but these monomers are not readily available.[7] For these reasons, it is of great interest to develop new nonfouling materials for a wide range of biological applications.

Recently, it has been demonstrated that zwitterionic materials such as sulfobetaine methacrylate (SBMA) and carboxybetaine methacrylate (CBMA) are excellent nonfouling materials.[8,9] An important characteristic of these materials is the fact that they have both a positively and negatively charged moiety within the same monomer side chain, while maintaining overall charge neutrality. It has also been demonstrated that mixed positively and negatively charged self-assembled monolayers (SAMs) of equal valence are highly resistant to protein adsorption.[10,11] On the basis of these studies and related work,[12,13] it was hypothesized that any material composed of a homogeneous blend of mixed charge components with balanced charge will present protein-resistant properties. Furthermore, it has recently been shown that nonfouling hydrogels can be formed from a variety of different homogeneous mixtures of charged monomers as long as the hydrogels maintain an overall neutral charge.[14] It is believed that these nonfouling characteristics are a result of the formation of a strong hydration layer around the terminal groups of the material. Molecular dynamics simulations have suggested that the disruption of this hydration layer during protein adsorption is energetically unfavorable, leading to a strong repulsive force acting on the protein.[12,13,15]

While hydrogel materials have a wide range of biological applications, there is a need to develop stable and robust surface coatings that also have nonfouling properties.[14] SAMs provide an excellent platform for completing fundamental studies. However, it has been demonstrated that polymer brushes have lower levels of fouling than their SAM counterparts from complex medium including serum, plasma, and bacterial adhesion and biofilm formation.[16] This improved resistance is due to the high surface packing densities that can be obtained for polymer brushes. Because of this, surface-grafted polymer brushes represent an excellent approach for developing nonfouling surface coatings. Surface-initiated atom transfer radical polymerization (ATRP) is a well-developed and well-characterized method for forming controlled polymer brushes on surfaces. Block copolymers can also be formed by ATRP using a multistep process where each monomer component is grafted to the surface in a stepwise fashion.[17] However, there are only a few reports on the formation and characterization of random, statistical copolymer brushes from mixtures of monomers in one reaction solution.[18]

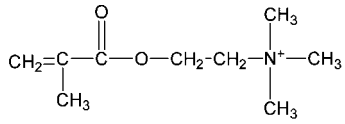
In this work we form polymer brush coatings via surface-initiated ATRP from mixtures of positively charged [2-(methacryloyloxy)ethyl]trimethylammonium chloride (TM) and negatively charged 3-sulfopropyl methacrylate potassium salt (SA), which are shown in Figure 1. ω-Mercaptoundecyl bromoisobuyrate (−Br thiol) was synthesized and characterized previously and used as an ATRP initiator on gold-coated surfaces.[8,19] The charged monomers were polymerized from mixtures containing varying molar ratios of the two monomers. The following molar ratios in the reaction solution were examined (TM:SA): 1:0, 2:1, 1:1, 1:2, and 0:1. The protein-resistant properties were determined by measuring the adsorption of single protein solutions of fibrinogen (FBG), bovine serum albumin (BSA), and lysozyme (LYZ) onto the polymer brush-coated surfaces using a surface plasmon resonance (SPR) sensor. FBG is a typical protein used to evaluate the nonfouling characteristics of materials due to its roles in the inflammatory response and its ability to easily adsorb to a wide range of materials.[5,7] Because FBG has a negative charge (pI 5.8) under physiological conditions, positively charged LYZ (pI 11) was also used as a test protein.[5] BSA was included due to its natural abundance in the body.

Materials and Methods

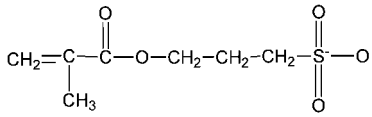
Materials. TM, SA, tetrahydrofuran (THF), methanol, isopropanol (IPA), acetone, 2,2′-bipyridine (BPY), copper(I) bromide [Cu(I)Br], phosphate buffered saline (PBS), FBG, BSA, and LYZ were purchased from Sigma (St. Louis, MO) and used as received. Absolute ethanol (200 proof) was purchased from AAPER Alcohol and Chemical Co. (Shelbyville, KY) and used as received. PBS

* Corresponding author. E-mail: sjiang@u.washington.edu.

10.1021/ma800185y CCC: $40.75
Published on Web 05/22/2008



[2-(methacryloyloxy) ethyl] trimethyl ammonium chloride (TM)



3 sulfopropyl methacrylate potassium salt (SA)

Figure 1. Monomers used to create mixed charge copolymers via surface-initiated ATRP.

buffer was made by dissolving PBS in 18.2 MΩ cm H_2O (150 mM, pH 7.4) before use. Protein solutions (1 mg/mL) were made by dissolving the proteins in PBS buffer. BK-7 glass chips (Schott North America Inc., Elmsford, NY) and P(100) Si wafers (University Wafer, South Boston, MA) were coated with a 2 nm Ti adhesion layer and a 48 nm Au layer by electron beam evaporation. P20 7/17 primer, AZ 1512 photoresist, and AZ 351 developer were purchased from MicroChemicals (Ulm, Germany). TFA gold etchant was purchased from Transene Co., Inc. (Danvers, MA).

Self-Assembled Monolayer Preparation. Bromine-terminated SAMs were prepared by the adsorption of −Br thiol on gold-coated substrates, following previously reported procedures.[8] Briefly, all gold-coated substrates were rinsed with ethanol, dried with filtered air, irradiated under ultraviolet for 20 min, rinsed with 18.2 MΩ cm water, rinsed with ethanol, and dried with filtered air. SAMs were formed by soaking the cleaned substrates overnight in a 0.1 mM alkanethiol solution in ethanol at room temperature. Finally, the −Br-terminated SAM-coated chips were washed with ethanol, THF, and ethanol, and dried with filtered air just prior to undergoing the surface-initiated polymerization.

Atom Transfer Radical Polymerization. ATRP was completed using previously published procedures.[8] Briefly, −Br thiol-coated SPR gold chips and gold patterned Si wafers were placed in a reaction tube with Cu(I)Br and purged with nitrogen. At the same time, solutions of BPY in methanol and monomers in a 1:1 water: methanol mixture were also nitrogen purged. Following purging, the monomer and BPY solutions were moved to the reaction vessel using a syringe, and the reaction proceeded for 1 h. After 1 h, the samples were removed from the reaction tube and immersed in PBS overnight before use in either protein adsorption or polymer characterization studies. This overnight soaking step was completed to ensure that all of the unreacted monomers and reaction solvents were removed from the polymer brush before further analysis. The final reaction concentrations were as follows: 1:3 water:methanol solution, 0.05 M Cu(I)Br, 0.1 M BPY, and ≤1.125 M total monomer.

Polymer Brush Thickness by Atomic Force Microscopy (AFM). Gold-coated Si wafers were patterned using standard photolithography techniques. Briefly, a ∼20 μm layer of P20 7/17 primer and a ∼100 μm layer of AZ 1512 photoresist were spin-coated onto the gold-coated wafers. The wafers were then placed under a photolithography mask that was patterned with 25 μm lines, spaced 25 μm apart. Following exposure to ultraviolet light, the photoresist was developed using an AZ 351 developer. Then the underlying gold layer was etched away using TFA gold etching solution. Following this etching step, the remaining photoresist was removed by rinsing the sample with IPA, followed by sonication in acetone. SAMs were formed on the patterned gold-coated Si wafers and then samples underwent ATRP. Following the PBS soaking stage, the patterned samples were rinsed extensively with 18.2 MΩ cm water, dried with filtered air, and placed in a desiccator overnight before imaging. Tapping mode AFM images were acquired using a Dimension 3100 AFM (Digital Instruments/Veeco, Woodbury, NY) operated in air. Commercial Si cantilevers (DI) with a resonant frequency of ∼300 kHz were used. Cross-sectional slices were taken from the images that were obtained using NanoScope 6.13R1 (Digital Instruments/Veeco) and analyzed with Origin Pro 7.0 (OriginLab Corp., Northampton, MA).

Composition by Electron Spectroscopy for Chemical Analysis (ESCA). Patterned Au-coated wafer samples were prepared as described above. Following the PBS soaking stage, the samples were rinsed extensively with 18.2 MΩ cm water, dried with filtered air, and placed in a desiccator overnight before analysis. ESCA spectra were taken on a Surface Science Instruments S-probe spectrometer with monochromatized Al Kα X-rays. The spot size for these acquisitions was ∼800 μm. The pass energy for the survey spectra was 150 eV. Detailed scans were completed with an identical pass energy for both the N and S peaks to more accurately quantify smaller amounts of those elements. The takeoff angle was 55°, resulting in a ∼50 Å sampling depth. The Service Physics ESCAVB Graphics Viewer program was used to determine the peak areas, which were used to calculate the elemental compositions of the samples. The peak areas were normalized by the number of scans, points/electronvolt, Scofield photoionization cross section, and sampling depth for this calculation.[20]

Protein Adsorption by SPR. Protein adsorption was measured with a custom-built four-channel SPR sensor (Institute of Radio Engineering and Electronics, Academy of Sciences, Prague, Czech Republic) based on the Kretschmann geometry of the attenuated total reflection method and wavelength modulation.[21] Following the PBS soak, chips were rinsed extensively with 18.2 MΩ cm H_2O, dried with filtered air, and then mounted to a coupling prism using refractive index matching fluid (Cargille, Cedar Grove, NJ). A baseline signal was established by flowing PBS at a rate of 50 μL/min through the sensor for 10 min. Following this, fresh 1 mg/mL protein solutions of FBG, LYZ, and BSA were flowed through independent channels for 10 min to measure the nonspecific adsorption of these proteins to the polymer brush-coated surfaces. To remove unbound protein molecules and to reestablish the baseline, PBS buffer was flowed for an additional 10 min. The protein adsorption was quantified by measuring the change in wavelength in the buffer baseline, and this was converted to an adsorbed amount. For the SPR sensor used in this study, a 1 nm shift in wavelength starting at 750 nm represents a surface coverage of ∼15 ng/cm^2 of adsorbed protein.

Results and Discussion

Prior to analyzing the nonfouling characteristics of the polymer brushes, efforts were taken to characterize the brushes that were formed during the two-component ATRP reaction. The polymer brushes were characterized with ESCA and AFM to determine the composition and thickness of the polymer brushes that were formed, respectively. The ratio of the atomic percentages of nitrogen and sulfur was used to quantify the ratio of the TM monomer to the SA monomer in the polymer brush, and these ratios are summarized in Table 1. Nitrogen can only be found in the TM monomer and is representative of the amount of this monomer present. While sulfur is present in both the −Br thiol and the SA monomer, the sulfur in the SAM layer is not detected by ESCA due to the surface sensitivity of this technique (∼50 Å sampling depth). This was confirmed in the compositional analysis for the 1:0 TM:SA samples, where there was no detectable S present in five of the six analysis spots (the remaining spot had only 0.09% S). The N/S ratio allowed for the determination of reaction conditions that resulted in the formation of a homogeneous mixed polymer brush with overall charge neutrality and nonfouling characteristics. In Table 1 it can be seen that the ratio of N/S decreases as the amount of TM is reduced from 1:0 to 0:1. Furthermore, the reaction ratio of 1:1 resulted in the formation of a 1:1 statistical copolymer mixture of the two monomers based on the calculated N/S ratio of 0.95 ± 0.1. It should be mentioned that there was a slight nitrogen signal present in two of the six analysis spots for the

Table 1. Polymer Brush Characterizations (Average ± SD)

TM:SA monomer ratios	1:0	2:1	1:1	1:2	0:1
N/S ratio by ESCA ($n = 6$)	NA[a]	1.25 ± 0.1	0.95 ± 0.1	0.53 ± 0.1	0.10 ± 0.2
brush height by AFM (nm) ($n = 9$)	15.6 ± 3	24.3 ± 7	35.4 ± 12	39.6 ± 7	35.1 ± 14

[a] This ratio does not have a numerical value because sulfur was only detected in one of the six spots (N/S = 45.8).

0:1 mixture which resulted from contamination, as there was no TM monomer in this reaction. However, the resulting N/S ratio is on par with the error for this sample and the total amount of nitrogen was less than 1% of the average atomic composition for this ratio. Representative ESCA spectra and a complete summary of the elemental compositions can be found in the Supporting Information.

The heights of the polymer brushes that were formed were measured by AFM. Au-coated Si wafers were patterned using standard photolithography techniques to get lines of Au with a width and spacing of ~25 μm and a measured step height of 48.2 ± 1 nm. These patterned wafers were then subjected to the ATRP reaction, and the step height was measured again to determine the height of the polymer brush coating. A representative AFM and cross-sectional image can be found in the Supporting Information. The AFM topographical analysis indicated that the ATRP reaction produced uniform and smooth polymer brush coatings. The average surface roughness of the polymer brushes was on the order of 2 nm. The average height of the polymer brushes was calculated for samples from three separate reactions for each monomer ratio, and these values are summarized in Table 1. In this table it can be seen that the polymer brushes formed under all of the reaction conditions had heights in the range of 15−40 nm. The farthest outlying height profile was for the brush formed from pure TM (1:0). With this exception, there are no distinguishable differences in the thicknesses of the polymer brushes. Because all of the polymer brushes have similar thicknesses, it is not expected that there will be any differences in the resistance to protein adsorption resulting from the thickness differences. This is based on previous studies which have shown that there are minimal differences in the adsorption of both FBG and LYZ to PC-based polymer brushes when the graft thickness varies by 15 nm.[7]

SPR is an ideal platform for measuring binding events on a surface, and it was used here to characterize the nonspecific protein adsorption from single protein solutions to the brush coatings. The adsorption of FBG, BSA, and LYZ was measured simultaneously in a four-channel SPR. Representative SPR sensorgrams can be found in the Supporting Information, and the results are summarized in Figure 2. It can be seen that there are significant amounts of FBG and approximately a monolayer of BSA adsorbed to the brush formed from pure TM, but no significant adsorption of LYZ. The opposite trend was seen for the adsorption of proteins to the pure SA polymer brush, where there was significant LYZ adsorption, limited FBG adsorption, and no BSA adsorption to this surface. The importance of testing multiple proteins to determine nonfouling is emphasized by these two control points, which indicate that differences in the electrostatic interactions between proteins and surfaces can lead to vastly different adsorption behaviors.

In Figure 2, it can be seen that the amount of adsorbed protein drops when both polymers are grafted simultaneously from a mixture. When the monomers are reacted at a 1:2 ratio, the LYZ binding reduces to just below a monolayer of adsorbed protein (~210 ng/cm^2), while the FBG and BSA binding drops to less than 2 ng/cm^2 for both proteins. The reaction ratio of 2:1 has even better nonfouling characteristics, as there is only ~16 ng/cm^2 of adsorbed FBG and less than 2 ng/cm^2 of adsorbed BSA and LYZ. This indicates that both of these reaction conditions result in the formation of polymer brush coatings that are relatively lower fouling. On the basis of the ESCA compositional

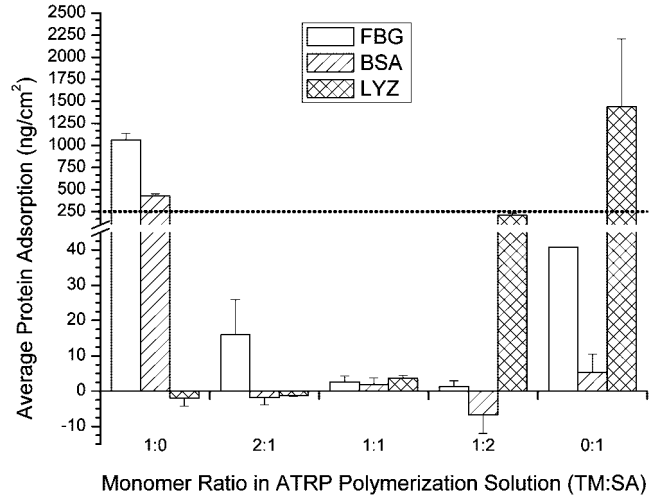


Figure 2. Adsorption of FBG (clear), BSA (diagonal lines), and LYZ (cross hatch) to polymer brush-coated surfaces as measured by SPR. Each bar represents the average ± SE for the adsorption of each protein to each polymer ratio, measured on three separate samples ($n = 3$). The dotted line represents a monolayer of adsorbed protein, ~250 ng/cm^2, based on the amount of FBG that adsorbs to a methyl-terminated SAM.[12]

analysis, it can be seen that these reaction conditions result in the formation of copolymers that have an N/S ratio of 1.25 ± 0.1 and 0.53 ± 0.1 for the 2:1 and 1:2 ratios, respectively. The N/S ratio would suggest that both brushes have a slight surface charge, which accounts for the respective protein binding to the surfaces. This ratio also indicates that the 1:2 polymer brush deviates further from the nonfouling N/S criteria of 1.00 as compared to the 2:1 ratio. This results in a higher surface charge and explains the greater levels of protein adsorption that were seen.

In Figure 2, it can also be seen that when the monomers are present in a 1:1 ratio during the reaction, a nonfouling statistical copolymer brush is formed from these two monomers. This surface coating had a N/S ratio of 0.95 ± 0.1, and no significant measurable protein adsorption from the single protein solutions (<4 ng/cm^2). These results strongly support the hypothesis that materials with nonfouling characteristics can be formed from neutral surface coatings formed from mixed charge components. It should be mentioned that it is likely that other monomers will require different reaction ratios in order to achieve a 1:1 surface ratio, depending on the polymerization reaction kinetics of the individual monomers.

Additional reaction conditions were also tested for the 1:1 monomer ratio, to confirm the hypothesis that the nonfouling characteristics are a result of the statistical copolymer brushes. Reaction conditions that were varied included the reaction time which ranged from 1 to 24 h and the reaction temperature which was varied from room temperature to 40 °C. All of these varying reaction conditions resulted in the formation of polymer brushes which were nonfouling to both FBG and LYZ. The average protein adsorption for these varying reaction conditions is summarized in Table 2 in the Supporting Information. This indicates that nonfouling polymer brushes can be formed from a mixture of mixed charge monomers over a wide range of reaction conditions as long as the monomers are present as a statistical copolymer in the resulting brush.

Conclusions

This study examined the nonfouling properties of five polymer brushes formed from charged monomers by surface-initiated ATRP. These polymer brushes were characterized by ESCA and AFM, and their resistance to protein adsorption was quantified by SPR. It was shown that when oppositely charged monomers are polymerized together, the polymer brush surface coatings become low fouling. The best nonfouling surface coating was found for the statistical copolymer brushes formed from a 1:1 homogeneous reaction mixture of the two oppositely charged monomers. It is believed that these surfaces exhibit nonfouling properties due to the formation of a strong hydration layer on the surface.

Acknowledgment. This work was funded by the Defense Threat Reduction Agency through Grant HDTRA1-07-1-0033 and the Office of Naval Research through Grant N000140410409. The photolithography and AFM work was completed at the University of Washington Center for Nanotechnology, one of the 12 nodes of the National Nanotechnology Infrastructure Network supported by the National Science Foundation. ESCA was carried out at the National ESCA and Surface Analysis Center for Biomedical Problems (NESAC/BIO) which is supported by NIBIB Grant EB-002027.

Supporting Information Available: Representative ESCA survey scans for each polymer mixture; a representative AFM height analysis for the 1:1 reaction ratio; representative SPR sensorgrams for each polymer mixture; a complete table of the ESCA compositional analysis; and a table summarizing the protein adsorption under varying reaction conditions. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Boozer, C. L.; Yu, Q.; Chen, S.; Lee, C. Y.; Homola, J.; Yee, S. S.; Jiang, S. *Sens. Actuators, B* **2003**, *20*, 22–30.
(2) Ratner, B. D.; Bryant, S. J. *Annu. Rev. Biom. Eng.* **2004**, *6*, 41–75.
(3) Li, L.; Chen, S.; Jiang, S. *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 1415–1427.
(4) Li, L.; Chen, S.; Zheng, J.; Ratner, B. D.; Jiang, S. *J. Phys. Chem. B* **2005**, *109*, 2934–2941.
(5) Ostuni, E.; Chapman, R. G.; Holmlin, R. K.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 5605–5620.
(6) Herold, D. A.; Keil, K.; Bruns, D. E. *Biochem. Pharmacol.* **1989**, *38*, 73–76.
(7) Feng, W.; Zhu, S.; Ishihara, K.; Brash, J. L. *Langmuir* **2005**, *21*, 5980–5987.
(8) Zhang, Z.; Chen, S.; Chang, Y.; Jiang, S. *J. Phys. Chem. B* **2006**, *110*, 10799–10804.
(9) Zhang, Z.; Chen, S.; Jiang, S. *Biomacromolecules* **2006**, *7*, 3311–3315.
(10) Chen, S.; Yu, F.; Yu, Q.; He, Y.; Jiang, S. *Langmuir* **2006**, *22*, 8186–8191.
(11) Holmlin, R. E.; Chen, X.; Chapman, R. G.; Takayama, S.; Whitesides, G. M. *Langmuir* **2001**, *17*, 2841–2850.
(12) Chen, S.; Zheng, J.; Li, L.; Jiang, S. *J. Am. Chem. Soc.* **2005**, *127*, 14473–14478.
(13) Hower, J. C.; He, Y.; Bernards, M. T.; Jiang, S. *J. Chem. Phys.* **2006**, *125*, 214704.
(14) Chen, S.; Jiang, S. *Adv. Mater.* **2008**, *20*, 335.
(15) Zheng, J.; Li, L.; Tsao, H. K.; Sheng, Y. J.; Chen, S.; Jiang, S. *Biophys. J.* **2005**, *89*, 158–167.
(16) Cheng, G.; Zhang, Z.; Chen, S.; Bryers, J. D.; Jiang, S. *Biomaterials* **2007**, *28*, 4192–4199.
(17) Edmonson, S.; Osborne, V. L.; Huck, W. T. S. *Chem. Soc. Rev.* **2004**, *33*, 14–22.
(18) Xu, C.; Barnes, S. E.; Wu, T.; Fischer, D. A.; DeLongchamp, D. M.; Batteas, J. D.; Beers, K. L. *Adv. Mater.* **2006**, *18*, 1427–1430.
(19) Jones, D. M.; Brown, A. A.; Huck, W. T. S. *Langmuir* **2002**, *18*, 1265–1269.
(20) Scofield, J. H. *J. Electron Spectrosc. Relat. Phenom.* **1976**, *8*, 129–137.
(21) Ladd, J.; Boozer, C.; Yu, Q.; Chen, S.; Homola, J.; Jiang, S. *Langmuir* **2004**, *20*, 8090–8095.

MA800185Y