Surface Grafted Sulfobetaine Polymers via Atom Transfer Radical Polymerization as Superlow Fouling Coatings

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One of the sulfobetaine methacrylate (SBMA) monomers, *N*-(3-sulfopropyl)-*N*-(methacryloxyethyl)-*N*,*N*-dimethylammonium betaine, was polymerized onto initiator-covered gold surfaces using atom transfer radical polymerization (ATRP) to form uniform polymer brushes. Self-assembled monolayers (SAMs) with ATRP initiators were characterized by X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). The thickness of grafted poly(SBMA) films was measured by ellipsometry. Fibrinogen adsorption on poly(SBMA) grafted surfaces was measured with a surface plasmon resonance (SPR) sensor. Two approaches were compared to graft ATRP initiators onto gold surfaces for surface polymerization and subsequent protein adsorption on these polymer grafted surfaces. The first was to prepare a SAM from ω-mercaptoundecyl bromoisobutyrate onto a gold surface. Superlow fouling surfaces with well-controlled poly(SBMA) brushes were achieved using this approach (e.g., fibrinogen adsorption <0.3 ng/cm^2). The second approach was to react bromoisobutyryl bromide with a hydroxyl-terminated SAM on a gold surface. Although protein adsorption decreased as the density of surface initiators increased, the surface prepared using the second approach was not able to achieve as low protein adsorption as the first approach. Key parameters to achieve superlow fouling surfaces were studied and discussed.

1. Introduction

Many hydrophilic surfaces can reduce protein adsorption. However, these surfaces are often not sufficient to prevent the undesirable adhesion of cells, bacteria, or other microorganisms. Even a small amount of proteins on a surface can lead to the adhesion and propagation of unwanted fouling.[1,2] For example, fibrinogen adsorption less than 5−10 ng/cm^2 is needed to inhibit platelet adhesion for blood compatibility,[2,3] and superlow fouling surfaces are required for these applications.

However, only a few candidates are regarded as "nonfouling materials" or "superlow fouling materials". Poly(ethylene glycol) (PEG) or oligo(ethylene glycol) (OEG) modified surfaces have been extensively studied to resist protein adsorption. The steric exclusion effect was considered as one of the reasons for PEG polymers to resist protein adsorption.[4−6] Recent studies of OEG self-assembled monolayers (SAMs) show that the appropriate surface density of OEG chains is needed for surface resistance to protein adsorption, and a tightly bound water layer around OEG chains is mainly responsible for large repulsive hydration forces.[7,8] However, PEG or OEG group decomposes in the presence of oxygen and transition metal ions found in most biochemically relevant solutions.[9−11]

Phophorylcholine (PC)-based polymers or surfaces have been shown to decrease protein adsorption.[12−17] They are considered as biomimetic fouling-resistant materials since they contain phophorylcholine headgroups, which are found in the outside layer of cell membranes. The hydration of PC-based materials is also thought to be the reason for their resistance to protein adsorption.[18] However, it is desirable to develop new materials other than PC for applications requiring long-term material stability due to the tendency of the phosphoester group to be hydrolyzed.[19] In addition, PC monomers, such as 2-methacryloyloxyethyl phosphorylcholine (MPC), are moisture sensitive and not easy to synthesize and handle.[20]

Similar to phophorylcholine-based polymers, sulfobetaine polymers belong to polybetaine polymers, in which both cationic and anionic groups are on the same monomer residue.[21] Compared with MPC, sulfobetaine methacrylate (SBMA) is easier to synthesize and handle. However, SBMA polymers were thought to be less fouling-resistant than PC polymers.[22,23] Since most previous studies of SBMA polymers concentrated on their copolymers with other hydrophobic monomers in order to attach them onto substrates or provide mechanical strength, the potential of sulfobetaines as nonfouling materials or biocompatible materials was underestimated. On the basis of previous studies,[8,9,16,24] we believe that the nonfouling properties of a surface are determined by both functional groups and their surface packing. Thus, if poly(SBMA) grafted surfaces are well controlled, they should be able to achieve superlow fouling.

In this study, sulfobetaine polymers were grafted from SAMs covered with initiators through atom transfer radical polymerization (ATRP). Two methods were applied to immobilize ATRP initiators onto gold surfaces (Scheme 1). Superlow fouling surfaces were achieved after well-controlled initiator formation and surface polymerization on gold surfaces. The effects of initiator immobilization and SBMA surface polymerization on protein adsorption were studied.

2. Experimental Section

Materials. Copper(I) bromide (99.999%), bromoisobutyryl bromide (BIBB 98%), pyridine (98%), *N*-(3-sulfopropyl)-*N*-(methacryloxyethyl)-*N*,*N*-dimethylammonium betaine (SBMA 97%), 11-mercapto-1-undecanol (**2**, 97%), 2,2′-bipyridine(BPY

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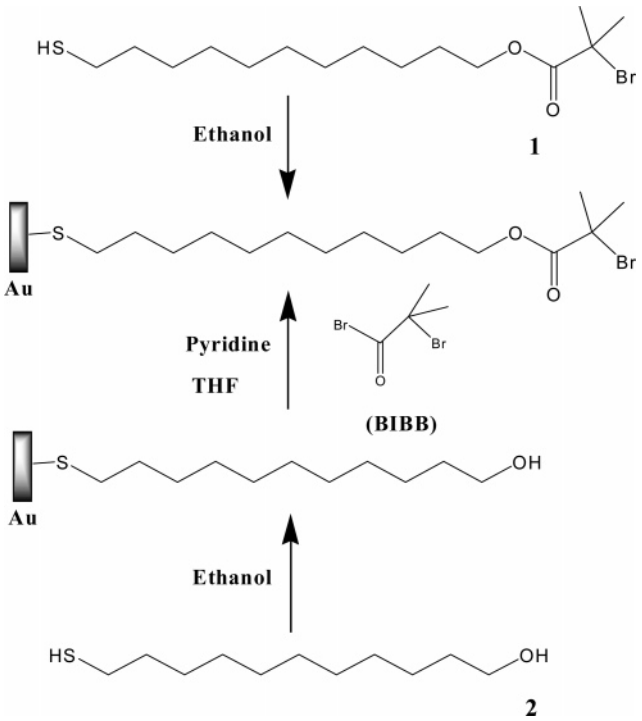
SCHEME 1: One-Step and Two-Step Methods for Preparing Initiator SAMs on Gold Surfaces



99%), and tetrahydrofuran (THF HPLC grade) were purchased from Sigma-Aldrich, Milwaukee, WI. Fibrinogen (fraction I from bovine plasma) and phosphate buffer saline (PBS, pH 7.4, 0.15 M, 138 mM NaCl, 2.7 mM KCl) were purchased from Sigma Chemical Co. Ethanol (absolute 200 proof) was purchased from AAPER Alcohol and Chemical Co. Water used in experiments was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ·cm. THF for reactions and washings were dried by sodium before use.

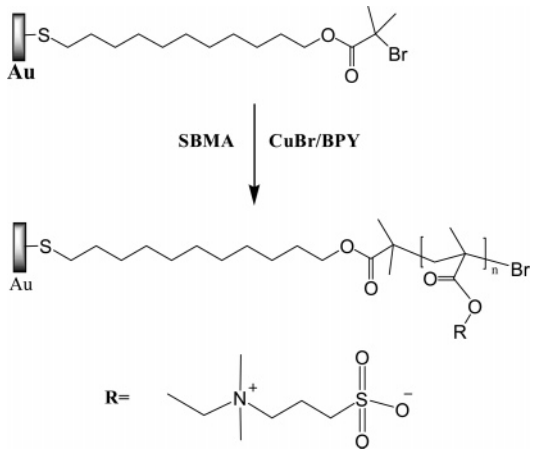
ω-Mercaptoundecyl bromoisobutyrate (**1**) was synthesized through reaction of BIBB and **2** using a method published previously.[25] ^{1}H NMR (300 MHz, $CDCl_3$): 4.15 (t, $J = 6.9$, 2H, OCH_2), 2.51(q, $J = 7.5$, 2H, SCH_2), 1.92 (s, 6H, CH_3), 1.57−1.72 (m, 4H, CH_2), and 1.24−1.40 (m, 16H, CH_2).

SAM Preparation and Initiator Immobilization. SPR glass chips or silicon wafers were coated with an adhesion-promoting chromium layer (thickness 2 nm) and a surface plasmon active gold layer (48 nm) by electron beam evaporation under vacuum. Before SAM preparation, the substrates were washed with pure ethanol, cleaned under UV light, and washed with water and pure ethanol. SAMs were formed by soaking gold-coated substrates in pure ethanol solution of thiols at room temperature after careful cleaning. In this work, two SAMs were formed on the substrates: initiator ω-mercaptoundecyl bromoisobutyrate (**1**) SAM (initiator SAM or Br-SAM) and 11-mercapto-1-undecanol (**2**) SAM (OH-SAM).

To prepare an initiator SAM on a gold surface and compare their effects on polymerization and protein adsorption, **1** solution with various concentrations and cleaning procedures were tested. If not specified, 1 mM **1** solution in pure ethanol was used to soak the substrates for 24 h. The substrates were rinsed with pure ethanol followed by THF and dried in a stream of nitrogen.

For preparation of a hydroxyl-terminated SAM, the gold substrates were soaked in 1 mM **2** ethanol solution for 24 h, and then the substrates were rinsed with ethanol and dried in a stream of nitrogen. Gold substrates with a hydroxyl-terminated SAM were reacted with BIBB under nitrogen protection with

SCHEME 2: Grafting Poly(SBMA) onto the Initiator-Covered Gold Surface via Surface-Initiated ATRP



anhydrous operation (Scheme 1). In this reaction, SAM-covered gold substrates were incubated in 25 mL of dry THF with 2.1 mL of pyridine (26.5 mmol), and then 3.1 mL of BIBB (25 mmol) was added dropwise with gently agitation. A white precipitate, likely pyridine hydrobromide, formed at the initial stage of reaction. After the reaction, the substrates were washed sequentially with THF, ethanol, and deionized water and dried in a stream of nitrogen.

SBMA Polymerization. CuBr and the substrate with immobilized initiators were placed in a reaction tube in a drybox under nitrogen protection. The tube sealed with rubber septum stoppers was taken out. Degassed solution (pure water and methanol in a 1:1 volume ratio) with SBMA and BPY were then transferred to the tube using syringe under nitrogen protection. After the reaction, the substrate was removed and rinsed with ethanol and water, and the samples were kept in water overnight. Usually rinsing with PBS buffer is also applied to remove unbound polymers before testing (Scheme 2).

SPR and Protein Adsorption. Protein adsorption was measured with a custom-built surface plasmon resonance (SPR) sensor, which is based on wavelength interrogation.[26,27] A SPR chip was attached to the base of the prism, and optical contact was established using refractive index matching fluid (Cargille). A dual-channel flow cell with two independent parallel flow channels was used to contain liquid sample during experiments. A peristaltic pump (Ismatec) was utilized to deliver liquid sample to the two channels of the flow cell. Fibrinogen solution of 1.0 mg/mL in PBS (0.15 M, pH 7.4) was flowed over the surfaces at a flow rate of 0.05 mL/min.

A surface-sensitive SPR detector was used to monitor protein−surface interactions in real time. In this study, wavelength shift was used to measure the change in surface concentration (mass per unit area).[28] We take the amount of adsorbed fibrinogen on a $HS(CH2)_{15}CH_3$ SAM (15 nm wavelength shift) as a monolayer (ML). The wavelength shift induced due to protein adsorption on measured surfaces was normalized to be %ML by that on a $HS(CH2)_{15}CH_3$ SAM.[9] %ML can be larger than 100% if the amount of adsorbed protein on a analyzed surface is greater than that on a $HS(CH2)_{15}CH_3$ SAM.

X-ray Photoelectron Spectroscopy (XPS). Gold-coated silicon chips were used for XPS analysis. The procedure for SAM preparation is the same as that for SPR chips. XPS analysis was performed using a Surface Science Instruments (SSI) S-Probe equipped with a monochromated Al Kα X-ray source. The energy of emitted electrons is measured with a hemispherical energy analyzer at pass energies ranging from 50 to 150

eV. Elemental composition present on the surface was identified from a survey scan. All data were collected at 55° from the surface normal takeoff angle. The binding energy (BE) scale is referenced by setting the peak maximum in the C 1s spectrum to 285.0 eV. Multiple samples were analyzed from each batch, and data were averaged. High-resolution C 1s spectra were fitted using a Shirley background subtraction and a series of Gaussian peaks. Data analysis software was from Service Physics, Inc.

Ellipsometry. Ellipsometry was performed using a spectroscopic ellipsometer (Sentech SE-850, GmbH). Sample preparation is the same as in XPS experiments. Five separate spots were measured at three different angles of incidence (50°, 60°, and 70°) in the visible region. The same batch of gold-coated chips was cleaned by UV-ozone cleaner for 20 min, washed with ethanol and Millipore water, and dried with nitrogen. The bare gold-coated chips were used as reference. The thicknesses of films studied were determined using the Cauchy layer model with an assumed refractive index of 1.45.

Tapping Mode Atomic Force Microscope (TM-AFM). The gold substrates for TM-AFM were prepared by the vapor deposition of gold onto freshly cleaved mica (Asheville-Schoonmaher Mica Co.) in a high-vacuum evaporator (BOC Edwards Auto306) at ca. 10^{-7} Torr. Mica substrates were preheated to 325 °C for 2 h by a radiator heater before deposition. Evaporation rates were 0.1−0.3 nm/s, and the final thickness of the gold film was ca. 200 nm. Gold-coated substrates were annealed in H_2 frame for 1 min before use. All TM-AFM images were acquired using a Nanoscope IV (Veeco, CA) AFM, equipped with E scanner. Si cantilevers (TESP, DI) with resonant frequencies of ∼270 kHz, force constants of 20−100 N/m, and tip apex radii of 5−10 nm were used.

3. Results and Discussion

SAMs on gold surfaces are an excellent platform for surface polymerization.[25,30−35] ATRP is well suited for polymerization on SAM surfaces since the reaction can be carried out at room temperature, the reaction media can be water or other polar solvents, and the molecular weight of the polymer is controllable. Two methods were used to immobilize ATRP initiators onto gold surfaces, as shown in Scheme 1. One approach was to prepare an α-bromocarbonyl-terminated thiol and to form a SAM from the thiol onto a gold surface. Jones et al. prepared surfaces covered with initiators of different densities using this method.[25,33] They found that the thickness of two methacrylate polymers increased with the density of the initiator and there was no optimal initiator density. The other approach was to graft the initiator on a hydroxyl-terminated SAM surface. Kim et al. studied this method and estimated that only 10% of the initiator on the surface initiated the polymerization of PMMA chains.[30] These two approaches were compared in this work for surface polymerization and subsequent protein adsorption.

Preparing Superlow Fouling Surfaces on an Initiator-Terminated SAM. Fibrinogen in PBS solution was flowed over a SPR sensor chip, followed by buffer solution to remove loosely bound proteins. The difference in wavelength before Fg injection and after the removal of weakly adsorbed proteins was an indication of Fg adsorption. Figure 1 gives the SPR signal change as a function of time on different surfaces. The bare gold surface has 109% ML adsorption (wavelength shift 16.4 nm) with respect to adsorption on a $HS(CH_2)_{15}CH_3$ SAM. After soaking in 1 mM **1** solution, 125% ML adsorption (wavelength shift 20.3 nm) was detected. Protein adsorption on the bare gold and the initiator **1** SAM surface is high. After the polymerization of SBMA through ATRP, protein adsorption was greatly

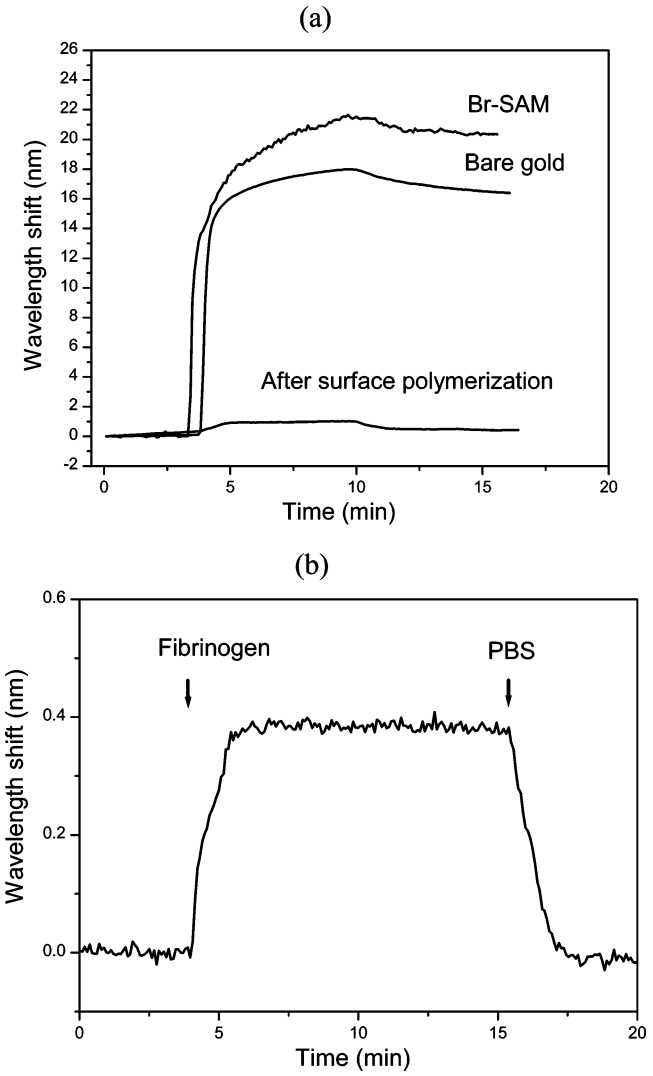


Figure 1. (a) Adsorption of 1 mg/mL fibrinogen in PBS buffer (0.15 M, pH 7.4) on a bare gold surface, a gold surface with initiator **1** (Br-SAM), and a surface grafted with poly(SBMA) from SPR measurements and (b) a typical nonfouling surface grafted with poly(SBMA). Polymerization occurred on the Br-SAM surface immersed in 25 mL of CH_3OH/H_2O containing 7.5 mmol of SBMA, 2 mmol of BPY, and 1 mmol of CuBr for 1 h. The wavelength shift of 1 nm in SPR is equivalent to 0.15 mg/m^2 adsorbed proteins.

decreased (Figure 1a) to less than 0.02 nm (0.1% ML or 0.3 ng/ cm^2 of fibrinogen adsorption), which is the lowest detection limitation of our SPR sensor. Lysozyme and bovine serum albumin (BSA) adsorption was also measured from SPR at the level similar to fibrinogen adsorption. A superlow fouling surface (protein adsorption < 5−10 ng/cm^2) covered with well-controlled poly(SBMA) brushes is achieved. Furthermore, the substrates grafted with poly(SBMA) were left in air or immersed in water at room temperature for more than 1 month without losing their superlow fouling properties. The parameters for controlling polymeric layers to achieve very low protein adsorption are discussed in the following sections.

Effects of Initiator-Terminated SAM Formation on Protein Adsorption. The quality of the initiator SAM is important to subsequent polymerization via ATRP and protein adsorption. A significant amount of unbound thiol molecules was found if the initiator SAM was washed only with pure ethanol as for the preparation of most SAMs. These unbound thiol molecules were completely removed if the initiator SAM was rinsed with ethanol followed by THF since THF is a better solvent for

TABLE 1: Elemental Compositions of a Gold Surface Covered with the Initiator 1 SAM from XPS Analysis

initiator concn (mM)	Br	S	O	C	Au
0.5[a]	3.1 ± 0.8	1.6 ± 0.3	10.0 ± 1.8	56.6 ± 2.9	28.7 ± 1.0
1[a]	3.4 ± 0.8	1.7 ± 0.2	9.3 ± 0.3	54.4 ± 2.7	31.3 ± 2.8
10[a]	4.0 ± 0.3	2.1 ± 0.5	9.4 ± 1.2	54.9 ± 1.9	29.5 ± 2.0
1[b]	2.4 ± 0.3	1.6 ± 0.3	10.5 ± 0.7	56.7 ± 0.3	28.8 ± 1.0
10[b]	2.1 ± 0.6	1.7 ± 0.3	10.6 ± 0.5	56.4 ± 1.5	29.2 ± 0.6

[a] The samples were washed with ethanol only. [b] The samples were washed with ethanol followed by THF.

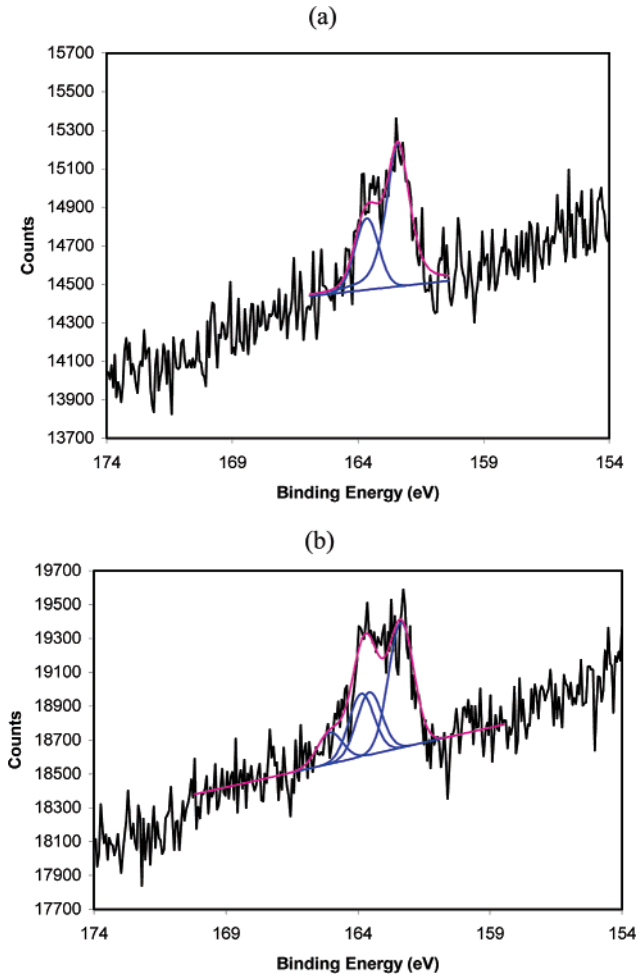


Figure 2. High-resolution XPS spectra of the S_{2p} region. The substrates were prepared by soaking in 10 mM **1** initiator solution for 24 h and then rinsed with ethanol and THF (a), for which no unbound sulfur peaks were observed, or with ethanol only (b), for which 37.1% unbound sulfur species were observed. The peaks were fit using two S 2p doublets with 2:1 area ratio and splitting of 1.2 eV.

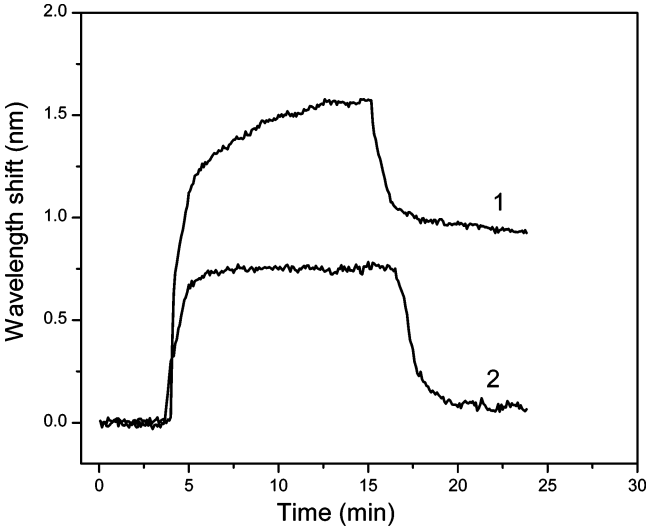


Figure 3. SPR sensorgrams of fibrinogen adsorption on poly(SBMA) grafted surfaces with unbound initiators (1) and without unbound initiators (2). The two substrates were placed into the same reactor for polymerization with 2.0 mmol of SBMA, 0.1 mmol of BPY, and 0.05 mmol of CuBr in 25 mL of CH_3OH/H_2O for 17 h.

compound **1** than ethanol. In this work, S_{2p} high-resolution spectra from XPS measurements were used to analyze unbound thiol molecules on the surfaces. For samples rinsed with both ethanol and THF, only one sulfur species was detected corresponding to the doublet structure with both $S_{2p3/2}$ and $S_{2p1/2}$ peaks. The binding energy of the $S_{2p3/2}$ peak was 162.4 eV, close to the reported binding energy of sulfur atoms bound to the gold surface as a thiolate species[36] (Figure 2a). AFM images show that the surface is featureless for the initiator SAM on gold except for defects and domains from the gold substrate, indicating a homogeneous monolayer without unbound thiol molecules on the gold surface (Figure 3). Elemental composition from XPS analysis (Table 1) also shows that bromine and sulfur signals do not depend on the concentration of the initiator if the samples were rinsed with THF followed by ethanol. For those surfaces washed only with ethanol, the S_{2p} cannot be fit with a single doublet. Another doublet structure ($S_{2p3/2}$ peak binding energy of 163.6 eV) was assigned to unbound thiol molecules (Figure 2b). There are about 21.7%, 26.1%, and 37.1% unbound initiators on the surfaces prepared from 0.5, 1.0, and 10 mM initiator solutions, respectively. In addition, it can be seen from Table 1 that bromine and sulfur signals slightly increase with the increasing concentration of the initiator solution if these samples are rinsed only with pure ethanol (Table 1).

Here we compare two slides covered with the initiator SAM but washed in two different ways (i.e., ethanol only and ethanol followed by THF) for polymerization via ATRP and subsequent protein adsorption. These two slides were put into a same reactor and polymerized for 17 h. After polymerization, the substrates treated with only ethanol had a visible polymer layer on the surface, indicating the formation of a thick polymer layer on the surface. This polymer layer cannot be removed even after being rinsed and soaked in water for 24 h and then in PBS buffer solution for 24 h. The thickness of the polymer is too thick to be measured accurately by ellipsometry. For the substrate treated with ethanol followed by THF, the polymer grafted is not visible. The thickness of the polymer film after soaking in water and PBS buffer for 24 h was measured to be 12 nm from ellipsometry. Figure 5 shows the difference in wavelength shift from SPR for fibrinogen adsorption on these two polymerized slides. The thicker polymer layer initiated from the surface with unbound thiols leads to some fibrinogen adsorption (0.9 nm shift in wavelength), corresponding to a 6% ML of adsorbed fibrinogen. The polymer layer initiated from the surface without unbound initiators has very low protein adsorption. Unbound thiol molecules can cause the formation of a thick polymer film. Strong intermolecular interactions

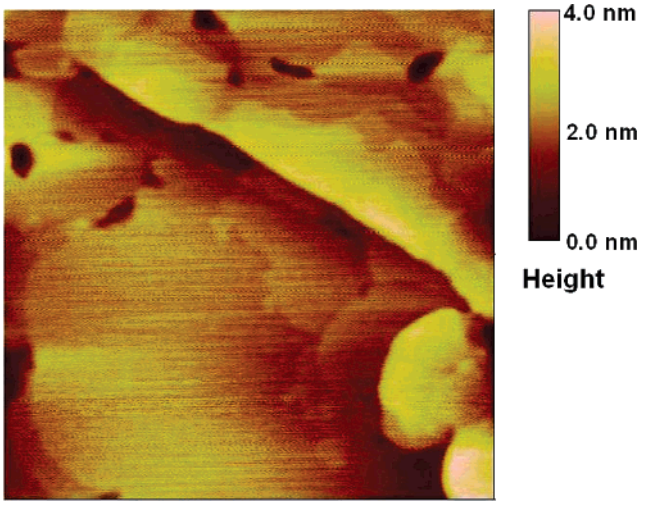


Figure 4. TM-AFM image of the initiator **1** SAM on a gold substrate (scan size: 1 μm × 1 μm). The surface was prepared in 10 mM initiator **1** solution for 24 h and then washed with ethanol and THF.

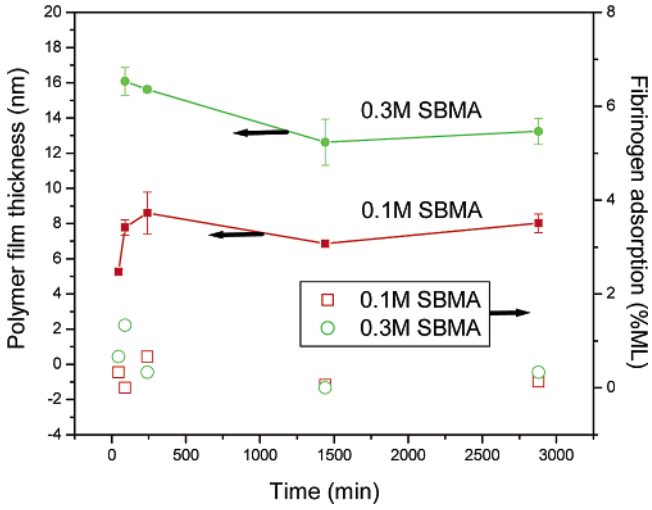


Figure 5. Thickness of poly(SBMA) (measured by ellipsometry, solid symbols) and corresponding fibrinogen adsorption on poly(SBMA) grafted surfaces (measured by SPR, open symbols) as a function of reaction time. For reaction with 0.1 M SBMA, 2.5 mmol of SBMA, 1 mmol of BPY, and 0.5 mmol of CuBr in 25 mL of CH_3OH/H_2O were used for polymerization. For reaction with 0.3 M SBMA, 7.5 mmol of SBMA, 2 mmol of BPY, and 1 mmol of CuBr in 25 mL of CH_3OH/H_2O were used for polymerization. % ML fibrinogen adsorption reported is with respect to that on a CH_3 SAM.

among zwitterionic groups via intra- and interchain ionic contacts lead to dehydration within the thick polymer film and thus protein adsorption. The ionically cross-linked networks are very common in polymeric betaines, leading to the insolubility of those polymers in pure water.[21] Usually, insoluble polybetaines in water can be dissolved in aqueous electrolyte solutions since electrolytes can penetrate into the ionic network and decrease the electrostatic interactions among polymer chains.[21] However, it is found that the thick polymer film formed cannot be removed even with buffer solution. On the basis of the results

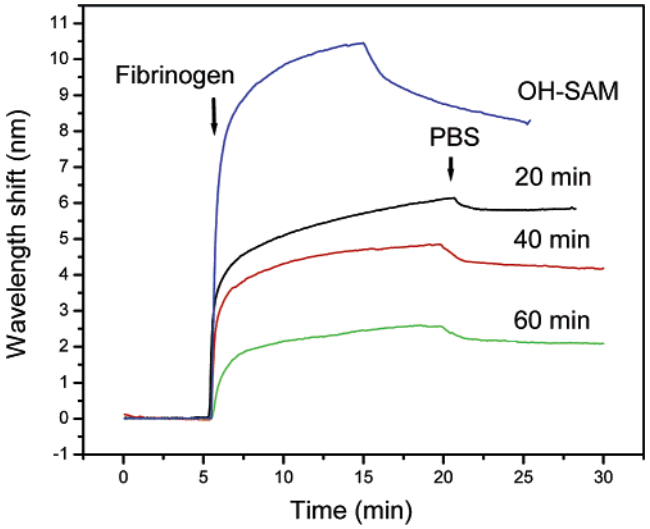


Figure 6. SPR sensorgrams for fibrinogen adsorption on an initiator-modified OH-SAM grafted with poly(SBMA). The SAM surfaces were reacted with 25 mmol of BIBB for 20, 40, and 60 min, respectively, in 25 mL of dry THF with 26.5 mmol of pyridine. The substrates were placed in a reactor for polymerization with 2.0 mmol of SBMA, 0.1 mmol of BPY, and 0.05 mmol of CuBr in 25 mL of CH_3OH/H_2O for 17 h.

above, it can be seen that the treatment of the initiator SAM with appropriate solvents is necessary to achieve superlow fouling surfaces.

Effects of Surface Polymerization on Protein Adsorption. Figure 5 shows polymer thickness as a function of polymerization time for different SBMA concentrations. After polymerization, substrates were washed with both water and PBS buffer. The thickness of both substrates was measured by ellipsometry. For reaction with a SBMA concentration of 0.1 M, the thickness of the polymer film increased rapidly at the beginning of the reaction and leveled off at ~8 nm after 4 h, at which a termination might occurs.[39] Reaction with a SBMA concentration of 0.3 M leveled off at ~12 nm rapidly (Figure 5). Reactions with higher concentrations lead to thick and uneven polymer films on surfaces. A longer reaction time may even result in gelation throughout the solution, which makes it difficult to measure film thickness by ellipsometry. In this work, the CuBr/BPY complex was used to catalyze the polymerization, but no catalyst deactivator or free initiator was used to control the polymerization as reported previously in some studies.[32,37,38] Thus, polymer brushes grew fast in a short period of time. Figure 5 also shows fibrinogen adsorption on poly(SBMA)-covered surfaces measured by SPR. All substrates were washed with pure water and PBS buffer extensively before SPR experiments. It is shown that all the surfaces with polymer film thickness ranging from 5 to 12 nm highly resist fibrinogen adsorption.

Immobilization of Initiators on a Hydroxyl-Terminated SAM and Its Effect on Protein Adsorption. A two-step method was reported to immobilize the ATRP initiator onto a surface.[30] This method was also used in this work. A hydroxyl-terminated SAM was first formed from 11-mercapto-1-undecanol (**2**) onto a gold surface, and the initiator groups were then

TABLE 2: Elemental Compositions of a Hydroxyl-Terminated SAM Reacted with 25 mmol of BIBB from XPS Analysis

reaction time	Br	S	O	C	Au	Si
0	0.0 ± 0	1.9 ± 0.4	8.8 ± 0.2	56.0 ± 1.7	33.3 ± 1.2	0.0 ± 0
20 min	3.2 ± 0.4	1.6 ± 0.3	9.9 ± 1.0	51.3 ± 2.1	34.0±1.0	0.0 ± 0
60 min	5.4 ± 1.2	1.8 ± 0.1	8.4 ± 0.7	44.7 ± 2.3	39.7 ± 3.1	0.0 ± 0
9 h	2.3 ± 0.7	0.7 ± 0.2	29.2 ± 5.2	40.2 ± 2.3	25.8 ± 6.2	1.8 ± 0.8
4 d	4.3 ± 0.7	0.0 ± 0	49.8 ± 3.0	34.6 ± 1.5	0.7 ± 0.1	10.6 ± 1.2

grafted onto the surface via the reaction of BIBB with the hydroxyl group (Scheme 1). Table 2 presents data from XPS elemental analysis of the surface as a function of reaction time. Results show that in the case of reaction with BIBB for 20 min Br and O compositions increase while S and Au compositions remain unchanged. It was reported previously that thiolate SAMs could be unstable in the presence of acid bromide.[30] However, there is no evidence that a reaction time of 20 min affects the quality of SAMs in this work. For a reaction time of 60 min, the Br composition increased. When reaction time was 9 h or longer, the concentration of Br, S, and Au decreased, the concentration of O increased significantly, and the Si peak appeared. Long-time reaction not only destroyed the SAM film but also removed the gold layer from the silicon wafer. The gold layer was mostly removed after a reaction for 4 days possibly due to the erosion of the adhesion Cr layer.

SBMA was polymerized on the substrates described above using ATRP. Figure 6 presents fibrinogen adsorption on the substrates as a function of reaction time from SPR measurements. Fibrinogen adsorption decreased for reaction time from 20 to 60 min. But, no further decrease was obtained for reaction time after 60 min. The two-step method does not achieve as low protein adsorption as the one-step method, which uses milder reaction conditions without degrading the initiator SAM.

4. Conclusions

It is demonstrated in this work that poly(SBMA) grafted surfaces via ATRP highly resist protein adsorption. The quality of initiator SAMs is important to the effective reduction of protein adsorption. Two methods are compared for immobilizing ATRP initiators onto gold surfaces. One is to directly assemble the initiator-terminated SAM from ω-mercaptoundecyl bromoisobutyrate onto a gold surface while the other is to react BIBB with a hydroxyl-terminated SAM. Results show that the former method is better than the latter one due to milder reaction conditions used. A high-quality initiator **1** SAM is obtained when it is washed with ethanol followed by THF. After poly(SBMA) is grafted onto the initiator **1** SAM surface followed by washing with PBS, a superlow fouling surface is achieved. The effects of polymerization time and initiator concentration are also studied. It is shown that polymer layers prepared all have excellent protein-resistant properties, and their thickness ranges between 5 and 12 nm.

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