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RESEARCH ARTICLE

A Facile Method for Construction of Antifouling Surfaces by Self-Assembled Polymeric Monolayers of PEG-Silane Copolymers Formed in Aqueous Medium

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Self-assembled polymeric monolayers (PMs) on Si/SiO_2 wafers were prepared in water from a series of random copolymers of poly(ethylene glycol) methyl ether methacrylate (PEGMA) and 3-(trimethoxysilyl)propyl methacrylate (TMSMA), denoted as poly(TMSMA-*r*-PEGMA). Four polymers of poly(TMSMA-*r*-PEGMA) were synthesized by free radical polymerization with a systematic variation of co-monomer feed ratios. Regardless of PEG grafting density in the copolymers, all PMs formed approximately 1 nm-thick film as measured by ellipsometry. However, the PMs with a higher grafting density of PEG resulted in more hydrophilic surfaces in terms of water contact angle. The protein resistance of the PMs was evaluated using bovine serum albumin (BSA) as a model protein. Analyses by ellipsometry, atomic force microscopy (AFM), and X-ray photoelectron spectroscopy (XPS) showed that the PMs of the copolymers markedly reduced the nonspecific adsorption of proteins compared to the unmodified Si/SiO_2 wafers. The study also revealed that the PMs prepared from the copolymers with a higher PEG grafting density were more effective in resisting the nonspecific protein adsorption.

Keywords: PEG Copolymer, Antifouling, Polymeric Monolayer, Bioactive Surface, Protein Resistance.

1. INTRODUCTION

The construction of protein- and cell-resistant surfaces has been of a crucial requirement for the preparations of medical or analytical devices and drug delivery vehicles in direct contact with biological fluids.[1,2] A popular strategy for rendering surfaces antibiofouling is to modify the outermost surface of the materials with PEG, a biocompatible polymer known for its protein-repellent property.[3–4] Among a variety of materials, oxide-based substrates such as SiO_2 and indium tin oxide (ITO) glasses have been the subject of much attention because these oxide substrates are widely used in the manufacture of biomedical devices.[5–8] Direct PEG modification of oxide-based surfaces could be achieved using various PEG-grafted copolymers either by physical adsorption[9] or by electrostatic interaction.[10] In our previous papers, we reported a facile method for the direct PEG modification of oxide substrates by the formation of self-assembled polymeric monolayers (PMs) via multiple covalent bonds.[11,12] The key material in the approach was a random copolymer composed of an 'anchor part' (trimethoxysilyl group) and a 'function part' (PEG), denoted as poly(TMSMA-*r*-PEGMA). The surface-reactive trimethoxysilyl group allowed the copolymer to form multiple covalent bonds onto SiO_2 surfaces along with multiple PEG immobilizations and consequently resulted in high protein- and cell-resistant surfaces. As compared to the monovalent PEG-Silane systems, this multivalent PEG-Silane exhibited a faster, simpler, and a more reliable surface modification by forming self-assembled polymeric monolayers along with the better antifouling performance. Although the surface density of PEG is known to play an important role in preventing protein adsorption,[13] such consideration was not given in our previous report. On the other hand, self-assembled monolayers on the SiO_2-based substrates have been commonly prepared in the anhydrous and toxic organic solvents. Therefore, from the environmental point of view, generation of the PEG-based polymeric monolayers in aqueous medium would be beneficial. Such benign coating process using water as a solvent would be especially advantageous in the engineering of the surface for *in vivo* use.[14] Since poly(TMSMA-*r*-PEGMA) is water-soluble, we anticipated

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that the polymeric monolayers could also be prepared even in water in the similar way as previously achieved in methanol. In this paper, we report a facile preparation method for the self-assembled polymeric monolayers of the PEG-based copolymers on SiO_2 substrates in water and the protein resistances of the resulting ultrathin PEG films. In addition, the ratio of PEG to silane in the series of poly(TMSMA-*r*-PEGMA) copolymers was systematically varied to examine the effect of the PEG-Silane ratio on the wettability, morphology, and protein resistance of the PMs prepared in water.

2. EXPERIMENTAL DETAILS

2.1. Materials

3-(Trimethoxysilyl)propyl methacrylate, poly(ethylene glycol) methyl ether methacrylate (average $M_n = ca.$ 475), and 2,2′-azobisisobutyronitrile (AIBN) were purchased from Aldrich Chemical Co. (Milwaukee, WI). All organic solvents were used as received. The Si/SiO_2 wafers used herein was first cleaned using detergent, followed by washing with deionized water and methanol several times. Prior to form the polymeric films, all the substrates were treated with O_2 plasma for 1 min to generate -OH groups as well as to clean the surfaces.

2.2. Measurements

^{1}H NMR (300 MHz) spectra were recorded on a JEOL JNM-LA300WB FT-NMR (Tokyo, Japan). Organic phase gel permeation chromatography (GPC) was performed using a Waters 1515 series isocratic pump, a Rheodyne model 7725 injector with a 100 μl injection loop at a flow rate of 0.4 ml/min. The thicknesses of the monolayer films were measured with a Gaertner L116A ellipsometer (Gaertner Scientific Corporation, IL) at a 70° angle of incidence. A refractive index of 1.46 was used for all films, and a three-phase model was used to calculate thicknesses. A phoenix300, contact angle & surface tension analyzer (Surface electro optics, Kyonggi, Korea) equipped with video camera and monitor was used to measure contact angle. X-ray photoelectron spectroscopy (XPS) spectra were obtained using a Kratos AXIS Ultra Imaging X-ray photoelectron spectrometer with a monochromatized Al K X-ray source. Scanning force micrographs (1.0×1.0 μm^2) were performed in tapping mode on a NanoScope III Dimension (Veeco Instruments Inc., Woodbury, NY) in air.

2.3. Synthesis of the Copolymer Comprising 3-(Trimethoxysilyl)Propyl Metharylate (TMSMA) and Poly(Ethylene Glycol) Methyl Ether Methacrylate (PEGMA): Poly(TMSMA-*r*-PEGMA).

Detailed synthetic procedure of the copolymers is described elsewhere.[11] Initial feed ratios of two monomers were 1:1 for poly **1**, 1:2 for poly **2**, 1:4 for poly **3**, 1:9 for poly **4**, respectively. Actual ratios of two monomer units incorporated in the final copolymers were calculated from comparison of the integration value of the peak at $\delta = 4.13$ (CO_2-CH_2 at PEGMA) with that of the peak at $\delta = 3.92$ (CO_2-CH_2 at TMSMA) in the ^{1}H NMR spectra. Poly **1** (1:1 of Silane/PEG, $M_n = 13540$ with $M_w/M_n = 1.89$), poly **2** (1:1.6 of Silane/PEG, $M_n = 21789$ with $M_w/M_n = 2.21$), poly **3** (1:3.6 of Silane/PEG, $M_n = 15680$ with $M_w/M_n = 2.17$), poly **4** (1:7.5 of Silane/PEG, $M_n = 32590$ with $M_w/M_n = 2.78$).

2.4. Formation of the Self-Assembled Polymeric Monolayers on Si/SiO_2 Wafer

The self-assembled polymeric monolayers (PMs) of the copolymers onto oxygen plasma-treated Si/SiO_2 wafers as a model oxide surface were prepared by immersing the substrates in the aqueous solution of the copolymer (10 mg/ml) for 1 h at the ambient temperature followed by washing with the same solvent. The resulting PMs were further cured at 110 °C for 10 min to assure covalent bond formations via dehydration between the copolymer and the outer surface.

2.5. Protein Adsorption on Control (Unmodified) and the Self-Assembled Polymeric Monolayers (PMs) on Si/SiO_2 Wafer

The PMs on wafers were placed in a bovine serum albumin (0.25 mg/ml in PBS, pH 7.4) for 2 h, after which the surface of wafer was gently washed with PBS and assessed by ellipsometer and by XPS to obtain the amount of protein adsorption onto the surface.

3. RESULTS AND DISCUSSION

3.1. Synthesis of the Copolymers Comprising Different Ratios of Monomer

We have synthesized a series of random copolymers, poly(TMSMA-*r*-PEGMA)s with four different initial feed ratios of two co-monomers, TMSMA as a surface anchoring group and PEGMA as a protein repelling group as shown in Figure 1: poly **1** = 1:1, poly **2** = 1:2, poly **3** = 1:4, and poly **4** = 1:9, respectively. We found that the compositions of the copolymers were well matched to the initial feed ratios. The molecular weights of four copolymers synthesized herein ranged from 13,000 to 32,000 with a polydispersity index of *ca.* 2.

3.2. Formation and Characterization of the Self-Assembled Polymeric Monolayers

The mechanism of the PMs formation in water is proposed as illustrated in Figure 1. Once the trimethoxy moiety in the copolymer is hydrolyzed to the trihydroxysilane,

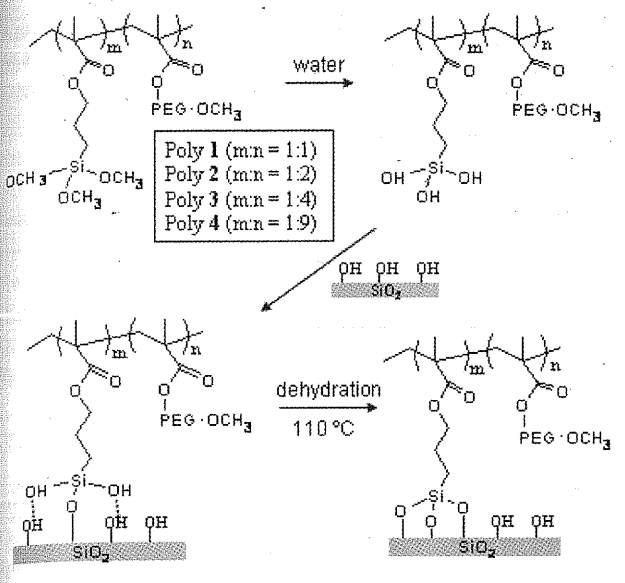

Fig. 1. Chemical structure of poly(TMSMA-*r*-PEGMA) copolymers synthesized at different feed ratios of two monomers (m:n) and a proposed mechanism on formation of the self-assembled polymeric monolayers on O_2 plasma-pretreated Si/SiO_2 surfaces in water.

$-Si(OH)_3$, the PMs can be formed through multiple hydrogen bonding interactions at the interface of the polymer and the hydroxyl-presenting surface of silicon wafer. The PMs could then be rendered more robust through multiple covalent bond formations in the curing process after dehydration at high temperature.

The self-assembled PMs formed from poly **1** through poly **4** were characterized by ellipsometry and the contact angle measurement to measure the thickness and the hydrophilicity of the PMs, respectively (Table I). The ellipsometric thickness data clearly indicated that ultrathin films were successfully generated from the copolymers on the surface with average thickness of *ca.* 1 nm, suggesting that the films exist in the form of polymeric monolayers as similar as those prepared in methanol previously,[11] not in the form of polymeric multilayers. Contrary to our initial expectation, PMs of the four polymers had almost the same thickness with the difference of less than 1.5 Å. This similarity is presumably because the thickness was measured in a dry state of each PMs, which otherwise may give different film thickness in aqueous phase.[15] Although poly **4** had only 10% of anchor part, stable PMs were successfully formed, suggesting that the present copolymer system effectively anchors to the surface presumably due to its covalent bond nature.

Table I. Thickness and static water contact angle of the self-assembled polymeric monolayers constructed on Si/SiO_2 wafer in water.

	Poly **1**	Poly **2**	Poly **3**	Poly **4**
Thickness (nm)	1.09±0.04	1.08±0.03	0.97±0.14	0.96±0.13
Static CA (°)	35±0.5	34±0.3	26±0.5	21±0.5

The wettability of the PMs was attained by static water contact angle measurement at the ambient temperature. As expected, the contact angle decreased with the increasing ratio of hydrophilic PEG in the copolymers. Assuming that a lower contact angle corresponds to the presence of more PEG in the PMs, the contact angle data indirectly confirm the formation of the desired PMs with right PEG ratio. The PMs formed from poly **4** (90% of PEG as a molar ratio in the polymer) was much more hydrophilic than that from poly **1** (50% of PEG) with significant angle difference about 15°.

These results clearly suggest that
(1) the PMs of poly(TMSMA-*r*-PEGMA) copolymers can be spontaneously formed in water, and
(2) PEG density on the surfaces can be controlled with ease by employing the present copolymer system.

3.3. Protein Adsorption Evaluated by Ellipsometry, AFM, and XPS

The degrees of nonspecific protein adsorption onto the PMs were measured by various analytical instruments including ellipsometry, atomic force microscope (AFM), and X-ray photoelectron spectrometry (XPS). The relative protein-resistance were compared each other with respect to PEG ratios in the copolymers.

Table II presents the thickness of the PMs measured by ellipsometry after BSA adsorption. The thickness of each PMs after exposure to BSA remained almost the same as the initial thickness, while a significant increase in the thickness by ca. 4.5 nm was observed for the unmodified silicon wafer as a control. This clearly demonstrates that the PMs formed in water are also highly resistant to nonspecific BSA adsorption.

The surface topography of the PMs on Si/SiO_2 wafers after BSA adsorption was also examined by tapping mode atomic force microscopy (AFM) (Fig. 2). Three different positions per substrate were taken. The AFM images clearly revealed there was very little or no BSA adsorption onto the PMs formed from each copolymer on Si/SiO_2 wafers, though few aggregates were observed in the cases of poly-**3** and poly-**4**. However, the amount of adsorption seemed negligible when compared to that of adsorption on the control Si/SiO_2 wafer. In fact, the surface topography of the PMs after BSA adsorption turned out to be almost same as those formed in water before BSA adsorption (data not shown). The AFM results

Table II. Thickness of the control (unmodified) and the self-assembled polymeric monolayers on Si/SiO_2 wafer after bovine serum albumin (BSA) adsorption.

	Control (unmodified)	Poly **1**	Poly **2**	Poly **3**	Poly **4**
Thickness (nm)	4.48±0.06	1.15±0.06	1.10±0.02	0.96±0.13	0.96±0.13

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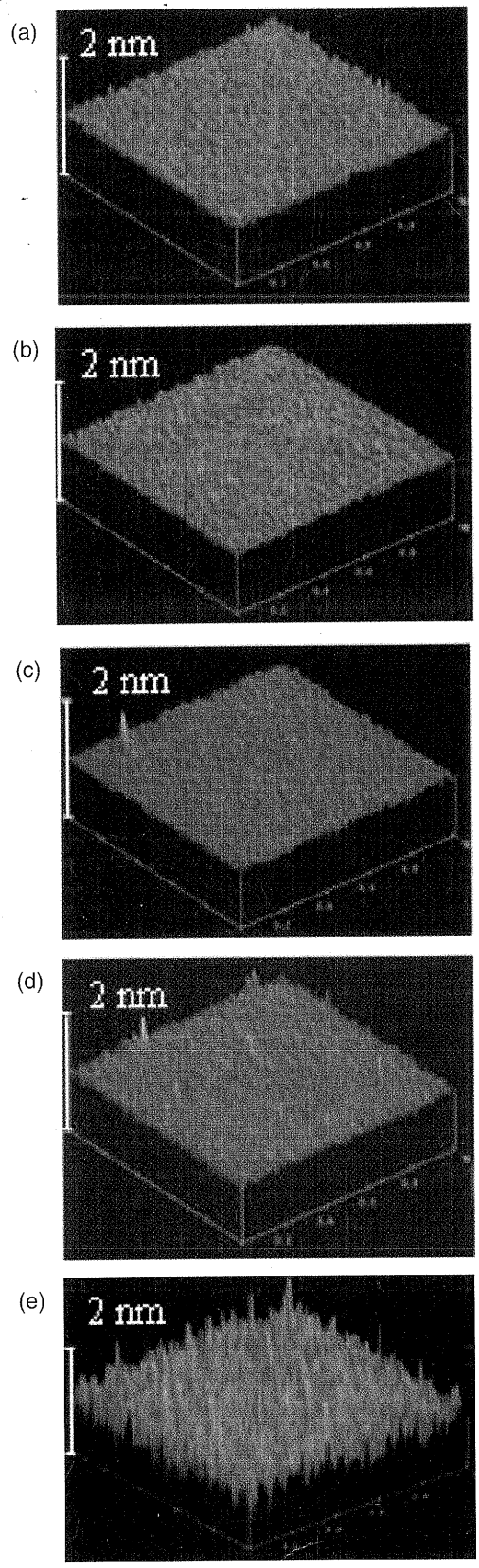

Fig. 2. AFM height images of the control (unmodified) and the self-assembled polymeric monolayers on Si/SiO_2 wafer after protein adsorption: (a) Poly **1**, (b) Poly **2**, (c) Poly **3**, (d) Poly **4**, and (e) control (unmodified).

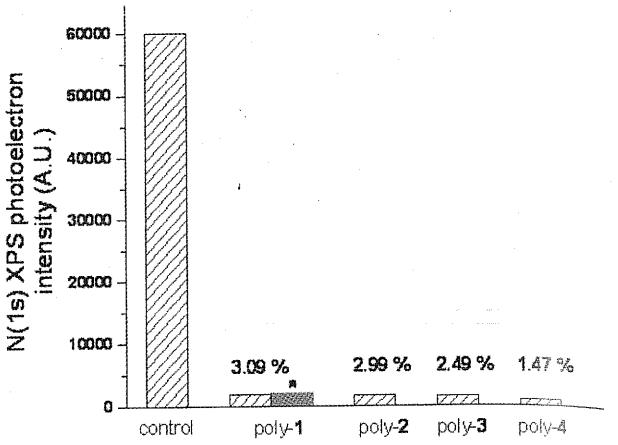

Fig. 3. High-resolution N(1s) XPS intensity on the control (unmodified) and the self-assembled polymeric monolayers on the Si/SiO_2 wafers measured after bovine serum albumin (BSA) adsorption. The filled box marked as asterisk represents the N(1s) intensity measured after incubation for 2 weeks. The relative amount (denoted as percentage) of protein adsorption on each surface is calculated by comparison of unmodified, bare Si/SiO_2 surface as 100% of protein adsorption.

were in satisfactory aggrement with the results obtained by ellipsometry. However, the effect of PEG density on the protein-resistance is not clear because of the limitation of AFM and ellipsometry as means of quantitative analysis.

To obtain more quantitative data on the protein-resistance of each PMs containing different PEG density, we analyzed the surfaces of the PMs by high resolution N(1s) XPS after BSA adsorption (Fig. 3). According to the N(1s) intensities of XPS, all four PMs showed significantly lower protein adsorption (up to 1.47%) in comparison to a control, unmodified Si/SiO_2 wafer (taken as a 100% of adsorption). As increasing PEG ratio in the copolymer, relative amount of protein adsorption slightly decreased as expected: poly **1** (lowest PEG density; 3.09%) and poly **4** (highest PEG density; 1.47%), respectively. Although it seems the effect of PEG density on protein-resistance was not significant within the PEG/Silane ratios of the present copolymer system, it is obvious higher PEG density improves the protein-resistance, making the surface more anti-biofouling. In addition, the long term stability and the protein resistance of the PMs were also examined using the PMs of poly **1.** When the N(1s) intensity of the PMs of poly **1** was measured after incubation in BSA solution for 2 weeks, there was no increase in the amount of BSA adsorption during that period, suggesting the PMs formed in water are stable enough to retain its protein-resistance up to at least two weeks.

4. CONCLUSION

In conclusion, we have demonstrated that protein-resistant surfaces can be easily prepared on bare Si/SiO_2 wafers by forming the self-assembled polymeric monolayers in

aqueous phase. Furthermore, we have examined the effect of PEG density in the polymeric monolayers on the resistance to protein adsorption. Lower nonspecific adsorption of proteins was obtained with a higher PEG density in the copolymer. In addition, the polymeric monolayers on Si/SiO_2 wafers formed from aqueous polymer solutions were stable enough to retain its anti-biofouling property up to two weeks in the protein solution. The use of water for the anti-biofouling coating may be of beneficial for the *in vivo* use from the environmental and biocompatibility points of view. Therefore, the present copolymer systems may have warrant applications in the preparation of anti-biofouling surfaces of medical and analytical devices.

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