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Surface modification using silanated poly(ethylene glycol)s

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Abstract

Surface-grafted poly(ethylene glycol) (PEG) molecules are known to prevent protein adsorption to the surface. The protein-repulsive property of PEG molecules are maximized by covalent grafting. We have synthesized silanated monomethoxy-PEG (m-PEG) for covalent grafting of PEG to surfaces with oxide layers. Two different trialkoxysilylated PEGs were synthesized and characterized. The first trialkoxysilylated PEG was prepared by direct coupling of m-PEG with 3-isocyanatopropyltriethoxysilane through a urethane bond (silanated PEG I). The other silanated PEG (silanated PEG II) containing a long hydrophobic domain between PEG and a silane domain was prepared by reacting m-PEG with 1,6-diisocyanatohexane and 10-undecen-1-ol in sequence before silylation with 3-mercaptopropyl trimethoxysilane. Silanated PEGs I and II were grafted onto glass, a model surface used in our study. The PEG-grafted glass surfaces were characterized by contact angle, X-ray photoelectron spectroscopy (XPS), and atomic force microscopy (AFM). Although contact angle did not change much as the bulk concentration of silanated PEG used for grafting increased from 0.1 to 20 mg/ml for both PEGs I and II, the surface atomic concentrations from XPS measurements showed successful PEG grafting. Surface PEG grafting increased concentration of surface carbon but decreased silicone concentration. The high resolution C1s spectra showed higher ether carbon with lower hydrocarbon compositions for the PEG-grafted surfaces compared to the control surface. AFM images showed that more PEG molecules were grafted onto the surface as the bulk concentration used for grafting was increased. AFM images of the dried surfaces showed that the surfaces were not completely covered by PEG molecules. After hydration, however, the surface appears to be covered completely probably due to the hydration of the grafted PEG chains. Glass surfaces modified with silanated PEGs reduced fibrinogen adsorption by more than 95% as compared with the control surface. Silanated PEGs provides a simple method for PEG grafting to the surface containing oxide layers.

Keywords: Poly(ethylene oxide); Silanated PEG; Surface grafting; Contact angle; X-ray photoelectron spectroscopy; Atomic force microscopy

1. Introduction

Materials in contact with biological fluids often develop a problem of biofouling resulting from protein adsorption and cell adhesion [1–5]. Surface modification using hydrophilic polymers has been shown to be effective in prevention of protein adsorption and cell adhesion [6–10]. Biomaterial surfaces have been modified with various molecules, such as albumin [11–13], heparin [14–20], poly(ethylene glycol) (PEG) [21–30], self-assembled monolayer (SAM) [24,31–35], and phospholipids [36–38]. Of these, PEG has been used most widely for surface modification because of its unique properties such as hydrophilicity, flexibility, high exclusion volume in water, nontoxicity, and nonimmunogenecity [39]. Methods for surface modification by PEG range from simple physical adsorption to chemical bond formation, such as chemical coupling [21,40–42] and graft polymerization [43,44]. Since the PEG on the surface should not be removed from the surface for long-term effect, covalent grafting is most preferred. One of the simplest approaches for PEG grafting is to introduce functional groups either to the surface or to the hydroxyl group of PEG for chemical reaction between PEG and the surface [21,45]. This approach, however, results in poor grafting efficiency when the molecular weight of PEG is larger than 800 Da [46]. This is probably due to the lack of contact between the end hydroxyl groups of PEG and the surface. PEG is in a random coil shape in bulk solution and the hydroxyl groups do not have any driving force to adsorb to the surface. Consequently the density of the

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grafted chains is usually low. Thus, in many cases the effect of PEG grafting is not fully realized due to the low density of the grafted PEG chains.

We explored the self-assembly of silane coupling agents to surfaces with oxide layers, such as Al_2O_3, SiO2, and glass, for covalent grafting of PEG [47–49]. This is similar to the self-assembling of oligo(ethylene glycol) alkanethiolates on gold surfaces [24,34]. We coupled alkoxyalkyl silane to monomethoxy-PEG (m-PEG) with a hydrophobic spacer to provide self-assembling and covalent grafting properties. Incorporation of a hydrophobic link between PEG and alkoxyalkyl silane is expected to generate extra stabilization of the grafted polymer chains through hydrophobic interactions among them. PEG grafting was characterized by contact angle, X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM), and protein adsorption.

2. Experimentals

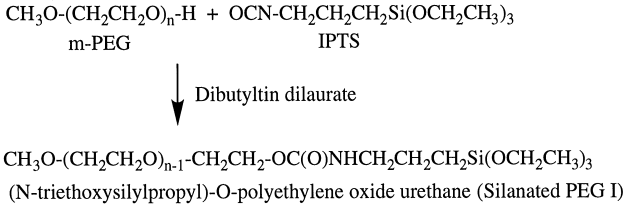
2.1. Materials

Monomethoxy poly(ethylene glycol) (m-PEG; MW 5000), 1,6-diisocyanatohexane (DIH), 10-undecen-1-ol, lithium aluminum hydride ($LiAlH_4$), dibutyltin dilaurate, azobisbutyronitrile (AIBN) phosphorous pentoxide, and anhydrous toluene were obtained from Aldrich Chemical Co. (Milwaukee, WI). Tetrahydrofuran (THF), hexane, benzene were purchased from Malinckrodt (Paris, KY). THF was purified by refluxing with lithium aluminum hydride overnight and distilling from $LiAlH_4$ to remove water and impurities. Distilled anhydrous THF were kept in a storage bottle and used in three days. 3-isocyanatopropyltriethoxysilane (IPTS; 95%), 3-mercaptopropyl trimethoxysilane, *n*-propyltrimethoxysilane (PTMS), and triethoxysilylpropyl ethylcarbamate (TESPEC) were purchased from United Chemical Technologies Inc. (Bristol, PA) and used without further purification. All other chemicals were of reagent grade and used without further purification.

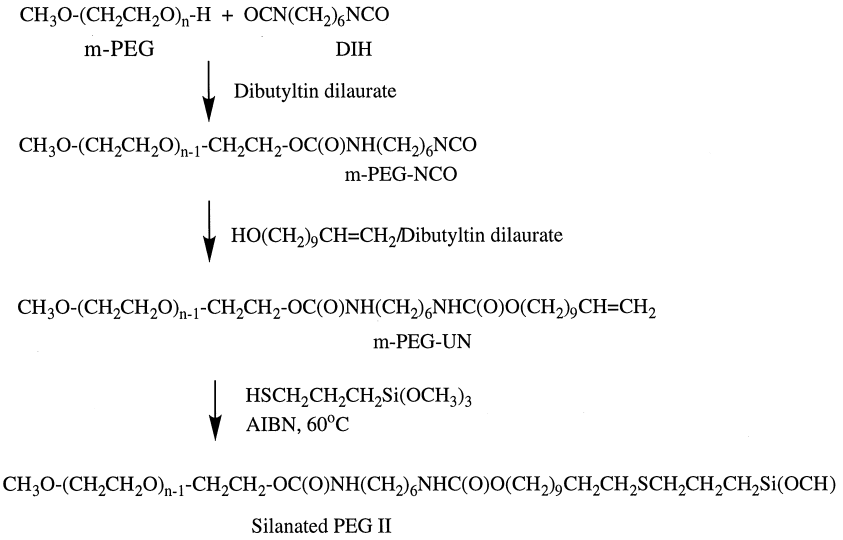
2.2. Synthesis of (N-triethoxysilylpropyl)-O-monomethoxy PEG urethane (Silanated PEG I)

Forty grams of m-PEG (0.008 mol) were dissolved in 250 ml of benzene. One hundred milliliters out of 250 ml of benzene were distilled off to remove water on PEG by forming azeotropic mixture. Residual benzene was further distilled out under reduced pressure. As shown in Scheme 1, triethoxysilylpropyl m-PEG carbamate (Silanated PEG I) was synthesized from m-PEG and 3-isocyanatopropyltriethoxysilane (IPTS) using dibutyltin dilaurate as a catalyst.

m-PEG solution (40 g of m-PEG in 200 ml of THF) was prepared in a 1000 ml three-necked, round-bottomed flask under dry nitrogen. IPTS and dibutyltin dilaurate,



Scheme 1. Synthesis of triethoxysilylpropyl m-PEG carbamate (Silanated PEG I) from m-PEG and 3-isocyanatopropyltriethoxysilane (IPTS) using dibutyltin dilaurate as a catalyst.



Scheme 2. Synthesis of *N*-{*O*-[11-(3-trimethoxysilylpropylthio) undecyl] *N*-*n*-hexyl carbamyl} *O*-monomethoxy PEG urethane (Silanated PEG II).

a catalyst, were added to the m-PEG solution. The molar ratios of IPTS and dibutyltin dilaurate to PEO were 2.5 and 0.1, respectively. After adding chemical reagents, the mixture was stirred continuously for 48 h under dry nitrogen. After the reaction, silanated PEG I was precipitated from THF with hexane twice, and dried in vacuo.

^{1}H-NMR ($CDCl_3$): δ 0.53 (t, $-CH_2Si$), 1.13 (t, $-OCH_2\underline{CH_3}$), 1.52 (m, $-CH_2\underline{CH_2}CH_2Si$), 3.20–3.90 (m, $-(\underline{CH_2}\,\underline{CH_2}O)_n-$), 4.11 (t, $-\underline{CH_2}OC(O)NH-$), 4.98 (br, $-OC(O)\underline{NH}-$).

Elemental analysis. Calculated for $C_{239}H_{481}N_1O_{119}Si_1$: C, 54.15; H, 9.14; N, 0.26; Si, 0.53. Found: C, 54.18; H, 9.26; N, 0.24; Si, 0.50.

2.3. Synthesis of N-[O-[11-(3-trimethoxysilylpropylthio) undecyl] N-n-hexyl carbamyl] O-monomethoxy PEG urethane (Silanated PEG II)

The synthetic route for the silanated PEG II is shown in Scheme 2. m-PEG and 1,6-diisocyanatohexane (DIH) were reacted to form isocyanato terminated m-PEG. To this was added a hydrophobic moiety, 10-undecen-1-ol, and finally silylated.

2.3.1. Preparation of N-(6-isocyanatohexyl) O-monomethoxy PEG urethane (m-PEG-NCO)

m-PEG-NCO was prepared by following the method used by Zalipsky et al. [50] with minor modification. Forty grams of m-PEG was dried by distillation of 250 ml of benzene. A 500 ml three necked, round bottomed flask was charged with 150 ml of THF and 5 times molar excess of DIH (0.040 mol) over m-PEG. Under nitrogen atmosphere, 400 ml of 10% m-PEG in THF and 0.5 ml of dibutyltin dilaurate were added dropwise to 1,6-diisocyanatohexane (DIH) solution. Reaction mixture was stirred magnetically for 16 h at room temperature. After the reaction, m-PEG-NCO was precipitated by adding *n*-hexane, and dried overnight in a vacuum oven in the presence of phosphorous pentoxide.

2.3.2. Preparation of N-[O-(10-undecenyl) N-n-hexyl carbamyl] O-monomethoxy urethane (m-PEG-UN)

Seven grams of m-PEG-NCO and 150 ml of THF were charged to a 250 ml round bottomed, three-necked flask. After adding 2.5 ml of 10-undecen-1-ol and 0.1 ml of dibutyltin dilaurate to the m-PEG-NCO solution, the reaction mixture was stirred overnight at room temperature. After the reaction, the product was precipitated with n-hexane, and purified further by recrystallization with ethanol.

2.3.3. Preparation of N-[O-[11-(3-trimethoxysilylpropylthio) undecyl] N-n-hexyl carbamyl] O-monomethoxy PEG urethane (Silanated PEG II)

Silylation of m-PEG-UN was carried out by free-radical addition of a thiol to a double bond [33,51,52]. Five grams m-PEG-UN, 1.5 ml of 3-mercaptopropyl trimethoxy silane, 0.041 g of azobisisobutyronitrile (AIBN), and 50 ml of benzene were charged to a 250 ml round bottomed flask. Reaction mixture was degassed by using a mechanical pump, and purged with dry nitrogen. The temperature of reaction solution was adjusted to 60–65°C using an oil bath, and kept for 1 d with magnetic stirring. The product was precipitated by anhydrous diethylether. Silanated PEG II was collected by filtration and extensively washed with large volume of anhydrous ether to remove residual AIBN and unreacted 3-mercaptopropyl trimethoxy silane.

^{1}H-NMR ($CDCl_3$): δ 0.68 (t, $-CH_2Si$), 0.9–2.2 (m, $-CH_2CH_2CH_2CH_2-$ from DIH and 10-undecen-1-ol), 3.40–3.60 (m, $-(\underline{CH_2}\,\underline{CH_2}O)_n-$), 3.94 and 4.12 (t, $-\underline{CH_2}OC(O)NH-$), 4.70 and 4.84 (br, $-OC(O)\underline{NH}-$).

Elemental analysis. Calculated for $C_{254}H_{510}N_2O_{121}S_1Si_1$: C, 54.59; H, 9.20; O, 34.64; N, 0.50; Si, 0.50. Found: C, 54.57; H, 9.51; N, 0.54; Si, 0.53.

2.4. PEG grafting

Glass coverslips (9 mm × 5 mm, no. 1 thickness; Bellco, Vineland, NJ) were cleaned by soaking in chromic acid overnight and washing extensively in running distilled water. They were further washed three times with deionized distilled water for 5 min each in an ultrasonic cleaner (Model 2200, Branson, Danbury CT). Clean glass coverslips were grafted with silanated PEGs and *n*-propyltrimethoxysilane (PTMS) under two different conditions. First, ethanol solutions of the silanated PEGs and PTMS were made by dissolving them in a 95 : 5 ethanol : water mixture and adjusting the pH of the final solution to pH 2.0 with concentrated HCl. The concentration of silanated PEGs was varied from 0.1 to 20 mg/ml.

In the second condition, PEG was grafted under anhydrous toluene by refluxing overnight at 70°C. After dipping glass substrates into 4 ml of silanated PEG solution in test tubes (16 mm × 100 mm) for 2 h, the coverslips were sequentially rinsed with ethanol and water three times each. The washed coverslips were then dried overnight at 70°C. Each coverslip was then washed by soaking in 5 ml of 1% SDS solution overnight and washed thoroughly with deionized distilled water in an ultrasonic bath. After the reaction, treated surfaces were cured for 1 d at 70°C.

2.5. Contact angle measurements

Advancing and receding contact angles of surface-modified coverslips were measured by the Wilhelmy plate method. These contact angle data were evaluated with the Autotensiomat® surface tension analyzer (Model 215, Fisher Scientific, Pittsburgh, PA) at a controlled speed of 2.54 cm/min at room temperature. Four samples were used for each modification.

2.6. X-ray photoelectron spectroscopy (XPS)

For the XPS experiment, circular glass coverslips (12 mm diameter, no. 1 thickness, Bellco, Vineland, NJ) were cleaned and grafted with silanated PEGs as described above. Silanated PEG in anhydrous toluene (10 mg/ml) was prepared separately and 5 ml of the polymer solution was added to a test tube (16 mm diameter × 100 mm length). After transferring glass substrate into the test tube, the solution was refluxed in an oil bath overnight at 80°C. After the reaction, glass coverslips were washed consecutively with chloroform, chloroform-methanol (1 : 2) and methanol for 5 min each. Then PEG-grafted glass was annealed at 70°C for 1 d. After grafting, modified glass was washed extensively as described above.

XPS analysis was performed on a Surface Science SSX-100 spectrometer (Mountain View, CA) equipped with a monochromatic Al K_α source, which permitted analysis of the outermost 100 Å of a sample in an elliptical area whose long axis can be adjusted from 150 to 1000 μm. The energy of the emitted electrons was measured with a hemispherical energy analyzer at pass energy of 50 eV for high resolution spectra and 150 eV for elemental quantification. The binding energy (BE) scale was referenced by setting the C–C peak maximum in the C 1s spectra of the samples to 285 eV. An electron flood gun set at 5 eV was used to minimize surface charging of the samples. Typical pressures in the analysis chamber during spectral acquisition were 10^{-9} Torr. All samples were analyzed at a photoelectron take-off angle of 55°. Angle-dependent XPS data were collected at 0, 55, and 80° corresponding to sampling depths of 90, 50, and 20 Å, respectively.

2.7. Atomic force microscopy (AFM)

PEG-grafted glass surfaces were visualized by an atomic force microscope (Nanoscope III; Digital Instrument, Santa Barbara, CA). Images were obtained by scanning surface for 10 min in a tapping mode using a single crystal silicon probe (model TESP). For hydration studies, modified coverslips were hydrated in a liquid cell for 30 min using deionized distilled water. After hydration, surface was observed in a tapping mode using a silicon nitride probe (model DNP). The spring constant was 0.58 N/m.

2.8. Fibrinogen adsorption on to surfaces

Glass tubes (1.5 mm diameter × 100 mm length; Kimble, Vineland, NJ) were cleaned by soaking in chromic acid overnight and washing extensively in running distilled water. They were further washed three times with deionized distilled water for 10 min in an ultrasonic cleaner. Silanated PEGs and two silanes, PTMS and TESPEC, were used to modify inner surface of glass tubes. Surface modifying solutions were made by dissolving the silanes in an ethanol : water (95 : 5) solution and adjusting the pH of the final solution to 2.0 with concentrated HCl. The tubes were first filled with the 95% ethanol : water solution using a syringe. The solution was replaced with silanated PEG solution (at the concentration of 10 mg/ml). After 2 h of grafting, the tubes were rinsed with alcohol and water three times each. The washed tubes were then dried in vacuo for 1 h at 50°C. Each tube was then washed by soaking in 1% SDS solution overnight and rinsed extensively with deionized distilled water.

Protein adsorption was studied by following the method used by Tseng et al. [27] with a minor modification. Briefly, fibrinogen (Sigma type I, St. Louis, MO) was purified by the Laki method [53] and labeled with ^{125}I (Amersham, Arlington Heights, IL) using Enzymobead reagent (Bio-Rad, Rockville Center, NY). Fibrinogen solution at the concentration of 0.15 mg/ml was prepared by mixing one part of ^{125}I-labeled fibrinogen with nine part of non-labeled fibrinogen. The fibrinogen was allowed to adsorb onto the surfaces for 1 h at room temperature, and then surfaces were rinsed with phosphate buffered saline (PBS; pH 7.4). The surface fibrinogen concentration was determined by measuring the radioactivity of ^{125}I-labeled fibrinogen using a gamma counter (Gamma 5500B, Beckman, Arlington Heights, IL). Four samples were used for the calculation of the surface fibrinogen concentration.

3. Results

Silanated PEGs were synthesized by coupling silanes to PEG through urethane bonds. Silanated PEG I was obtained by direct coupling of 3-isocyanatopropyltriethoxysilane to m-PEG. Silanated PEG II was prepared by inserting a hydrophobic domain with 19 carbon length between hydrophilic PEG and silane by attaching DIH and 10-undecen-1-ol through urethane bonds. Proton NMR spectrum of silanated PEG I showed a triplet peak at 4.11 ppm, which was assigned to methylene protons next to urethane bonds. When m-PEG was incorporated with hydrophobic linkers through urethane bonds, triplets from methylene protons next to urethane bond characteristically showed up at 3.95 and 4.13 ppm. Coupling of silanes was supported by triplets at 0.53 and 0.68 ppm for silanated PEG I and II, respectively. Those peaks were from methylene protons next to triethoxysily and trimethoxysilyl, respectively. In the case of silanated PEG I, the triplet from methylene next to triethoxysilyl group shifted 0.07 ppm to upfield with the formation of a urethane bond. The NMR spectrum of silanated PEG II exhibits a presence of hydrophobic spacer by showing broad methylene proton peaks of DIH and 10-undecen-1-ol ranging from 0.9 to 2.2 ppm. Two urethane bonds

Table 1
Advancing (θ_a) and receding (θ_r) contact angles on glass surfaces modified with *n*-propyltrimethoxysilane, silanated PEG I, and silanated PEG II[a]

Concentration (mg/ml) used for grafting	Contact angle	
	θ_a	θ_r
n-Propyltrimethoxysilane		
1.0	60.3 ± 5.9	38.6 ± 3.0
5.0	102.4 ± 3.1	65.1 ± 2.1
10.0	105.9 ± 1.0	69.2 ± 3.2
Silanated PEG I		
0.1	30.0 ± 2.2	17.9 ± 2.4
1.0	27.2 ± 1.7	12.9 ± 5.2
5.0	23.4 ± 2.9	9.6 ± 2.1
10.0	32.3 ± 2.9	18.5 ± 2.0
20.0	28.5 ± 5.4	15.1 ± 1.6
Silanated PEG II		
0.1	43.0 ± 0.6	20.1 ± 3.8
1.0	45.5 ± 2.9	18.4 ± 2.9
5.0	46.1 ± 1.1	21.8 ± 3.5
10.0	49.3 ± 0.6	24.4 ± 2.5
20.0	49.8 ± 2.3	22.9 ± 2.3

[a]Average ± standard deviation ($n = 4$).

for the extension of the hydrophobic spacer were also identified by proton peaks at 4.70 and 4.84 ppm.

The contact angles on the PEG-grafted surfaces are shown in Table 1. Glass surfaces modified with *n*-propyltrimethoxysilane (PTMS) showed advancing contact angles of 60 and up to more than 100°. The receding contact angles were between 40° and 70° depending on the concentration of PTMS used. When the slanated PEG was grafted to glass, contact angles were much less than those observed on PTMS-modified glass. Both advancing and receding angles were smaller on the glass surfaces grafted with silanated PEG I than those with silanated PEG II. Advancing and receding contact angles of PEG I-grafted glass surfaces ranged from 23.4 to 32.3° and from 9.6 to 18.5°, respectively. On the other hand, those for PEG II-grafted glass surfaces ranged from 43.0 to 49.8° and from 18.4 to 24.4°, respectively. The presence of a hydrophobic part in silanated PEG II resulted in higher contact angles compared to those on the PEG I-grafted surfaces. For both silanated PEGs, however, there were no significant changes in contact angles as the bulk concentration of silanated PEGs used for grafting increased from 0.1 to 20 mg/ml. The increase in contact angle by silanated PEG treatment indicates that PEG was grafted successfully onto glass.

Table 2 summarizes atomic concentrations of surfaces grafted with silanated PEGs in two different conditions. The main surface components were Si, O, and C. PEG grafting decreased surface silicon concentration from 28.4 to less than 20%. PEG grafting also decreased

Table 2
Elemental composition of glass modified with silanated PEGs[a]

Sample	% Composition				
	Si	O	C	N	Other atoms
Control glass	28.3	54.2	12.2	—	5.3
Silanated PEGI in 95% EtOH	18.0	39.4	35.1	0.3	7.2
Silanated PEG II in 95% EtOH	19.4	40.8	33.7	1.1	5.8
Silanated PEG I in toluene	19.0	51.3	23.3	0.6	5.8
Silanated PEG II in toluene	19.4	42.1	31.0	1.0	6.5

[a]The concentration for silanated PEG grafting was 10 mg/ml.

Table 3
High resolution XPS C1s composition at a 55° take-off angle[a]

Sample	Composition (%)			
	CH_2	C–O	O–C(O)–NH	C–O/CH_2
Control glass	84	11	5	0.13
Silanated PEG I in 95% EtOH	40	58	2	1.45
Silanated PEG II in 95% EtOH	56	41	3	0.73
Silanated PEG I in toluene	11	82	6	7.45
Silanated PEG II in toluene	40	56	4	1.4

[a]The binding energy of hydrocarbon was corrected to 285.0 eV and the binding energy of ether carbon was resolved into 286.8 eV. The concentration for silanated PEG grafting was 10 mg/ml.

surface oxygen concentration. On the other hand, carbon concentration increased from 12.2 to more than 30% except for surfaces grafted with silanated PEG I in toluene. The presence of carbon on the control glass is most likely due to the adsorption of hydrocarbon species from the atmosphere [54–57]. PEG has a nominal composition of 67 atomic percent carbon and 33 atomic percent oxygen, while glass has a nominal composition of 67 atomic percent oxygen and 33 atomic percent silicon. Thus, the decrease in silicon and oxygen concentrations as well as the increase in carbon concentration observed by the grafting of silanated PEGs is consistent with the presence of PEG on the surfaces. Although the changes in atomic concentration were in the direction expected for the grafting of PEG, the extents of changes in the silicon and oxygen concentration were rather small. Only slight change in the surface silicon concentration may indicate that the surface coverage by the grafted PEG was incomplete. The high resolution C1s spectra showed that PEG grafting increased the percent concentration of ether carbon coming from PEG, while the percent concentration of hydrocarbon decreased (Table 3). Theoretical values of the C–O/CH_2 ratio are 97 and 13 for

Table 4
Angular dependent elemental composition of glass modified with silanated PEG I in 95% ethanol and with silanated PEG II in anhydrous toluene[a]

Take-off angle	Sampling depth (Å)	% Composition			
		Si	O	C	N
Silanated PEG I					
0	90	22.9	52.5	24.4	0.3
55	50	21.1	45.1	33.5	0.3
80	20	19.7	31.6	48.5	0.2
Silanated PEG II					
0	90	20.3	54.4	24.2	1.2
55	50	17.0	44.2	37.0	1.5
80	20	7.0	29.9	60.1	2.6

[a]The concentration for silanated PEG grafting was 10 mg/ml.

silanated PEG I and II, respectively. Table 3 showed a big difference between theoretical and experimental values of C–O/CH_2 ratio. Even though silanated PEG grafting in anhydrous toluene showed larger values of carbon ratio than grafting in the presence of water, experimental values clearly suggest that the surface coverage was incomplete. Surface modification with silanated PEG I resulted in higher concentration of ether carbon than the modification with silanated PEG II because of higher ratio of ether carbon to hydrocarbon. Angle-dependent elemental composition was calculated for the surface modified with silanated PEG I in the presence of water (Table 4). Grafting of silanated PEG I resulted in decreased oxygen concentration and increased carbon concentration as the take-off angle increased. The silicone concentration decreased only slightly. This also indicates that the surface coverage was not complete. The formation of complete PEG layer on top of glass would have decreased silicon concentration dramatically with the increase of take-off angle and silicon atom would have disappeared when sampling depth is equal to thickness of PEG layer. In the case of silanated PEG II grafting in anhydrous toluene, silicon concentration decreased rather substantially in comparison with the grafting of silanated PEG I in 95% ethanol (Table 4). As sampling depth decreased from 9 to 2 nm, silicon concentration decreased from 20.3 to 7.0%. On the other hand, carbon concentration increased from 24.2 to 60.1%. This result showed silanated PEG II grafting in anhydrous toluene formed a PEG layer of approximately 2 nm thickness in the dries state.

The surface topography of PEG-grafted surfaces was examined by AFM. The control glass surface was rough with a large number of small peaks and valleys (Fig. 1A). Panels B–E in Fig. 1 show images of the surface grafted with different concentrations of silanated PEG I. As the bulk concentration of silanated PEG I for grafting increased, the surface became relatively smoother with occasional high peaks of PEG aggregates. The image analysis of Fig. 1B showed that the average peak height was about 1 nm. The height in Fig. 1C ranged from 2 to 5 nm. When 5 mg/ml of silanated PEG I was used (Fig. 1D), substantial number of peaks were higher than 2 nm. This may be due to the higher degree of oligomerization with the increase in bulk silanated PEG I concentration. At 10 mg/ml concentration (Fig. 1E), the surface topography was not much different from the grafting at 5 mg/ml. Grafting of PEG I in anhydrous toluene (Fig. 1F) resulted in smoother surface compared with grafting in 95% ethanol (Fig. 1E). The AFM images of surfaces grafted with silanated PEG II were much different from those in Fig. 1. In general, as shown in Fig. 2, the surface was much rougher than those grafted with silanated PEG I. The size of each PEG peak was much larger than that seen in Fig. 1. Even at the bulk concentration of only 0.1 mg/ml of silanated PEG II, the surface was covered with PEG of 4 nm height (Fig. 2B). As the polymer concentration increased to 1 mg/ml, the grafted PEG peaks were as high as 4 nm (Fig. 1C). More PEG grafting was clear as the bulk concentration of silanated PEG II increased to 5 mg/ml and above (Fig. 2D and E). PEG aggregates were clearly shown and each aggregate was about 150 nm wide and 10 nm high. Comparison of Figs. 1 and 2 show that PEG II resulted in larger aggregates on the surface than those by PEG I. Grafting of PEG II in anhydrous toluene also resulted in much smoother surface (Fig. 2F) compared with the grafting in 95% ethanol (Fig. 2E). When the PEG-grafted surfaces were exposed to water, the grafted PEG layer swelled significantly as shown by the increase in height (Fig. 3). Surfaces grafted with silanated PEG I and II in 95% ethanol resulted in rough surfaces even after swelling in water (Fig. 3A and B). Numerous PEG peaks were apparent and the size of the PEG peaks was substantially larger for PEG II. When silanated PEG was grafted in anhydrous toluene, however, the surface appeared relatively smoother after hydration in water (Fig. 3C and D). This is probably due to the minimized aggregation of silanated PEG's in bulk solution before grafting to the surface.

The effect of grafted PEG on prevention of protein adsorption was examined using fibrinogen as a model protein. On control glass, the surface fibrinogen concentration was 0.47 μg/cm^2. The surface fibrinogen concentration became 0.60 and 0.40 μg/cm^2 when the surface was modified with PTMS and TESPEC, respectively. The surface fibrinogen concentration for a monolayer coverage is 0.4 and 1.7 μg/cm^2 for side-on and end-on conformations [58]. Thus, the values obtained for the control and the modified surfaces represent monolayer fibrinogen adsorption. The surface fibrinogen concentration was reduced to 0.01 and 0.02 μg/cm^2 after grafting

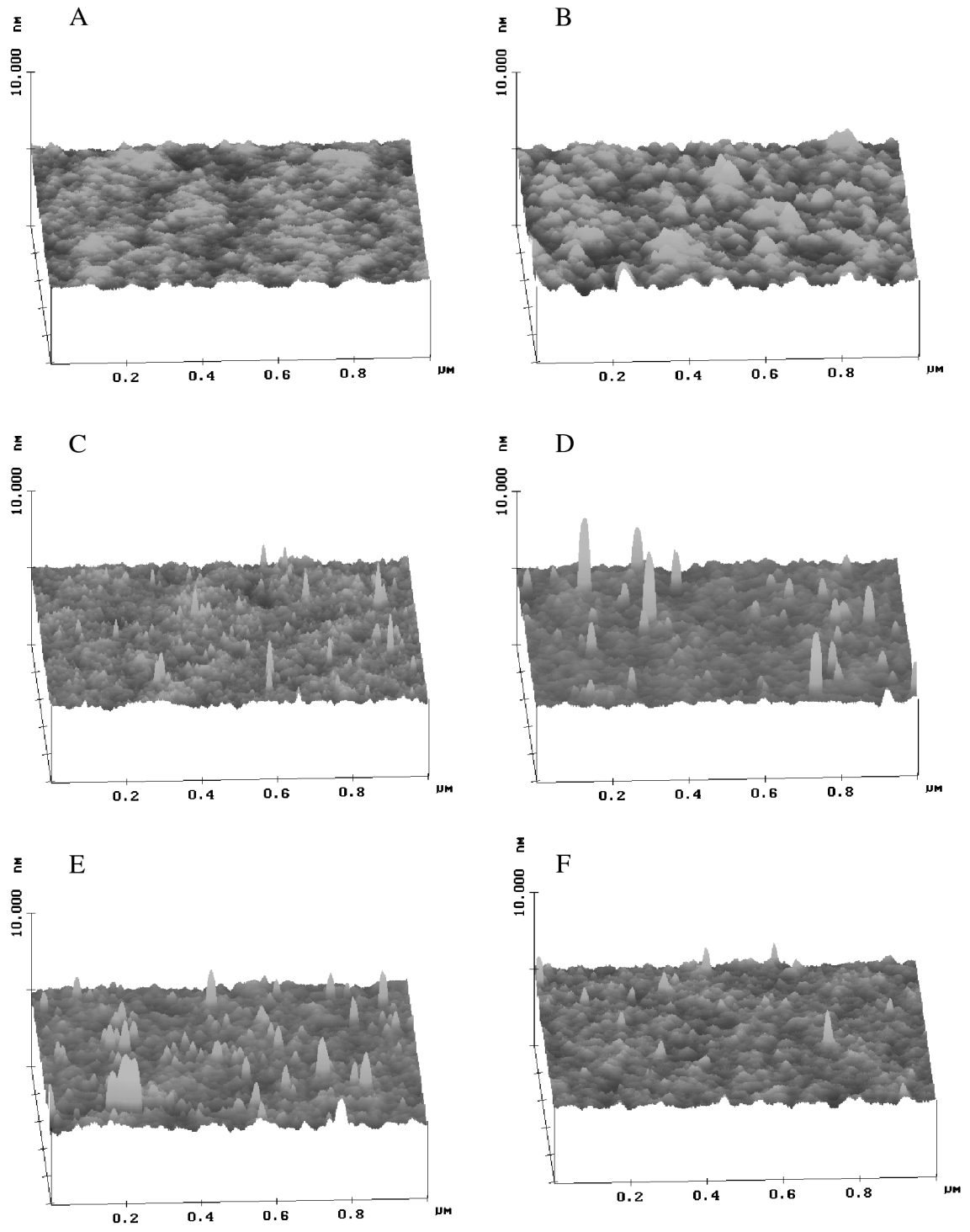


Fig. 1. AFM images of glass surfaces modified with silanated PEG I at different bulk concentrations. The bulk concentration of silanated PEG I was 0 mg/ml (A), 0.1 mg/ml (B), 1 mg/ml (C), 5 mg/ml (D), and 10 mg/ml (E) and (F). Silanated PEG I was grafted either in 95% ethanol (B)–(E) or in anhydrous toluene (F).

with silanated PEG I and II, respectively (Fig. 4). This is almost complete prevention of fibrinogen adsorption by the grafted PEG. It is interesting to note that surface grafted PEG I was as effective as PEG II in prevention of fibrinogen adsorption, despite lower surface coverage. Thus, it appears that the grafted PEG can prevent protein adsorption as long as the PEG layer can cover the entire surface in the hydrated state.

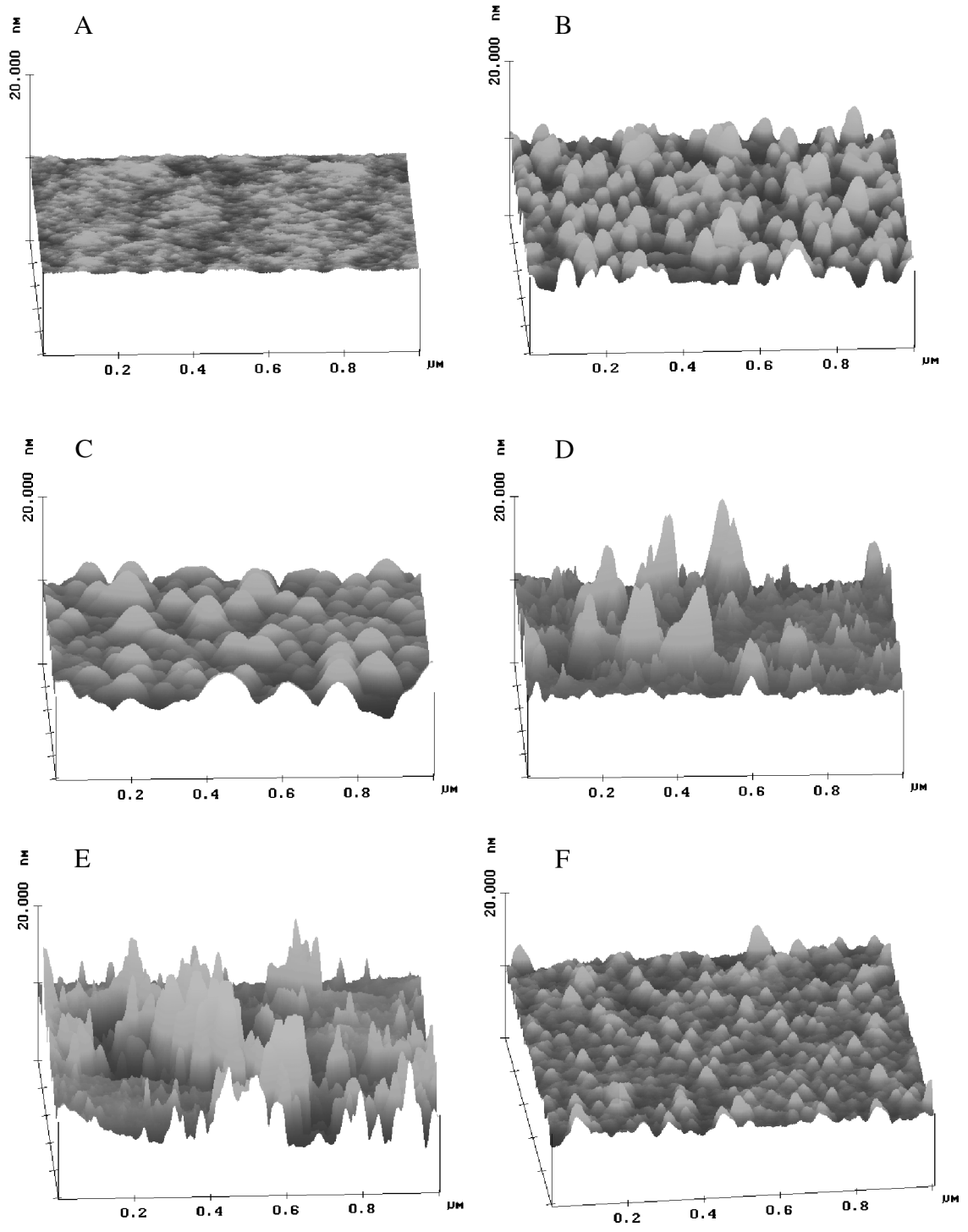


Fig. 2. AFM images of glass surfaces modified with silanated PEG II at different bulk concentrations. The bulk concentration of silanated PEG II was 0 mg/ml (A), 0.1 mg/ml (B), 1 mg/ml (C), 5 mg/ml (D), and 10 mg/ml (E) and (F). Silanated PEG I was grafted either in 95% ethanol (B)–(E) or in anhydrous toluene (F).

4. Discussion

Silanes with functional groups, such as amine, allyl, chloro, bromine, isocyanate, or mercapto group, are commercially available from Aldrich Chemical Co. (Milwaukee, WI) and United Chemical Technologies (Bristol, PA). A few silane PEGs, such as (*N*-triethoxysilylpropyl)-*O*-polyethylene oxide urethane (United Chemical Technologies, Bristol, PA) and silane PEG with a urea bond (Shearwater Polymers Inc., Huntsville, AL),

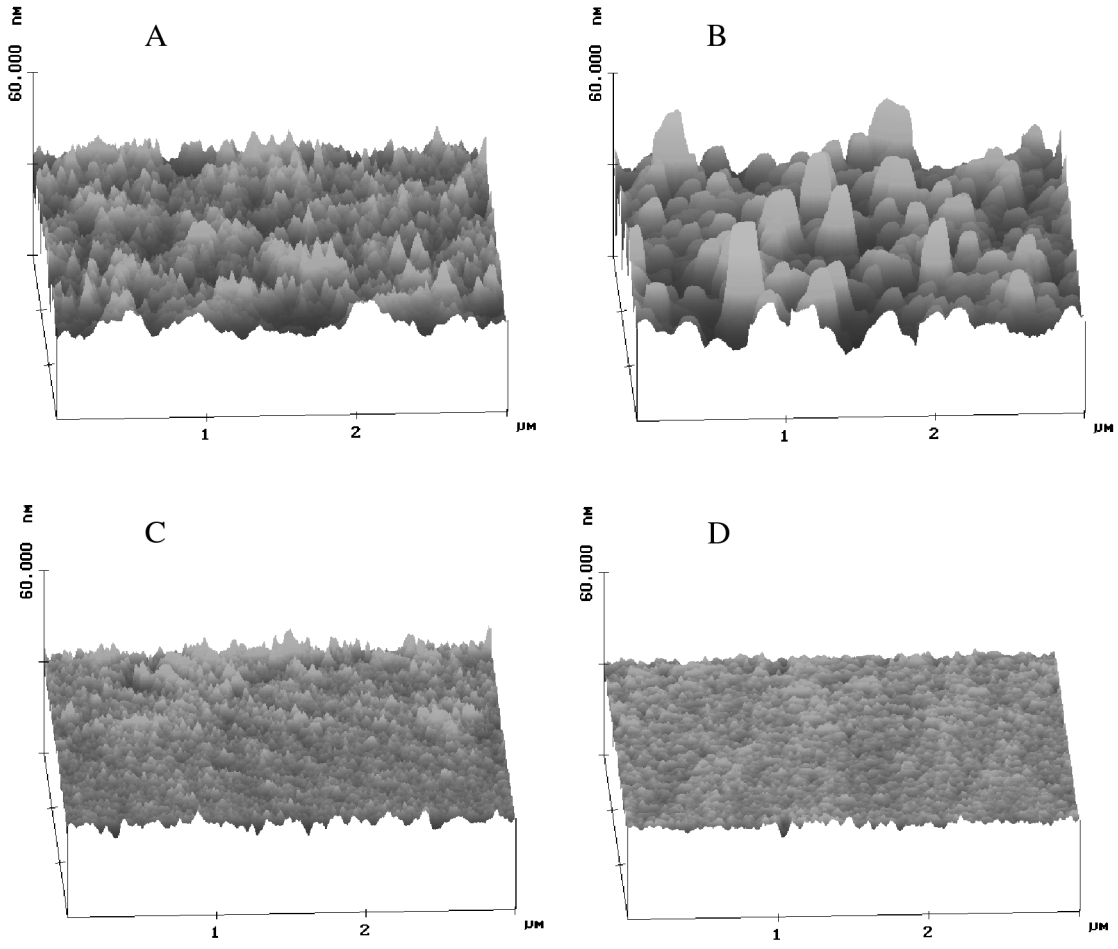


Fig. 3. AFM images of PEG-grafted glass surfaces after hydration for 30 min in a liquid cell of AFM. Silanated PEG I (A) and (C) and PEG II (B) and (D) were grafted at the bulk concentration of 10 mg/ml. Silanated PEGs were grafted either in 95% ethanol (A) and (B) or in anhydrous toluene (C) and (D).

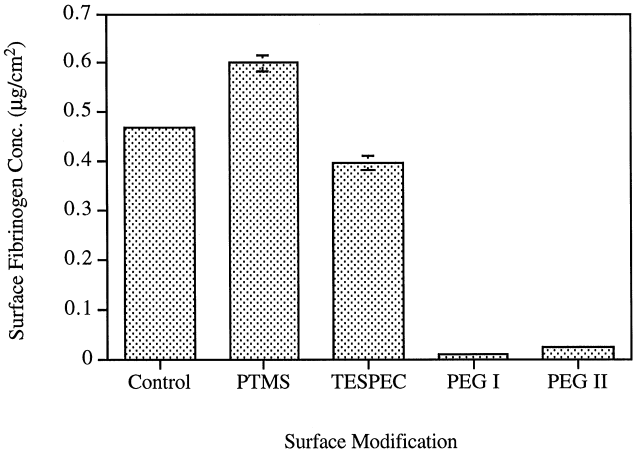


Fig. 4. Fibrinogen adsorption on control and PEG-grafted glass surfaces. Clean glass (Control) and glass modified with *n*-propyltrimethoxysilane (PTMS) or triethoxysilylpropyl ethylcarbamate (TESPEC) were used controls. The surface fibrinogen concentrations on glass surfaces grafted with silanated PEG I (PEG I) and silanated PEG II (PEG II) were 0.01 and 0.03 ± 0.01 μg/cm^2.

are also commercially available. Those modified PEGs have been grafted to the surface to reduce electroosmosis in electrophoretic process and to improve the efficiency of capillary electrophoresis [41,59]. Silanated PEG II synthesized in our study is rather unique because of its hydrophobic spacer. Design of silanated PEG with long hydrophobic chain was based on the formation of self-assembled monolayer by oligo(ethylene glycol) alkanethiolates on gold surfaces and by alkyl silanes on metal surfaces. In our synthesis of silanated PEG, urethane bond was used because of its stability in hydration. To attach rather long hydrophobic chain between PEG and silane, ω-alkenol of large carbon number was used after the formation of PEG-NCO with diisocyatohexane. Various diisocyanates, difunctional alkane such as diaminododecane, and ω-alkenol such as 10-undecen-1-ol can be utilized for the preparation of silanated PEG. The addition of silane to double bond was performed by free-radical addition of thiol [52]. This synthetic process can also be applied for the silylation of other hydrophilic polymers with a functional group, such

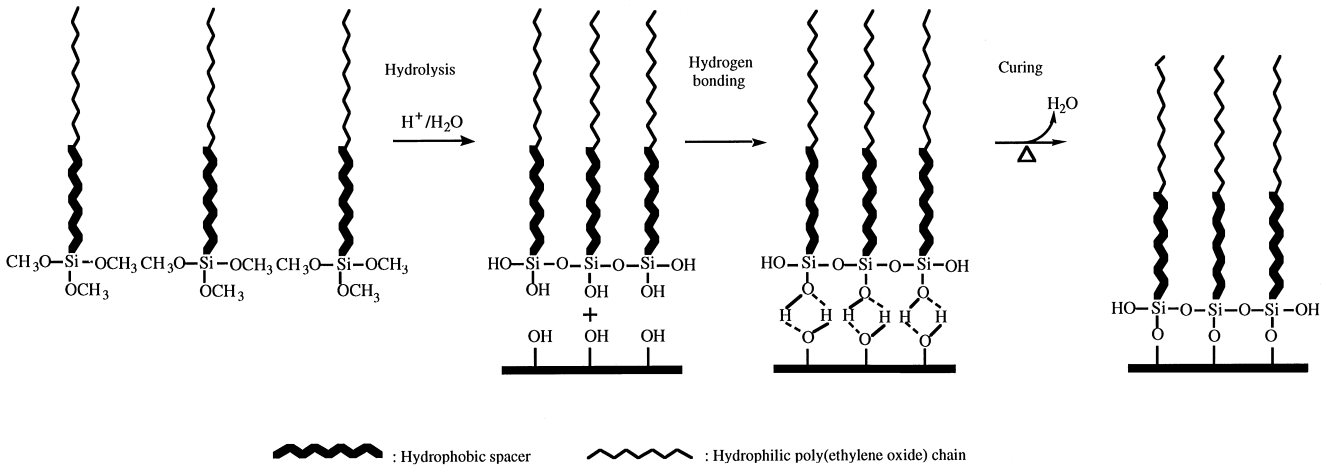


Fig. 5. Proposed mechanism of ideal grafting of silanated PEG II to glass surfaces.

as amine or hydroxyl group. Hydroxy-terminated polyvinylpyrrolidone, poly(vinyl alcohol), and poly(hydroxyethyl methacrylate) can be used to prepare silanated hydrophilic polymers with a minor modification of synthetic route for silanated PEG II. In the case of polymers with multifunctionality, diisocyanato alkane should be reacted with ω-alkenol first. The use of 1,4-diisocyantobenzene would result in the silanated polymers with a bulky hydrophobic spacer. The bulky hydrophobic spacer would facilitate the formation of organized monolayer on the surface.

Silanated PEGs were covalently grafted to the surfaces with oxide layers. Fig. 5 shows a possible mechanism for grafting of silanated PEGs onto surfaces such as glass in the presence of water in the bulk solution. The mechanism shown in Fig. 5 is for the ideal grafting, and in reality, silanated PEGs are expected to form aggregates in the bulk solution, especially in the presence of water in the bulk solution. The hydrolyzable moieties (i.e., methoxy groups) of silanated PEO are hydrolyzed in aqueous solution by acid catalysis. Such hydrolysis is expected to result in oligomerization of silanated PEO in the bulk solution before the remaining silanol groups form hydrogen bonds with hydroxyl groups of oxide layers. Curing at an elevated temperature leads to dehydration at the surface and formation of covalent bonds between silanated PEO and the surface silanol groups. The oligomerization of silanated PEGs in bulk solution may result in patchwise surface coverage due to the steric hindrance by the grafted PEGs. The incomplete surface coverage is expected to be more pronounced with silanated PEG II, since it may form larger oligomers by hydrophobic interaction between hydrophobic segments of the polymers. Even though the bulk concentration of silanated PEG II was increased to 10 mg/ml, the surface was still not completely covered by the grafted PEG. Since the oligomerization of silanated PEG in the bulk solution was thought to cause incomplete surface coverage, PEG grafting in anhydrous condition was expected to result in more homogeneous and better surface coverage. As shown in Fig. 3, PEG grafting under anhydrous condition resulted in more homogeneous surface compared with grafting in 95% ethanol. Therefore the controlled grafting of silanated PEG-II in an anhydrous condition may achieve an organized PEG layer after optimizing the lengths of PEG and hydrophobic spacer in silanated PEG-II.

The surface wettability of the PEG-grafted surfaces was reduced compared with control glass as indicated by the increase in contact angle (Table 1). It is common to observe increase in contact angle to more than 30° after PEG modification of the surface [21,23,24,33, 42–44,60,61]. Contact angle of surfaces modified with PEG was as high as 90° [61] depending on the nature of the substrate. While the extent of increase in contact angle depends on the nature of the grafted PEGs, the surface modification step itself makes the surface more hydrophobic. The extent of surface modification and the molecular weight of the grafted PEG [21] also affect the change in surface hydrophilicity. Thus, if the surface coverage by the grafted PEG is not high, the PEG grafting process itself may result in more hydrophobic surface, which in turn adsorb more protein adsorption. For this reason, it is important to make sure that the surface concentration of the grafted PEG is high enough. Since it is not easy to measure the exact surface concentration of the grafted PEG, alternative test, such as prevention of fibrinogen adsorption, should be done to make sure the enough PEG molecules are grafted to the surface.

The incomplete surface PEG coverage observed in the dried state and the increase in contact angles by PEG grafting deserve further discussion. Both of these factors should not be used against the efficacy of the grafted PEG in prevention of protein adsorption. When the

PEG-grafted surface is exposed to aqueous solution, the grafted PEG molecules swell to present more surface coverage (Fig. 3), even though the surface is less covered in the dry state (Figs. 1 and 2). The more surface coverage by the hydrated PEG layer can effectively prevent protein adsorption as a result of high chain flexibility and it is not surprising to observe effective prevention of fibrinogen by the surface-grafted PEG as seen in Fig. 4. Grafting of PEG to glass using silanated PEG is simple and quick in the presence of water and acid (catalyst). The surface grafted with silanated PEG was rather inhomogeneous but effective enough to prevent protein adsorption in the hydrated state. The surface concentration of the grafted PEG may be controlled by adjusting polymer concentration and/or reaction time under anhydrous grafting.

The use of silanated polymers for surface modification may have advantages over using other conventional methods. Silanated polymers can simplify modification procedure with fewer steps. Unlike conventional polymer grafting that required multistep heterogeneous reactions, the application of silylated polymer can accomplish the grafting with one step reaction in 95% ethanol with catalyst or anhydrous refluxing. Silanated polymers can be used to graft on various substrates with oxide layers, such as silicates, silicon wafer, ceramic, aluminum [62], stainless steel [63,64], and NiTi alloy [65,66]. Since these materials are commonly used as biomaterials, PEG grafting with silanated PEG may present a useful new method for surface modification for improved biocompatibility.

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