Polyethylene glycol–coated biocompatible surfaces

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Abstract: Surfaces covered with polyethylene glycol (PEG; $HO–(CH_2–CH_2–O)_n–H$) have been shown to be biocompatible because PEG's properties yield nonimmunogenicity, nonantigenicity, and protein rejection. To produce a biocompatible surface coating, we have developed a method for grafting PEG onto activated silica films. We first deposited an amorphous silica film by plasma-enhanced chemical vapor deposition from SiH_4 and O_2 gases, which provided the flexibility to coat diverse materials with different chemistries and shapes. The silica films were activated by exposure to water plasma, increasing the number of silanol groups (Si–OH) on their surface. The surface silanol groups were then chemically reacted with the hydroxyl end of PEG to form an ester bond, Si–O–C, and to cover the surface with PEG. The surface reactions were monitored using attenuated total reflection Fourier transform infrared spectroscopy. The vibrational absorption bands of the C–O and $–CH_2$ bonds increased with time and saturated, indicating that PEG was adsorbed to saturation coverage on the surface. Simultaneously, the Si–OH absorption band decreased, showing that the surface silanols reacted with PEG and were depleted. The PEG-covered surfaces were physically characterized by atomic force microscopy, Auger electron spectroscopy, ellipsometry, and contact angle measurements. These characterization techniques provided additional evidence for the existence of chemically bonded PEG on the surfaces. Efficacy of protein rejection on PEG-covered surfaces was studied through measurements of the fluorescence intensity of Texas red–labeled bovine serum albumin brought in contact with such surfaces in solution. Significantly less protein adsorption was observed on surfaces covered with PEG compared to uncovered surfaces. © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 51, 343–351, 2000.

Key words: polyethylene glycol; silica, silicon oxide; chemical grafting of PEG; biocompatible surface; biocompatible coating

INTRODUCTION

The ability to design, fabricate, and optimize surfaces tailored for a particular biological application is of crucial importance to the synthesis of biocompatible materials.[1] For example, one of the more difficult problems in bioengineering science is finding versatile materials to be used in constructing artificial parts that are able to reproduce the properties and functionality of human tissues and organs.[2] Biomaterials that are in demand include materials for temporary and long-term therapeutic devices, prostheses for bone, skin, joints, cartilage, teeth, heart valves, blood vessels, and contact and intraocular lenses, and encapsulation for drug delivery systems.[3–5] Although efforts devoted in the past to developing biomaterials have resulted in recovery of specific functions or prolongation of life for many patients, successful applications are limited in scope, and there are many problems yet to be solved. One of the major problems is the undesired interactions of foreign materials introduced into the body with the immune system (e.g., leukocytes, phagocytes) and biomolecules such as lipids, proteins, fats, and enzymes. This problem is generally referred as bioincompatibility.[6,7]

The two most desirable characteristics of prosthetic devices are precise medicofunctionality and excellent biocompatibility. The former characteristic defines the performance or function of the device needed to realize a medical aim efficiently. The second characteristic is essential for any prosthesis that comes into contact with the human body and specifically with the immune system. When any foreign material is introduced into an organism, disturbances are generated at the contacting interfaces. Examples of these disturbances are blood coagulation, cytotoxic effects, immune response deficiency, injuries of biological membranes, and tissue damage. Furthermore, the device

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itself can suffer deterioration such as chemical attack, physical deformation, and mechanical dysfunction.[8]

One of the most successful approaches to producing surfaces that are able to resist protein adhesion and biological attack has been to use polyethylene glycol (PEG) as a surface protector. For example, it has been found that grafting PEG to solid surfaces reduces protein adsorption and cell adhesion.[9–13] Furthermore, it was demonstrated both *in vitro* and *in vivo* that PEG coatings suppress platelet adhesion, leading to reduced risk of thrombus formation, tissue damage, and other cytotoxic effects.[14] Others have found that some substances covered with a PEG coating do not show antigenic activity.[11–13] PEG is unusually effective at excluding other polymers from its presence in aqueous solution. This property is thought to be directly related to its ability to repel proteins.[15] PEG forms two-phase systems with other polymers, it is nontoxic, it exhibits immunogenicity and nonantigenicity, and it does not harm active proteins or cells even when it interacts with them directly.[9] In addition, it attaches to surfaces with very little effect on their chemistry; it is soluble in water and most organic solvents such as toluene and chloroform, and it increases the solubility of large molecules irrespective of their size. Furthermore, molecules and surfaces coupled or coated with PEG often gain the favorable characteristics mentioned above. For example, PEG-coated surfaces become hydrophilic and protein rejecting.[16]

The inert character of PEG is based on its molecular conformation in aqueous solution, where PEG exposes uncharged hydrophilic groups and shows very high surface mobility (steric exclusion).[10,12] The solubility of PEG in water and some other polar compounds is due to the fact that it has a similar molecular structure to water and can participate in strong hydrogen bonding with the oxygen in ethers and hydrogens in water.[15] The ability of a PEG-coated surface to prevent proteins and other biomolecules from approaching the surface is thought to be further enhanced by a steric stabilization force.[8,17] There are two main contributions to this repulsive force: an excluded volume component and a mixing interaction component.[18,19] The former is an elastic response from the loss of conformation entropy. When a protein gets close to a PEG-covered surface, the available volume for each polymer segment is reduced, and consequently, a repulsive force is developed owing to loss of conformational freedom of the PEG chains. The second is the osmotic interaction between the protein and the PEG-covered surface. In this case, the number of available conformations of the PEG segments is reduced owing to either compression or interpenetration of the protein chains generating an osmotic repulsive force.[8,18] Whether the dominant effect is compression or interpenetration depends on the grafting density of PEG on the surface.[10] Compression is preferred for dense grafting.[19] On the other hand, interpenetration is likely to dominate at low grafting densities. Therefore, the functionality of PEG as a surface protector is interpreted as biocompatibility and PEG can be used for "camouflaging" drugs, implants, artificial medical devices, medical instruments, etc.[9]

The term "biocompatible surfaces" is used to define surfaces that are introduced in the human body without causing any allergic or rejective reactions. To produce such surfaces, it is necessary to form a permanent chemical bond between a material surface (substrate) and PEG. If PEG is only physically absorbed, it will eventually be removed by biofluids because it is soluble in water and in a great variety of organic solvents.[9]

Previous attempts at grafting PEG on surfaces of materials involved two or more reaction steps. One of the earliest works in this field was presented by Bückmann et al.,[20] who prepared PEG ligands with bromide, amine sulfonate and *N*-hydroxysuccinimide. Zalipsky et al.[21] described the synthesis of amino-, isocyanato-, and carboxylated PEG for attachment to drugs. Gombotz et al.[22] reported the covalent immobilization of high-molecular-weight polyethylene glycol on polyethylene terephthalate (PET) films. The PET films were produced by using a radiofrequency glow discharge polymer deposition process, and subsequently modified by introducing amino or hydroxyl groups with plasma-chemical coupling of allyl alcohol or allyl amine compounds. Finally, those surfaces were activated with cyanuric chloride and reacted with bisaminopolyethylene glycol. As a final example, Lea et al.[23] tried to react and coat aldehyde terminated monomethoxy-PEG on silicon nitride films. In that case, they used a silicon nitride surface that had been activated with 3-aminopropyltriethoxisilane. All of the above schemes used to attach PEG to surfaces involve either the functionalized derivatives of PEG (e.g., cyanuric chloride-activated PEG or PEG-silyl reagent) or functionalized substrate surfaces (e.g., silica sol with an amino reagent or an alkyl silylation reagent) in two-step reactions or more.[12,13,18,20,24] Moreover, these methods have a considerable disadvantage in that they work for only specific substrate materials and reactive schemes.

Our goal was to develop a general surface coating procedure that could be used with a large class of materials to produce biocompatible interfaces between body fluids and materials such as polymers, ceramics, and metals which may be used in artificial implants. In this article we demonstrate a versatile approach that allows the grafting of PEG on water-plasma "activated" silica films through an Si–O–C ester linkage. Silica films are produced by plasma-enhanced chemical vapor deposition (PECVD), which provides flexibility, because silica can be homogeneously deposited on many different materials (e.g.,

ceramics, metals, polymers) with complex shapes. The ability to coat arbitrary shapes and common materials that may be used in constructing functional parts is the most important advantage of our synthesis approach. Activation with water plasma produces a large number of hydroxyl groups on the silica surface, which can react with the alcohol end group of PEG. Silica was selected because its surface can be readily modified to create hydroxyl groups by exposing it to water plasma; it is nontoxic at low concentrations, and it is easy to deposit.[25–28] The surfaces produced by this method were characterized using several techniques to understand the effect of deposition parameters and water plasma conditions on the final surface properties.

MATERIALS AND METHODS

Experimental method

Our aim was to be able to graft PEG chemically to any desired substrate. We did this by initially coating the desired substrate with an amorphous silicon dioxide (i.e., silica) film deposited by PECVD. This silica film was then made reactive by exposure to water plasma which was used to hydroxylate the silica surface layer fully. That is, a saturated surface of hydroxyl or silanol (SiOH) groups was produced [Fig. 1(a)]. The silanol-covered silica surface was exposed to PEG 400 molecular weight (M_w) vapor in vacuum. The SiOH groups on the silica surface subsequently reacted with the end alcohol group of PEG, and a PEG film was created through an Si–O–C ester linkage on the silica layer [Fig. 1(b)]. During the reaction, the temperature of the substrate was maintained constant at about 100°C.

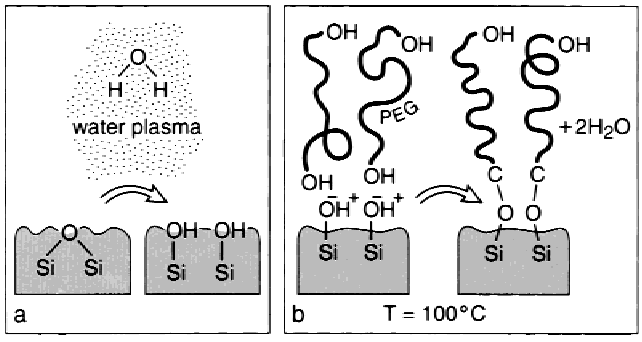

Figure 1. Schematic of the method used to create a chemisorbed PEG coating. **(a)** A silica film, deposited from PECVD, is treated with water plasma. This treatment produces a high density of silanol groups on the surface. **(b)** The water plasma–treated surface is exposed to low-molecular-weight PEG vapor under vacuum. The silanol groups on the silica surface react with the end alcohol group of the PEG to form an Si–O–C linkage. A uniform, dense, and stable PEG surface is thereby produced.

Materials

Polyethylene glycol

Polyethylene glycol is a linear neutral polyether. Its chemical structure representation is $HO–(CH_2CH_2O)_n–CH_2CH_2OH$. PEG is also known as polyethylene oxide (PEO), polyoxyethylene (POE), and polyoxirane. We used PEG of low molecular weight (400 g/mol) from Sigma (P-3265). PEG 400 is a viscous liquid which is transported as vapor using a turbomolecular pump to react with the silica surface.

Silica

Silica is used as an abbreviation for silicon dioxide in all its forms, such as crystalline, amorphous, hydrated, and hydroxylated, with the general formula SiO_2 or $SiO_2 \times H_2O$. The silica structure consists of interlinked SiO_4 tetrahedral arrays. At the surface, the silica structure terminates either in a siloxane group (Si–O–Si) with the oxygen at the surface or in one of several forms of silanol groups (Si–OH). The silanol groups can be divided into isolated groups, vicinal groups, or geminal silanols.[26] If the surface silicon atom has three bonds pointing into the bulk structure and the fourth is attached to a single OH group, it is defined as an isolated group or as a free silanol group. The vicinal silanols, which are also called bridged or associated silanols, occur when two silanol groups attached to different silicon atoms are close enough together to form a hydrogen bond.

The silica films used in these experiments are deposited by reacting SiH_4 and O_2 gases in a PECVD reactor (Fig. 2). The reactor is a six-way stainless-steel cross-vacuum chamber with a 16-in.-long, 2-in.-diameter Pyrex cylinder connected to the feedthrough port at the top. The plasma is produced by a helical resonator discharge source using a

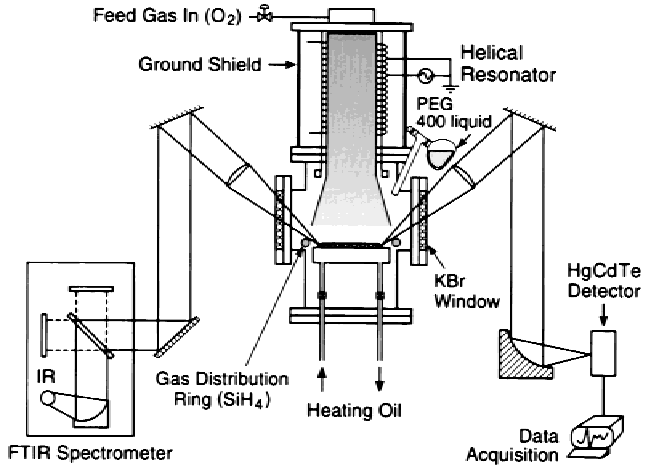

Figure 2. Schematic representation of the plasma enhanced chemical vapor deposition reactor with a plasma source, and *in situ* attenuated total reflection Fourier transform infrared spectroscopy apparatus used to detect PEG adsorbed on the silica surfaces and monitor the progress of the grafting reaction.

radiofrequency (rf)-powered copper coil at 13.56 MHz, which is surrounded by a grounded cylindrical copper shield enclosing the Pyrex tube. The rf power, provided by an RF Plasma Products Model RF5S power supply, maintains the plasma in the tube.[29]

This reactor also has an *in situ* attenuated total reflection Fourier transform infrared (ATR-FTIR) apparatus, which is used to monitor and analyze the deposition of the silica, the activation of silica with water plasma, and the reaction between PEG and silanol-saturated silica. The substrates used in this study were rectangular (50 × 10 × 0.71 mm) gallium arsenide (GaAs) wafers with 45° bevels at each of the short sides (i.e., trapezoidal ATR crystals).[30,31] GaAs crystal was used as the substrate because it is transparent above 770 cm^{-1} and allows monitoring of the C–O stretching vibration of PEG. Infrared radiation from an infrared spectrometer (Nicolet Magna 550) was focused on one of the beveled edges of the ATR crystal with a lens (KBr), traversed through the GaAs substrate, and exited from the opposite beveled edge undergoing multiple total internal reflections. The transmitted IR beam was collected by another KBr lens and focused on an HgCdTe detector by an off-axis parabolic mirror. Detailed descriptions of this reactor and ATR-FTIR apparatus were previously published by Han et al.[27] and Deshmukh et al.[29]

The oxygen gas was fed from the top of the Pyrex tube and flowed through the plasma where it was dissociated. The silane gas (SiH_4), diluted to 0.93% in argon (Ar), was introduced into the reactor through a gas injection ring surrounding the substrate on the electrode stage. The flow rate of gases were controlled by flow controllers (Edwards Model 825 or needle valves). A 300-L/s turbomolecular pump (Edwards, Model EXT351) backed with a two-stage mechanical pump (Edwards, Model E2M40) provided a base pressure of about 10^{-6} Torr and evacuated the reactor. The pressure in the reactor was controlled independently from the flow rates by a throttle valve (Edwards, Model 1850). The temperature of the substrate was held constant during the deposition by circulating heated oil through the substrate platter.

The deposition parameters used for producing silicon dioxide films of variable thickness were as follows. The system pressure for all depositions was 25 mTorr. The total flow rate was maintained constant at 50 sccm by varying the flow rates of SiH_4 and O_2. The rate of deposition was controlled between 14 and 100 Å/min by adjusting the SiH_4/O_2 flow rate ratio and the film thickness was controlled by adjusting the deposition time. The substrate temperature and rf power during each deposition were held constant at 250°C and 100 W, respectively. The mechanism of the deposition reactions was described in detail by Han et al.[27,28,32] In summary, PECVD of silica from SiH_4 and O_2 discharges produced oxygen excited molecules, atomic oxygen, and a mixture of silane molecular fragments, SiH_X ($0 \leq x \leq 3$), forming an oxide film. The surface of the as-deposited film was covered by SiOH or SiH species whose coverage depended on the SiH_4-to-O_2 flow rate ratio. Films used in this study were deposited under oxygen-rich conditions, which left the surface largely OH terminated. Full hydroxylation was ensured by following the deposition by water plasma treatment.

Water plasma treatment

After deposition of the silica layer, the sample was subjected to water plasma treatment to increase the hydroxyl group concentration on the surface controllably and reproducibly. The water plasma treatments were done in the PECVD reactor described above and in a capacitive parallel plate plasma reactor (not shown). Repeated experiments showed that both reactors produce similar hydroxylated silica surfaces. Deionized water vapor entered the reactor from the top of the plasma source and was dissociated by the plasma electrons as it flowed through the tube. Most of these treatments were done at low water vapor pressure (20 mTorr) and low rf power (10 W for 15 min). Such a gentle treatment was sufficient to saturate the surface with hydroxyl groups completely, without roughening the surface. We found that higher pressure or power resulted in rougher surfaces, as determined by AFM. In fact, water plasma treatment reduced surface roughness.[33]

RESULTS AND DISCUSSION

Silica surfaces

The PECVD silica films were deposited at different rates. The deposition rate depended on the ratio of SiH_4/O_2 gas flow rates.[27] The SiH_4/O_2 ratio ranged from 0.002 to 0.2 during deposition, which led to deposition rates between 14 and 100 Å/min. Several silica films were produced at different deposition rates to analyze the effect of the above parameters on the surface properties of the resulting silica films.

Silica films approximately 5000 Å thick were deposited at different deposition rates in five sets of three samples each. Table I shows thickness and root mean square (RMS) roughness values for these samples obtained from ellipsometry and AFM, respectively. The RMS roughness increased slightly with the deposition rate, although this dependence was weak. The rougher surface of sample 3 (14.8 nm) compared to other samples was most likely due to an initially damaged surface. From these data, we conclude that de-

TABLE I
Thickness and RMS Roughness Values for Different Deposition Rate Silica Films

Sample No.	Film Thickness (Å)	Deposition Time (min)	Deposition Rate (Å/min)	RMS Roughness (nm)
1	3860 ± 85	277	14	4.5 ± 1.2
2	5030 ± 20	152	33	6.6 ± 0.8
3	6370 ± 10	119	54	14.8 ± 2.1
4	4520 ± 30	62	73	6.9 ± 0.3
5	5070 ± 25	61	83	9.4 ± 2.5

position of bare silica at a low deposition rate is preferable for producing smooth surfaces.[33]

We also discovered that the water plasma treatment, originally planned solely to increase the amount of hydroxyl groups on the silica surface, had an additional beneficial effect. Water plasma treatment dramatically decreased the surface roughness of the silica films. A set of five samples of varying roughness (Table I) was exposed to the same water plasma treatment. As shown in Table II, the roughness of all five samples decreased to between 2 and 3 nm. Note that sample 3, which originally had a higher roughness than the other samples, also had a low roughness after the plasma treatment. The thickness of the films before and after the plasma treatment were the same within the experimental error (5–10%), indicating that the smoothing was not accompanied by significant etching of the film. The effects of water plasma treatment on silica film surfaces and mechanism of smoothing were discussed in a previous publication[33] and are outside of the scope of this article.

Grafting of PEG to activated silica

After depositing and hydroxylating the silica, we grafted PEG onto the activated surface. *In situ* ATR-FTIR (Fig. 2) was used to follow the species adsorbed on the activated silica surfaces, and thus the progress of the PEG reaction as a function of time. For these measurements, a thin amorphous film of silica (285 Å) was deposited at approximately 50 Å/min and exposed to water plasma until hydroxyl saturation was obtained at 100°C. Following this, the hydroxylated silica surface was exposed to PEG 400 for 43 h at 100°C.

The reaction between a surface silanol group and the end alcohol group on a PEG chain is a simple condensation reaction that produces an ester bond (Si–O–C). The mechanism of this reaction resembles the Ficher esterification mechanism, where a carboxylic acid is directly converted to an ester by heating it with an alcohol in the presence of a mineral acid. This mechanism is an acid-catalyzed nucleophilic acyl substitution which needs to be acid catalyzed because the carbonyl group of a carboxylic acid is not sufficiently electrophilic to be attacked by an alcohol. An acid catalyst protonates the carbonyl group and activates it toward nucleophilic attack, where loss of a proton gives the hydrate of an ester and loss of water from the hydrate of the ester yields ester and water as final products. In the same manner that the acid is the promoter of the Ficher esterification reaction, water plasma treatment was the promoter of this PEG-grafting reaction. Water plasma protonated the silicon dioxide surface forming silanol groups. The reactivity of the silanols can be compared with the reactivity of carboxylic acids, because both compounds had an acid end and the alpha species were strongly nucleophilic. The end alcohol group of the PEG molecule bonded to this protonated oxygen giving a metastable hydrate of an ester. Finally, loss of water occurred, and stabilization of the ester bond attached the PEG onto the silica surface covalently. The esterification reaction may have been driven to the ester side by using an excess of one of the reactants, heating the substrate, or removing one of the products. In our procedure, we used these three options simultaneously: Water was constantly removed because the reaction chamber was under vacuum, the substrate was maintained at 100°C, and PEG was continuously brought to the substrate surface. Consequently, saturation coverage of PEG on the surface was attainable.

TABLE II
Thickness and RMS Roughness Values for Water Plasma–Treated Silica Samples

Sample No.	Thickness before Plasma Treatment (Å)	Thickness after Plasma Treatment (Å)	RMS Roughness (nm)
1	3860 ± 85	3870 ± 25	2.3 ± 0.4
2	5030 ± 20	4625 ± 20	2.4 ± 0.3
3	6370 ± 10	6080 ± 40	2.9 ± 0.2
4	4520 ± 30	4290 ± 40	2.5 ± 0.3
5	5070 ± 25	5090 ± 10	3.3 ± 0.3

The surface reaction was monitored using *in situ* and in real-time ATR-FTIR spectroscopy. A background spectrum was taken after the water plasma treatment and used as reference for spectra taken during the PEG exposure and grafting reaction as a function of time. Figure 3(a) shows the infrared spectrum of the species adsorbed on the silica surface after 43 h of PEG 400 exposure. For comparison, Figure 3(b) shows the infrared spectrum of liquid PEG 400. The vibration bands in Figure 3(a) were assigned by comparing the absorption frequencies with values reported in the literature and the vibrational spectrum of liquid PEG [Fig. 3(b)]. The largest peak, at 1100 cm^{-1}, and the vibration band at 1300 cm^{-1} (antisymmetric stretch) in Figure 3(a,b) correspond to the C–O–C ether stretch. In the same manner, the bands peaking at 2960 and 2869 cm^{-1} corresponded to $–CH_2$ stretching vibrations. The area of the C–O–C and $–CH_2$ bands increased with time during exposure of the surface to PEG until saturation after 43 h at 100°C [Fig. 4(a,b)]. The absorption at 3740 cm^{-1} was assigned to the stretching vibration of isolated Si–OH on the silica surface[26] [Fig. 4(c)]. Similarly, the –OH peak decreased with time, corresponding to its reaction with the PEG chains, until a constant value was reached coinciding with the saturation of the C–O–C and $–CH_2$ peaks. This temporal evolution of the C–O–C, $–CH_2$ and –OH peaks was strong evidence that PEG was

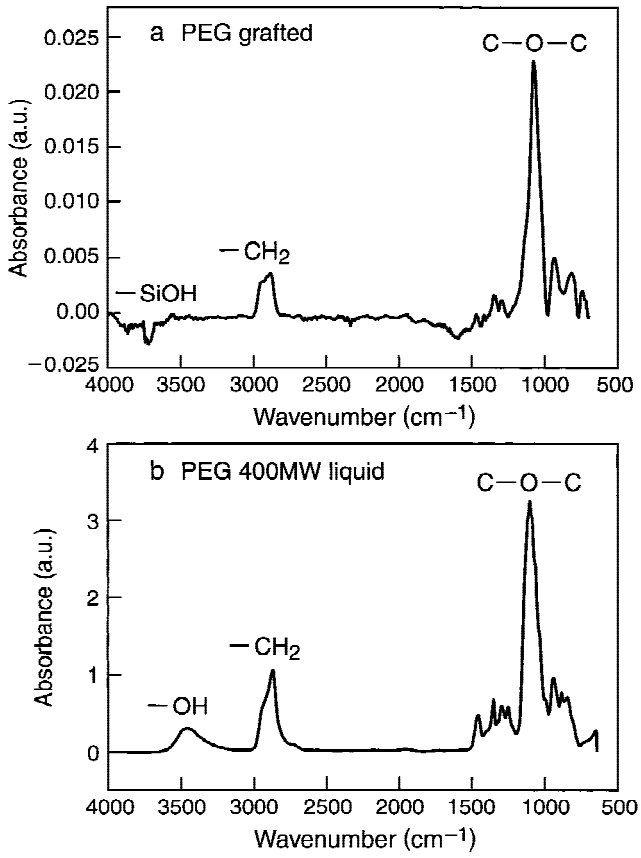

Figure 3. FTIR spectrum of the PEG covered silica and PEG. **(a)** FTIR spectrum of water plasma–treated silica film surface after 43 h of reaction with PEG. **(b)** FTIR spectrum of 400-M_w liquid PEG.

chemically grafted to the surface through a reaction of PEG with surface silanol groups. From other, similar experiments, we discovered that at higher surface temperatures (>150°C), the PEG starts to react with itself and also decomposes to form C=O and C=C compounds, although the surface grafting reaction saturates faster. The adsorption reaction is much slower at temperatures lower than 100°C. The reaction temperature of 100°C seems to be optimal for obtaining a saturated PEG-grafted surface in a reasonable amount of time.

Characterization of PEG-covered silica films

Contact angle measurements

The advancing contact angle of water on the surfaces provided further evidence for the presence of PEG. High contact angles were measured on as-deposited silica surfaces (58 ± 2°), indicating that these surfaces were hydrophobic. Water plasma treatment improved the wettability of the surfaces (45 ± 1°) by

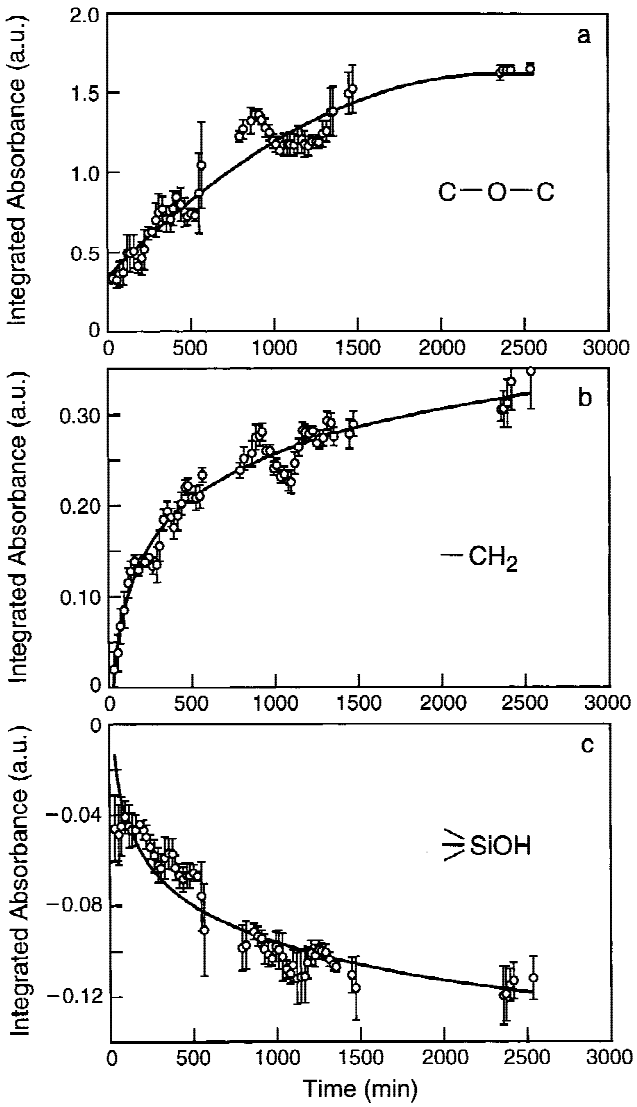

Figure 4. Integrated absorption of **(a)** the C–O–C vibration band, **(b)** the CH_2 vibration band, and **(c)** the SiOH vibration band as a function of time during reaction of activated silica surface.

increasing the hydroxyl groups on the surface. However, the surface covered with PEG had the lowest contact angle (25 ± 2°), as PEG is hydrophilic. In other words, a low contact angle confirmed the presence of PEG on the treated surfaces.

Auger spectroscopy

In addition to ATR-FTIR, we used Auger spectroscopy to further establish that PEG was indeed covalently bonded to the surface. Figure 5(a) shows an Auger electron spectrum of the surface of a 900-Å-thick silica film treated with water plasma, exposed to PEG, and rinsed with chloroform and water. Because PEG is soluble in those solvents, physically adsorbed PEG was expected to be washed off the surface

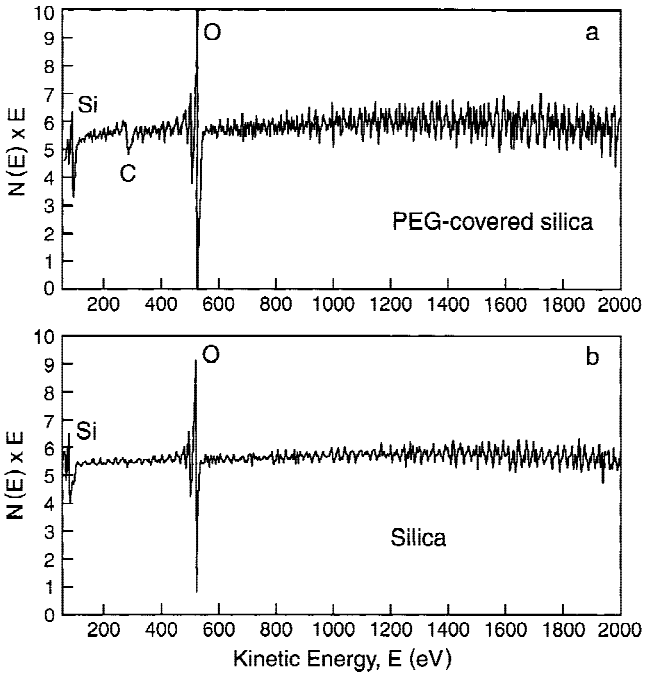

Figure 5. **(a)** PEG-covered silica surface Auger spectrum. This surface was treated with water plasma and exposed to PEG until saturation. It was then rinsed with chloroform ($CHCl_3$) and water. Oxygen (503 eV), silicon (92 eV), and carbon (272 eV) were detected on the surface, which form the PEG chains. **(b)** Auger electron spectrum of a silica surface. This surface was subjected to water plasma and rinsed with $CHCl_3$ and water, but it was not exposed to PEG. Oxygen and silicon were on the surface because they are the atomic species for silica; however, carbon was not found.

whereas covalently bonded PEG would remain on the surface even after vigorous rinsing in these solvents. The peak at 503 eV represented the characteristic energy of the Auger electron (KLL transition) of oxygen. The peak with low electron energy at 76 eV was characteristic of atomic silicon in an SiO_2 compound (LMM transition), and the peak at 271 eV was characteristic of atomic carbon (KLL transition). Therefore, we observed all the atomic species of the PEG molecule. To ascertain whether the carbon peak was due to contamination, characterization of a silica surface after the same treatment without the PEG exposure was also performed. Figure 5(b) shows the Auger electron spectrum of this control sample; whereas the same characteristic peaks for oxygen and silicon were detected owing to the silica, there was no carbon on the surface. Therefore, nonremovable carbon due to the PEG grafting $(CH_2–CH_2–O)_n$ was detected only in the spectrum of the PEG-covered silica surfaces.

AFM

The surface topology and smoothness of the bare silica and PEG-covered silica surfaces were determined with tapping mode AFM. The silica surfaces as-deposited showed an RMS roughness of 5.1 ± 0.2 nm [Fig. 6(a)]. After water plasma activation, the modified surface had an RMS roughness of 2.3 ± 0.2 nm [Fig. 6(b)]. However, after PEG was chemically bonded to the surface, the RMS roughness decreased further to 0.6 ± 0.2 nm and the surfaces appeared to be uniform [Fig. 6(c)]. We believe that PEG was filling the asymmetries on the silica, carpeting the surface completely. Consequently, PEG not only chemically modified the silica films, but also appeared to smooth the surface topography.

Fluorescence microscopy

We also investigated the biocompatibility of the PEG coating by measuring its ability to resist protein adsorption. PEG-coated silica surfaces, water plasma–treated silica surfaces, and bare silica films were all exposed to a 2-mg/mL solution of bovine serum albumin (BSA) labeled with a Texas red probe for 120 min at 37°C and pH 7.4 (imitating physiological conditions). Protein adsorption onto these surfaces was

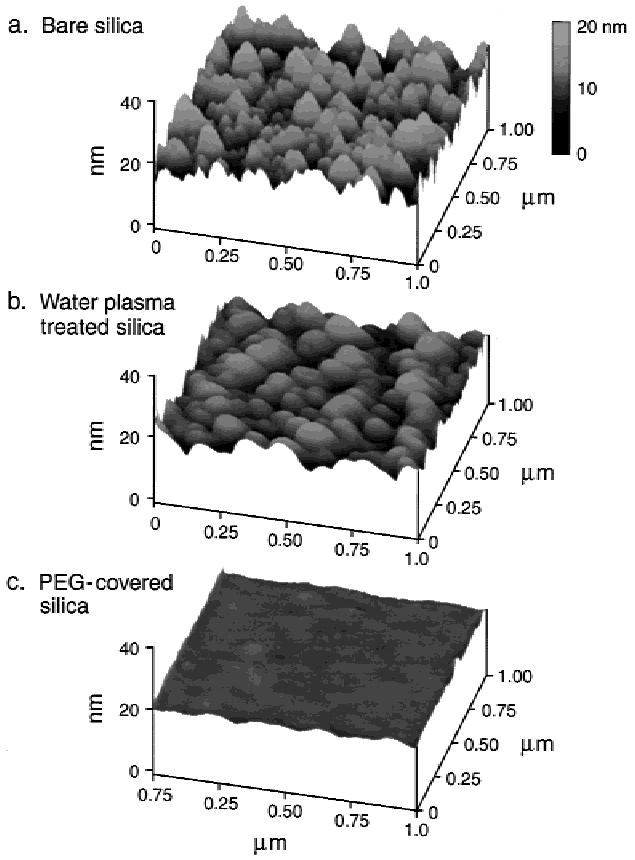

Figure 6. Three-dimensional AFM images (1 × 1 μm) of **(a)** as-deposited silica, **(b)** silica after water plasma treatment, and **(c)** PEG-covered silica. The root mean square roughness of the three films are 5, 2, and 0.5 nm, respectively.

detected with a CCD camera mounted on a fluorescence microscope. Figure 7(a–c) shows the fluorescence-intensity illumination difference from one sample of each of these three surface chemistries. The surface that was covered with PEG had very little fluorescence activity owing to low or absent protein adsorption [Fig. 7(c)]. Conversely, the bare silica surface was much brighter owing to relatively high amounts of adsorbed protein [Fig. 7(a)]. The surface treated with water plasma adsorbed some proteins, falling between the bare silica and PEG coated surfaces [Fig. 7(b)]. Quantitative analysis of protein adsorption was done by comparing the normalized adsorption intensity (NAI) of scans for the three different surfaces (scan size: 350 × 450 μm). The NAI is the percent ratio of the emission intensity of the surface and the emission intensity measured for the background. The background is taken on the surface that has not been ex-

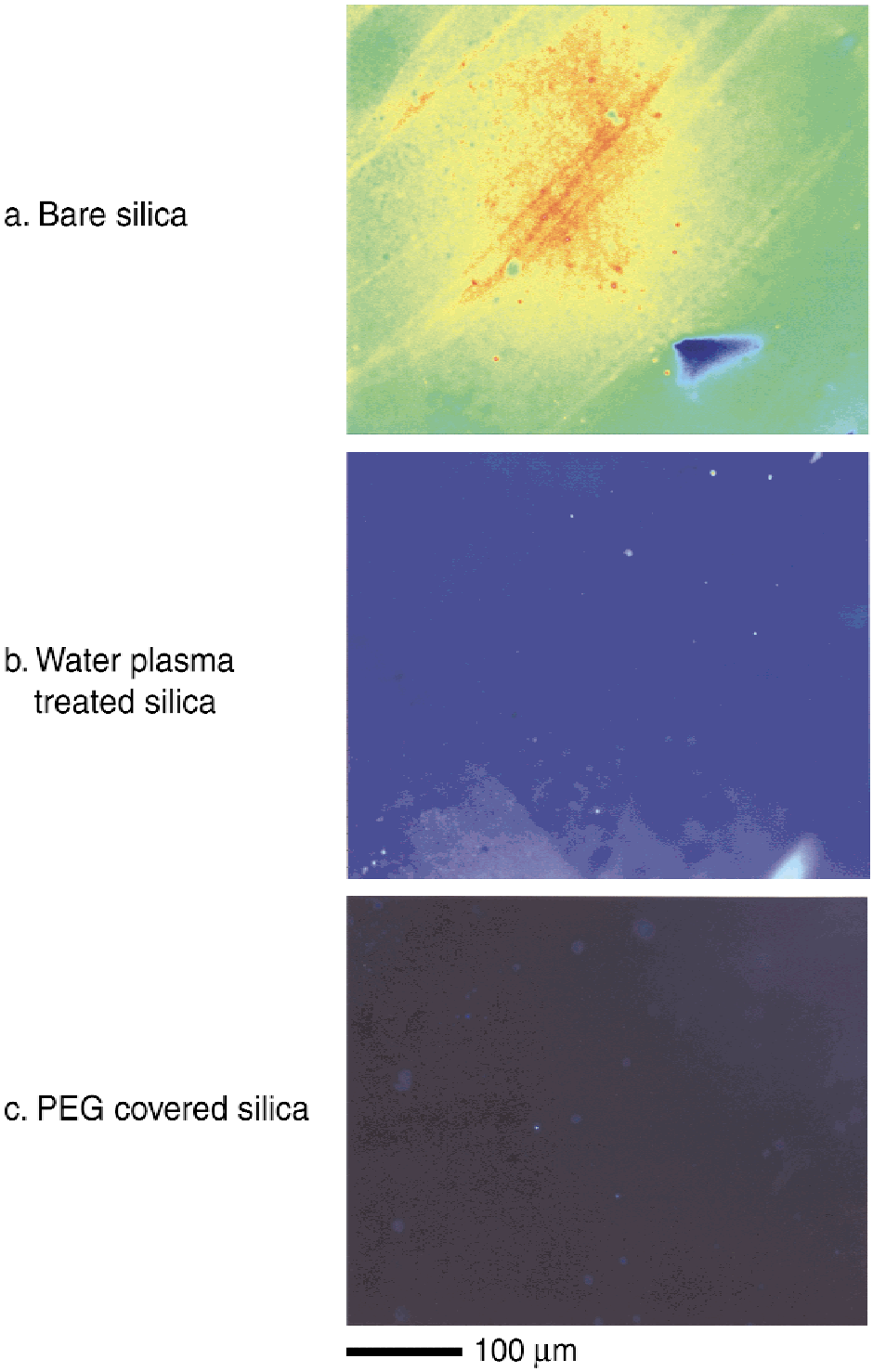

Figure 7. Fluorescence detection of adsorbed bovine serum albumin labeled with a Texas red probe. Incubation time was 120 min at 37°C and pH 7.4 (physiological conditions). **(a)** Bare silica with normalized adsorption intensity (NAI) of 71%. **(b)** Water plasma–treated silica with NAI of 62%. **(c)** PEG-covered silica surface with NAI of 7%.

posed to the protein. Bare silica exhibited the greatest NAI: 71%. The NAI for water plasma–treated silica was 62%. The silica surface that was reacted with PEG showed an NAI of only 7%. The adsorption of other proteins is currently under investigation.

SUMMARY AND CONCLUSIONS

A new PEG grafting method was optimized for producing biocompatible coatings. The coatings were produced by grafting PEG on a film of activated amorphous silica. Our approach is simple and flexible because silica can be easily deposited by PECVD on various materials, including metals and plastics. The proposed synthesis route is also efficient because PEG is grafted onto the surface by a direct condensation of its alcohol end group with the silanol groups on the silica surface. Surface characterization of the resulting films using infrared spectroscopy, Auger electron spectroscopy, contact angle measurements, and AFM corroborated that PEG was chemisorbed on the surface. The PEG films were smoother and more hydrophilic than either bare or activated silica surfaces. The measurements of fluorescence intensity showed that PEG-covered silica films prevented protein adsorption. Hence, PEG binding modifies the resulting surface topology, chemistry, and interactions, rendering surfaces coated with our protective layers biocompatible.

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