

Electrodeposition of amine-terminated poly(ethylene glycol) to titanium surface

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Abstract

The immobilization of poly(ethylene glycol), PEG, to a solid surface is useful to functionalize the surface, e.g., to prevent the adsorption of proteins. No successful one-stage technique for the immobilization of PEG to base metals has ever been developed. In this study, PEG in which both terminals or one terminal had been modified with amine bases was immobilized onto a titanium surface using electrodeposition. PEG was dissolved in a NaCl solution, and electrodeposition was carried out at 310 K with -5 V for 300 min. The thickness of the deposited PEG layer was evaluated using ellipsometry, and the bonding manner of PEG to the titanium surface was characterized using X-ray photoelectron spectroscopy after electrodeposition. The results indicated that a certain amount of PEG was adsorbed on titanium through both electrodeposition and immersion when PEG was terminated by amine. However, terminated amines existed at the surface of titanium and were combined with titanium oxide as N–HO by electrodeposition, while amines randomly existed in the molecule and showed an ionic bond with titanium oxide by immersion. The electrodeposition of PEG was effective for the inhibition of albumin adsorption. This process is useful for materials that have electroconductivity and a complex morphology.

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1. Introduction

The demand for metals in medical and dental devices is large. These materials are used because of their high durability, strength, and formability. Conventionally, metals are essential for orthopedic implants, bone fixators, artificial joints, and external fixators, since they can substitute for the mechanical function of hard tissues in orthopedics. In other words, metals in medical devices cannot be replaced with ceramics or polymers at present, mainly because the metallic materials have greater strength and toughness. On the other hand, metallic materials are generally not expected to be the biomaterials of the future at the research

level because they do not have bioactive and biofunctional properties.

However, metals with biofunctions have been required in the recent past. For example, stents are placed at stenotic blood vessels for dilatation, and blood compatibility or prevention of adhesion of platelets is necessary. In guide wires and guiding catheters, lubrication in the blood vessels is important for proper sliding and insertion. In addition, if metals are used as sensing devices, the control of cell adhesion is necessary. For these purposes, the fundamental property is the inhibition of protein adsorption. Poly(ethylene glycol), PEG, is a biofunctional molecule on which the adsorption of proteins is inhibited. Therefore, the immobilization of PEG to a metal surface is an important step towards the bio-functionalization of the metal surface.

The immobilization of biofunctional polymers on a noble metals such as gold is usually conducted by using the bonding $-SH$ or $-SS$ -group; however, this technique can only be used

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for noble metals. The adhesion of platelets and adsorption of proteins, peptides, antibodies, and DNA is controlled by modifications of the above technique. On the other hand, a class of copolymers based on poly(L-lysine)-g-poly(ethylene glycol), PLL-g-PEG, has been found to spontaneously adsorb from aqueous solutions onto TiO_2 , $\text{Si}_{0.4}\text{Ti}_{0.6}\text{O}_2$, and Nb_2O_5 to develop blood-contacting materials and biosensors [1,2]. In another case, TiO_2 and Au surfaces are functionalized by the attachment of poly(ethylene glycol)-poly(DL-lactic acid), PEG-PLA, copolymeric micelles. The micelle layer can enhance the protein resistance of the surfaces up to 70% [3]. Peptides with terminal cysteine residues were immobilized on maleimide-activated oxides [4–6]. A surface of stainless steel was firstly modified by a silane-coupling agent, SCA, (3-mercaptopropyl)trimethoxysilane. The silanized stainless steel, SCA-SS, surface was subsequently activated by argon plasma and then subjected to UV-induced graft polymerization of poly(ethylene glycol)methacrylate, PEGMA. The PEGMA graft-polymerized stainless steel coupon, PEGMA-g-SCA-SS, with a high graft concentration and, thus, a high PEG content was found to be very effective to prevent the absorption of bovine serum albumin and γ -globulin [7]. These processes require several steps but are effective for immobilization; however, no promising technique for the immobilization of PEG to a metal surface has been so far developed.

In this study, PEG in which both terminals or one terminal had been modified with amine bases was immobilized onto a titanium surface by electrodeposition in an attempt to develop a new technique that could be used for all metals and complexly designed surfaces. Furthermore, the mode of immobilization and chemical bonding state was characterized using X-ray photoelectron spectroscopy, XPS. The adsorption of plasma protein on the PEG-immobilized surface was evaluated.

2. Experimental

2.1. Electrodeposition

Both terminals of PEG were terminated with $-\text{NH}_2$ (B-PEG; PEG1000 Diamine, NOF Corporation, Japan), and only one terminal was terminated with $-\text{NH}_2$ (O-PEG; SUNBRIGHT MEPA-10H, NOF Corporation, Japan). The chemical structures of the PEGs are shown in Fig. 1. The molecular weights of both PEGs

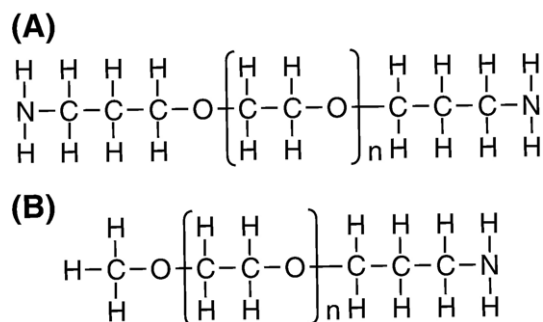


Fig. 1. Chemical structures of PEGs in which both terminals (A) and one terminal (B) were terminated with amine.

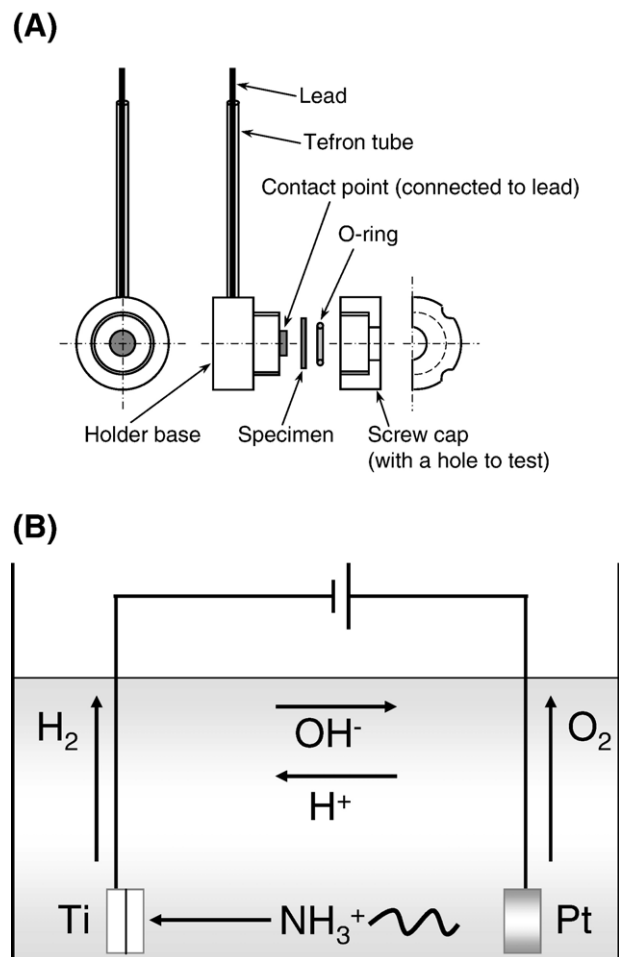


Fig. 2. Design of a polytetrafluoroethylene holder of titanium for electrodeposition (A) and schematic illustration of electrodeposition.

were about 1000. These terminated PEGs were dissolved in a 0.3-mol L^{-1} NaCl solution with a concentration of 2 mass%. In the solution, the $-\text{NH}_2$ terminal was dissociated and charged as $-\text{NH}_3^+$. The pH of the solution with B-PEG was 11.2, and that of the solution with O-PEG was 11.0. The resultant solution was used as an electrolyte for electrodeposition at 310 K.

A commercially pure titanium disk ($8\text{ mm}\phi \times 2\text{ mm}$ in thickness) with grade 2 was metallographically polished and ultrasonically rinsed in acetone and deionized water (Milipore). The titanium disk was fixed in a polytetrafluoroethylene holder that was insulated from the electrolyte except for an opening made for electrodeposition ($6.0\text{ mm}\phi$), as shown in Fig. 2(A). Therefore, the area exposed for electrodeposition was 28.3 mm^2 . The open circuit potential of titanium, E_{open} , vs. a saturated calomel electrode, SCE, before electrodeposition was measured as ca. -0.5 V . Thereafter, the cathodic potential was charged from E_{open} to -5 V vs. SCE with a sweep rate of 0.1 V s^{-1} and maintained at this potential for 300 s. During charging, the terminated PEGs were electrically migrated to the titanium cathode and deposited on it as shown in Fig. 2(B). This cathodic current was measured during electrodeposition and compared with the results from the NaCl solution without PEG. For comparison, titanium was immersed in the electrolyte containing B-PEG for 2 h and 24 h without any electric charge at 310 K. After electrodeposition,

specimens were rinsed in deionized water and dried with a stream of nitrogen gas (99.9%).

2.2. Ellipsometry

The thickness of the PEG layer deposited on titanium was determined with an ellipsometer (DVA-36Ls, Mizojiri Optical Co., Ltd.) in air. The use of an ellipsometer resulted in the underestimation of the thickness compared to that in solution. The light source was a He–Ne laser with a wavelength of 632.8 nm, and the incident angle to the titanium surface was 70°. The thickness was calculated by optical constants: the refractive index and absorption coefficient of titanium oxide with the titanium substrate were 2.209 and 3.079 [8,9], and those of the titanium substrate were 2.22 and 2.99 [10], respectively.

2.3. XPS

The deposition mode of PEG to the titanium surface and the chemical bonding state were characterized using XPS (SSI-SSX100). The take-off angle for photoelectron detection was 35° from the surface of the specimen. All binding energies given in this paper are relative to the Fermi level, and all spectra were excited with the monochromatized Al K α line (1486.61 eV). The spectrometer was calibrated against Au 4f_{7/2} (binding energy, 84.07 eV) and Au 4f_{5/2} (87.74 eV) of pure gold and Cu 2p_{3/2} (932.53 eV), Cu 2p_{1/2} (952.35 eV), and Cu Auger L₃M_{4,5}M_{4,5} line (kinetic energy, 918.65 eV) of pure copper. The energy values were based on published data [11]. In order to estimate the photoelectron peak intensities, the background was subtracted from the measured spectrum according to Shirley's method [12]. The composition and thickness of the surface oxide and the composition of the substrate were simultaneously calculated according to a method devised by one of the authors of this study [13,14]. Empirical data [15] and theoretically calculated data [16] of relative photoionization cross-sections were used for the quantification. The relative photoionization cross-sections used in this study are summarized in Table 1, where $\sigma_{ij}/\sigma_{O\ 1s}$ represents the relative photoionization cross-section of a level j electron of an element i to that of O 1s electrons.

2.4. Adsorption of albumin

The inhibition of plasma protein adsorption to a titanium surface with PEG deposition was evaluated. Albumin (fluorescein isothiocyanate conjugated bovine, A9771, Sigma) was dissolved into phosphate-buffered saline without calcium chloride and magnesium chloride (PBS: Dulbecco's PBS, D1408, Sigma) with a

Table 1
Photoionization cross-sections of level j of element i relative to that of O 1s, $\sigma_{ij}/\sigma_{O\ 1s}$

Photoionization cross-section			
Level	Ti 2p _{3/2}	C 1s	N 1s
σ_{ij}	1.28	0.34	0.62
Reference	14	15	15

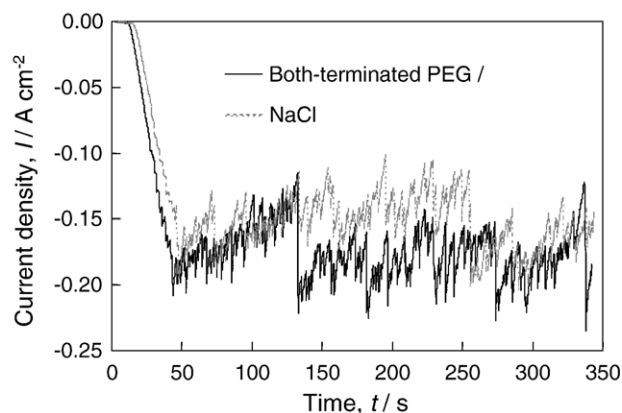


Fig. 3. Cathodic current during electrodeposition in NaCl solutions with and without PEG in which both terminals were terminated.

concentration of 4.5 g L⁻¹. Specimens were immersed in albumin-containing PBS for 30 min, rinsed with PBS and deionized water, and then dried with a stream of nitrogen gas (99.9%). The surface of specimens was observed with a fluorescence microscope (E-600, Nikon).

3. Results and discussion

3.1. Cathodic current

The cathodic current increased with the decrease of the potential in the cathodic direction and became almost constant after reaching the target potential, -5 V, with irregularities between -0.15 and -0.20 A cm⁻², as shown in Fig. 3. These current values contain a hydrogen evolution current because the charged potential was much lower than that of the hydrogen evolution potential according to the Pourvaix diagram [17]. The passive oxide film on titanium is not electrically reduced because hydrogen evolution is preferential even though the charged potential is within the range of immunity. In addition, the cathodic current in the NaCl solution without PEG was almost the same as that in

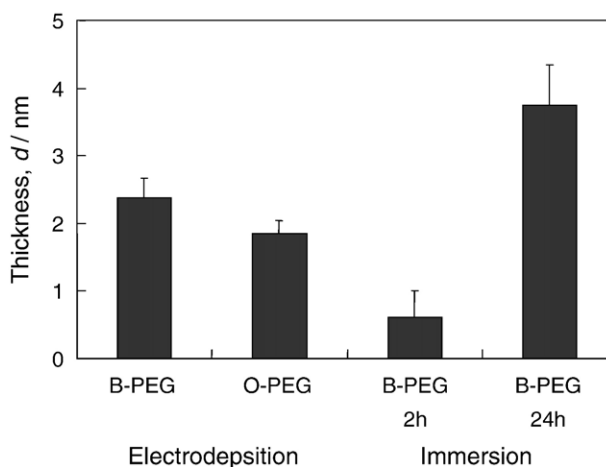


Fig. 4. Thickness of the PEG layer deposited on titanium by electrodeposition and immersion. B-PEG: both-terminal-modified PEG. O-PEG: one-terminal-modified PEG.

Table 2
Relative concentrations of elements determined by XPS

Deposition process	PEG termination Deposition time	Concentration (mass%)			
		C	O	N	Ti
Electrodeposition	Both-terminal 300 s	41.8 (0.4)*	44.4 (0.3)	1.3 (0.3)	12.5 (0.2)
	One-terminal 300 s	45.9 (1.0)	41.8 (0.8)	1.3 (0.1)	10.9 (0.3)
Immersion	Both-terminal 2 h	49.3 (1.5)	41.8 (0.5)	1.5 (0.0)	13.5 (1.0)
	Both-terminal 24 h	45.8 (1.7)	41.2 (1.3)	2.2 (0.0)	10.9 (0.5)

The bars in the table represent significant differences between the corresponding values ($p < 0.05$).

the solution with PEG. Therefore, an electrodeposition phenomenon did not clearly appear on the current.

3.2. Thickness of adsorbed PEG by ellipsometry

Fig. 4 shows the thicknesses of the PEG deposition layers determined by ellipsometry. These thicknesses are measured in air; therefore, the real thickness in solutions is larger than these values. The thickness of the deposition layer, in other words, the amount of deposited PEG, is the largest in this order: 24 h-immersion B-PEG, electrodeposition of B-PEG for 300 s, electrodeposition of O-PEG for 300 s, and 2 h-immersion B-PEG. This indicated that electrodeposition was more effective

than immersion for the deposition of PEG on the titanium surface. However, the PEG layer increased after a 24 h-immersion, indicating that the charged terminals of PEG attracted the electrostatically titanium surface that is covered by titanium oxide with a large number of hydroxyl groups [18,19]. In electrodeposition, the thickness of the B-PEG deposition layer was larger than that of the O-PEG deposition layer. This does not necessarily indicate that more B-PEG than O-PEG was deposited because ellipsometry was conducted in air and the PEG molecules collapsed on titanium. The B-PEG has more density per molecule after deposition on titanium surface than the O-PEG because both terminals are attracted to the titanium surface in the B-PEG. Therefore, the apparent thickness of the B-PEG in air was larger than that of the O-PEG.

3.3. Concentrations of elements by XPS

Carbon, nitrogen, oxygen, and titanium were detected using XPS. The relative concentrations of carbon, nitrogen, oxygen, and titanium in specimens were calculated assuming that the gross amount of these elements, as detected using XPS, was 100 mol%. Table 2 presents the concentrations of elements. Sodium and chlorine were not detected.

The carbon concentration was governed by the amount of PEG existing on titanium because carbon atoms exist in the whole molecule. Therefore, the difference in the carbon concentration represented that in the deposition amount of PEG. When a large amount of PEG was deposited on titanium, the apparent concentration of titanium decreased in the determination with XPS. The results of the titanium concentration are in good agreement

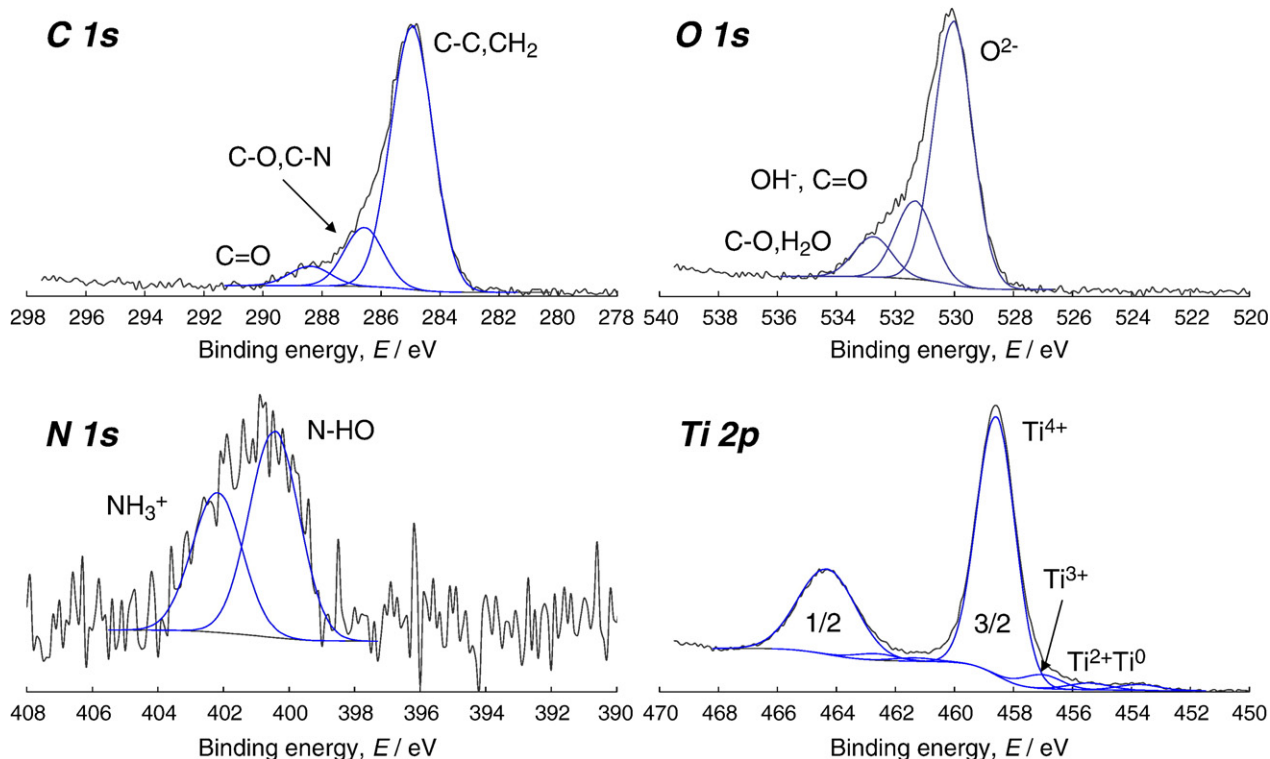


Fig. 5. C 1 s, O 1 s, N 1 s, and Ti 2 p electron energy region spectra by XPS and deconvolution of the peaks to component peaks.

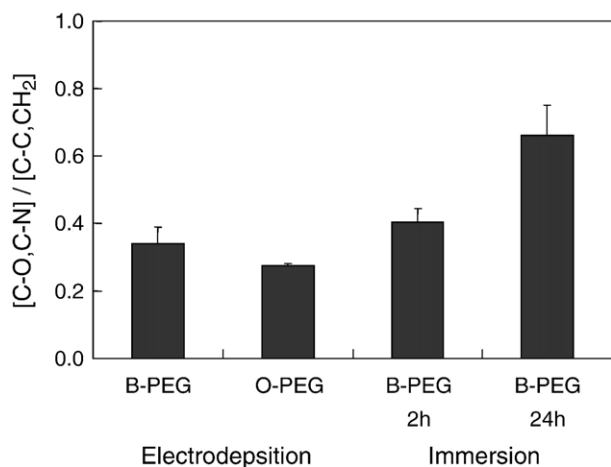


Fig. 6. Ratio, $[C-O, C-N]/[C-C, CH_2]$, obtained from the deconvoluted C 1s electron energy region peak. B-PEG: both-terminal-modified PEG. O-PEG: one-terminal-modified PEG.

with those of the carbon concentration from the viewpoint of the relative magnitude of both element concentrations. The carbon concentration in the electrodeposition of the O-PEG was significantly larger than that in the B-PEG, indicating that more O-PEG than B-PEG was deposited on titanium by electrodeposition. Probably, the B-PEG occupies more deposition sites, *e.g.*, hydroxyl groups on titanium oxide, than the O-PEG and less B-PEG could be deposited on titanium because B-PEG contains twice as many electric charges as O-PEG. More B-PEG adsorbed on titanium in a 24 h-immersion than in electrodeposition. In other words, PEG can be deposited on titanium only by long-time immersion whenever PEG is terminated by amine, independently of the mode of adsorption or chemical bond.

On the other hand, the nitrogen concentration in the 24 h-immersion specimen was significantly larger than those of other specimens. Nitrogen is originated from the terminal amines in PEG. The photoelectron signals detected by XPS from a deep site are weak because they decay while passing through molecules and solids. Therefore, nitrogen atoms in electrodeposited speci-

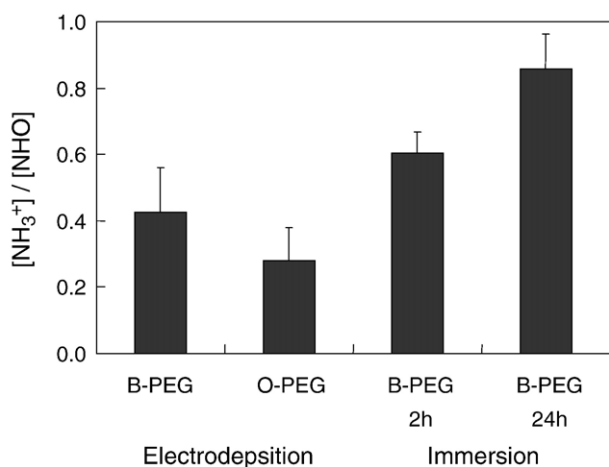


Fig. 7. Ratio, $[NH_3^+]/[NHO]$, obtained from the deconvoluted N 1s electron energy region peak. B-PEG: both-terminal-modified PEG. O-PEG: one-terminal-modified PEG.

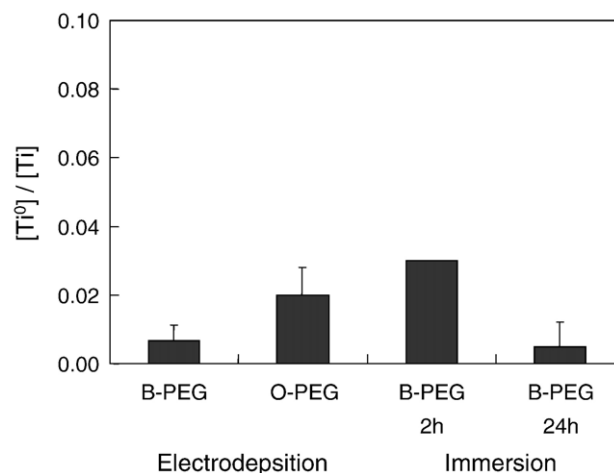


Fig. 8. Ratio, $[Ti^0]/[Ti]$, obtained from the deconvoluted Ti 2p electron energy region peak. B-PEG: both-terminal-modified PEG. O-PEG: one-terminal-modified PEG.

mens and 2 h-immersed specimens were located at deeper sites than 24 h-immersed specimens.

3.4. Chemical states of elements and bonding manner

Typical XPS spectra of C 1s, O 1s, N 1s, and Ti 2p electron energy regions and the deconvolutions of these peaks are shown in Fig. 5 [20–26].

The ratio, $[C-O, C-N]/[C-C, CH_2]$, in the C 1s peak in each specimen is shown in Fig. 6. C–C bonds existed in the entire molecule of PEG; therefore, this ratio is governed by the C–N bond. The photoelectron signals in XPS abruptly decayed depending on the depth direction. Therefore, the C–N bond was located inside of the deposited PEG layer by electrodeposition more often than by immersion because C–N bonds exist only at the terminal and C–O bonds exist in the whole molecule. In other words, nitrogen atoms exist at the interface between PEG and titanium.

The change in the ratios, $[NH_3^+]/[NHO]$, in the N 1s peak is shown in Fig. 7. This ratio is much smaller in electrodeposited

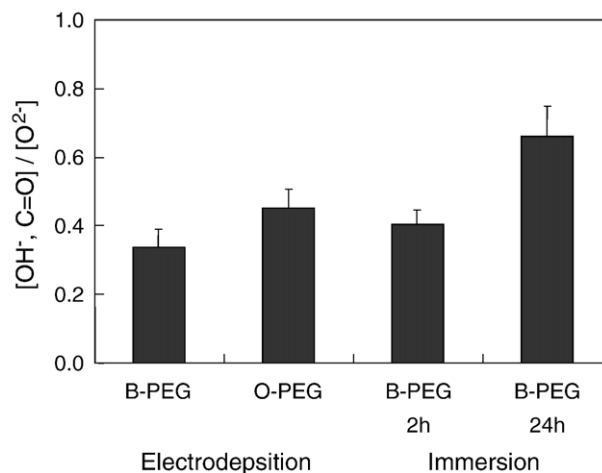


Fig. 9. Ratio, $[OH, C=O]/[O^{2-}]$, obtained from the deconvoluted O 1s electron energy region peak. B-PEG: both-terminal-modified PEG. O-PEG: one-terminal-modified PEG.

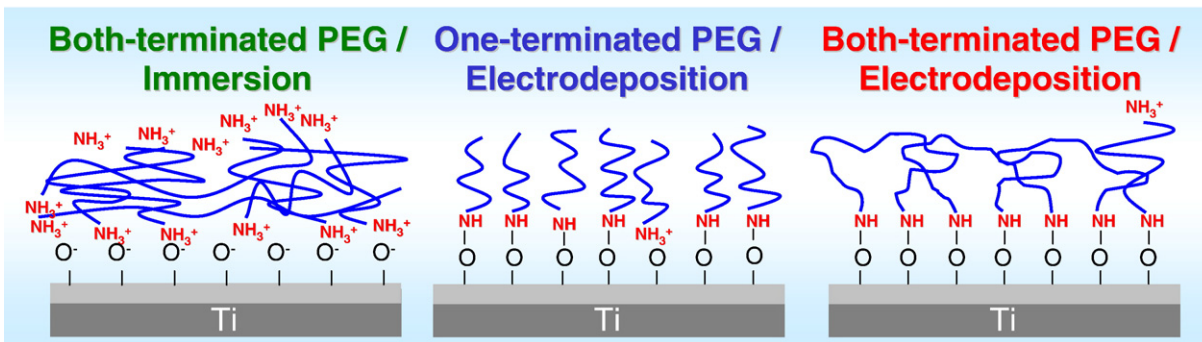


Fig. 10. Schematic model of the deposition mode and chemical bonding state of PEG by immersion and electrodeposition.

specimens than in immersed specimens. Nitrogen atoms in electrodeposited specimens existed as a N–HO bond rather than as NH_3^+ . This indicated that strong bonding between amine and titanium oxide was formed by electrodeposition, while ionic bonding was formed by immersion. In the Ti 2p region spectrum, the Ti^0 peak originates from the titanium metal substrate, and Ti^{2+} , Ti^{3+} , and Ti^{4+} peaks originate from titanium surface oxide. The integrated intensity of Ti^{4+} was the largest: titanium surface oxide is mainly TiO_2 . Although the proportion of Ti^{4+} among the Ti 2p_{3/2} peaks was almost constant, that of Ti^0 varied according to the specimen preparation conditions, as shown in Fig. 8. When the adsorbed layer of PEG was thick, the intensity of Ti^0 decreased because the signal from the titanium substrate was small. In this sense, the magnitude of the proportion shown in Fig. 8 is completely in accordance with the results from ellipsometry in Fig. 4.

From the decomposition of the O 1s peak, a large amount OH^- existed in the 24 h-immersion specimen (Fig. 9). Probably, adsorbed PEG contained a large amount of OH^- , whereas titanium oxide surface contains OH^- [18,19]. The amount was larger in the 24 h-immersed specimen than in the 2 h-immersed specimen, resulting in the difference in the amount of deposited PEG.

A certain amount of PEG is deposited on titanium through both electrodeposition and immersion when PEG is terminated by amine and charged in an aqueous solution. However, terminated amines exist at the surface of titanium and are combined with

titanium oxide as N–HO bond by electrodeposition, while amines randomly exist in the PEG layer and show an ionic bond with titanium oxide by immersion. In the B-PEG, the PEG deposited as a U-shape because both terminals combined with the titanium surface; in the O-PEG, PEG was deposited as a brush because only one terminal was combined with the surface. These results are illustrated in Fig. 10.

3.5. Albumin adsorption

Fig. 11 shows fluorescent images of albumin adsorbed on titanium before and after immobilization of PEG. The adsorption of albumin was inhibited with the deposition of PEG. Therefore, the electrodeposition of PEG was effective for the inhibition of the adsorption of albumin.

4. Conclusions

A certain amount of PEG was adsorbed on titanium through both electrodeposition and immersion when PEG is terminated by amine. However, terminated amines exist at the surface of titanium and are combined with titanium oxide as N–HO by electrodeposition, while amines randomly exist in the molecule and show an ionic bond with titanium oxide by immersion. The both-terminal-terminated PEG is deposited as a U-shape, and the one-terminal-terminated PEG is deposited as a brush. The

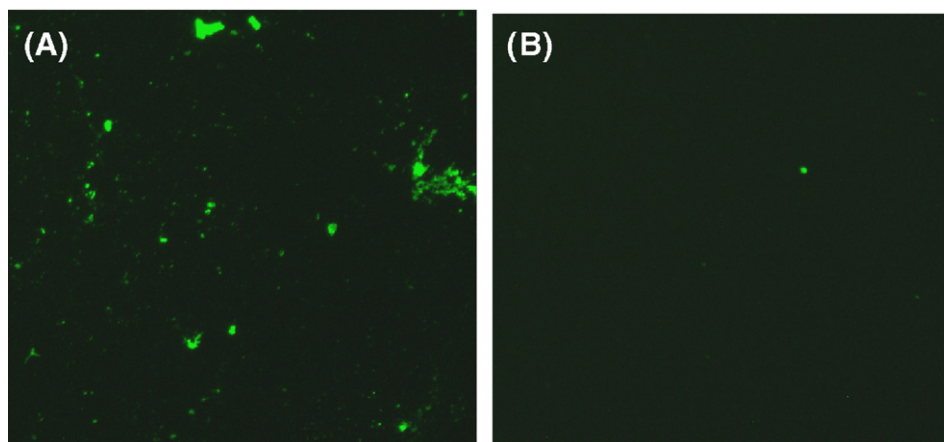


Fig. 11. Fluorescent images of albumin adsorbed on titanium before (A) and after (B) deposition of PEG.

electrodeposition of PEG was effective for the inhibition of albumin adsorption. This immobilization process is useful for all electroconductive and morphological materials.

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