Mechanism of Electrodeposition Process of Poly(Ethylene Glycol) Diamine to Titanium Surface

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Electrodeposition of biofunctional molecules is effective in adding biofunction to metals; however, the mechanism of electrodeposition remains unclear. We consider the electrodeposition process of poly(ethylene glycol) (PEG) to the pure titanium surface, in which the termination of both PEG terminals proceeds with NH_2 (PEG-diamine; MW: 1000). To elucidate this process, the thickness and mass change of the deposited layer and the electron transfer during electrodeposition was investigated using quartz crystal microbalance (QCM), ellipsometry, and cyclic voltammetry. Consequently, surface electric charge directly influenced the adsorption of PEG-diamine and unmodified PEG molecules. PEG-diamine was attracted to QCM electrode containing Ti (Ti-QCM electrode) and condensed on the surface by cathodic charge, by which electron transfer from PEG-diamine to the Ti surface occurred. Bonding of PEG-diamine with the Ti surface by electrodeposition was strong with no detachment from Ti. PEG-diamine was immediately adsorbed onto the Ti surface by the weak electrostatic force and bonded randomly via this force. Subsequent rearrangement and condensation occurred alongside an electrochemical reaction between the molecules and the Ti surface due to cathodic charge. Consequently, PEG-diamine molecules do not firmly remain on the Ti surface under electrodeposition, but shake near the Ti surface while undergoing repeated ionization and un-ionization. [doi:10.2320/matertrans.MT-M2020111]

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1. Introduction

At present, over 70% of the implant devices in medicine still consist of metals because of their high strength, toughness, and durability. Further, it is difficult to immediately replace the metals in medical devices with ceramics or polymers. A disadvantage of using metals as biomaterials is that they are typically artificial materials with no biofunction. To add biofunction to metals, surface modification is effective. Surface modification is an important and predominant technique for obtaining biofunction and biocompatibility in metals for biomedical applications. One surface modification technique is a process that changes the surface composition, structure, and morphology of a material, while not altering the bulk mechanical properties. A tremendous number of surface modification techniques using dry and wet processes to improve the hard tissue compatibility of titanium have been developed.¹⁻⁷⁾ A second approach is the immobilization of biofunctional molecules to the metal surface to control the adsorption of proteins and adhesion of cells, platelets, and bacteria.⁸⁾

Poly(ethylene glycol) (PEG) is an oligomer or polymer of ethylene oxide; however, historically, PEG has tended to refer to oligomers and polymers with a molecular weight below 20,000. It is coupled to hydrophobic molecules to produce non-ionic surfactants. This property, combined with the availability of PEGs with a wide range of end-functions, contributes to the wide use of PEGs in biomedical research in drug delivery, tissue engineering scaffolds, surface functionalization, and many other applications.⁹⁾ PEG is a biofunctional molecule onto which the adsorption of proteins is inhibited. Therefore, the immobilization of PEG to a metal surface is an important tool to biofunctionalize the metal surface.

We have employed an electrodeposition technique to immobilize PEG on the titanium (Ti) surface. For the electrodeposition of PEG to the Ti surface, both terminals of PEG are terminated with $-\text{NH}_2$ (PEG-diamine; MW: 1000). The cathodic potential was charged to Ti from the open circuit potential (OCP) to -5.0 V vs. a saturated calomel electrode (SCE) (V_{SCE}) and is maintained at this potential for 300 s. During charging, PEG-diamine electrically migrates to and is deposited on the Ti cathode. More terminated amines combine with Ti oxide as $\text{NH}-\text{O}$ bonds by electrodeposition, and PEG-diamine is immobilized as a loop-shape or U-shape. Meanwhile, more amines randomly exist as NH_3^+ in the PEG-diamine layer formed by immersion.^{10,11)} The amount of PEG-diamine immobilized on the metals is governed by the concentrations of the active hydroxyl groups on each surface oxide in the case of electrodeposition and governed by the relative permittivity of the surface oxide in the case of immersion.¹²⁾ The PEG-immobilized surface inhibits the adsorption of proteins and attachment of cells, as well as the adhesion of platelets¹³⁾ and bacteria,^{14,15)} indicating that this electrodeposition technique is useful for the biofunctionalization of metal surfaces. In addition, to immobilize RGD peptide on the electrodeposited PEG on Ti, PEG with an $-\text{NH}_2$ group and a $-\text{COOH}$ group ($\text{NH}_2\text{-PEG-COOH}$) was employed. One terminal group, $-\text{NH}_2$, must bind stably with a surface oxide on the metal. Meanwhile, the other terminal group, $-\text{COOH}$, can usefully bond to biofunctional molecules, such as RGD.¹⁶⁾ This RGD/PEG/Ti surface accelerates calcification by the MC3T3-E1 cell.¹⁷⁾ Calcification is strongest on the RGD/PEG/Ti surface, and bone formation on the RGD/PEG/Ti surface is accelerated compared to that on the RGD/Ti surface in rabbit.¹⁸⁾ Meanwhile, graft- and loop-type PEGs were formed on mirror-polished Ti surfaces using electrodeposition with mono- and diamine-functionalized PEGs, and a Velcro-like friction behavior could be induced by simply changing the

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Fig. 1 Chemical structure of PEG-diamine.

conformation of the PEGs.¹⁹⁾ In addition, electrodeposition is applied to 2-methacryloyloxyethyl phosphorylcholine (MPC) polymers to inhibit platelet adhesion to the Ti surface.^{20,21)}

As mentioned above, the electrodeposition of a biofunctional molecule is effective in improving the biofunction of metals. However, the mechanism of electrodeposition is still unclear; thus, we made the best effort to elucidate the electrodeposition process and mechanism through the investigation of the change in thickness and mass of the deposited layer, and the transfer of electrons during electrodeposition by quartz crystal microbalance (QCM), ellipsometry, and cyclic voltammetry techniques. This research will enhance our understanding of the electrodeposition phenomenon of functional molecules to metal surfaces and promote further advanced electrodeposition techniques.

2. Materials and Methods

2.1 Preparation of PEG-diamine solution

We have employed a characteristic PEG molecule in which both terminals of PEG were terminated with NH_2 (PEG-diamine; PEG1000 Diamine, NOF Corporation, Tokyo, Japan). The chemical formula of PEG-diamine is shown in Fig. 1. PEG-diamine was dissolved in 0.3-mol L^{-1} NaCl solution made from deionized water (Millipore) at a concentration of 0.02-mol L^{-1} , and the pH of the solution was adjusted to pH 3 and 11 by 1-mol L^{-1} HCl. The original PEG without termination (Unmodified-PEG; NOF Corporation, Tokyo, Japan) was used for comparison with the QCM technique. Unmodified-PEG was dissolved in deionized water with a concentration of 0.02-mol L^{-1} , and the pH of the solution was adjusted to pH 3 and 11.

2.2 Adsorption behavior of PEG-diamine using QCM and an ellipsometer

To investigate the adsorption behavior of PEG-diamine onto Ti when Ti is immersed in PEG-diamine solution, the mass change of Ti electrode during immersion in PEG-diamine solution and Unmodified-PEG solution was monitored using QCM. Commercially pure Ti was sputter-deposited on QCM electrode (quartz crystal of the A-T cut type, Hokuto Denko, Japan) with a fundamental resonant frequency of 6 MHz (Ti-QCM electrode). The exposed area of Ti to the solution was 1.33 cm^2 . When the crystal is made to oscillate at its resonant frequency, the fundamental frequency changes, as the mass is adsorbed onto or desorbed from the electrode. The frequency shift (Δf) was monitored by an electrochemical QCM controller (model HQ101B, Hokuto Denko, Tokyo, Japan) connected to an electrochemical analysis system (model HZ-3000, Hokuto Denko, Tokyo, Japan). Ti-QCM electrode was immersed in 0.3-mol L^{-1}

NaCl solution, and the Δf of the crystal was stabilized in the solution for 10 min before the injection of PEG-diamine solution. After 10 min, concentrated PEG-diamine solution was injected with a syringe at a concentration of 2 mass%. The Δf of the crystal at 37°C was monitored for 24 h.

After QCM measurement, specimens were rinsed in the deionized water and dried, followed by measurement using an ellipsometer (DVA-36LS, Mizojiri Optical Co., Tokyo, Ltd.) in air to determine the thickness of the adsorbed and remaining PEG-diamine molecule layer. The use of the ellipsometer resulted in the underestimation of the thickness compared with 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 TiO_2 with the Ti substrate were 2.209 and 3.079,^{22,23)} and those of the Ti substrate were 2.22 and 2.99,²⁴⁾ respectively.

2.3 Electrodeposition behavior of PEG-diamine using QCM

In this experiment, another type of QCM system was employed, the quartz crystal of the A-T cut type, exhibiting a fundamental resonant frequency of 10 MHz (Hokuto Denko, Tokyo, Japan). The exposed area of the electrode was 0.07 cm^2 . The immobilization behavior of PEG-diamine to Ti under charging cathodic potential was investigated using QCM. Platinum was used as the counter electrode and SCE as the reference electrode. In QCM, Ti-QCM electrode was immersed in the PEG-diamine solution for 10 min, followed by charging at -1 and -3 V_{SCE} for 20 min. Meanwhile, the Δf of the crystal was stabilized in 0.3-mol L^{-1} NaCl solution for 10 min before injection of the PEG-diamine solution. After 10 min, concentrated PEG-diamine solution was injected with a syringe at a concentration of 2 mass%. The Δf of the crystal was monitored for 20 min. Whole PEG-diamine molecules adsorbed onto the Ti surface were detected by QCM.

2.4 Change in the thickness of immobilized layer of PEG-diamine using an ellipsometer

A commercially pure Ti disk ($8\text{ mm}\phi \times 2\text{ mm}$ in thickness) with grade 2 was metallographically polished and ultrasonically rinsed in acetone, ethanol, and deionized water (Millipore). The Ti disk was fixed in a chamber for simultaneous characterization using the potentiostat and ellipsometer, as illustrated in Fig. 2. The OCP of Ti vs. SCE was measured before electrodeposition. Thereafter, the cathodic potential, -3 V_{SCE} , was charged for 300 s, followed by the measurement using the ellipsometer *in situ*. The thickness of the PEG-diamine layer deposited on Ti was determined with the ellipsometer. These electrodeposition and thickness measurement were repeated four times, respectively. In addition, the anodic potential $+0.1\text{ V}_{\text{SCE}}$ higher than that of OCP was charged for 300 s, followed by the measurement using the ellipsometer *in situ*. This process was repeated three times. The experimental flowchart is shown in Fig. 3. The reflective index of the solution was 1.33. The refractive index and absorption coefficient of the Ti substrate in the solution were 2.40 and 3.04, respectively.

Fig. 2 Electrochemical cell chamber of ellipsometry *in situ* measurement.

Fig. 3 Flowchart of *in situ* measurement process of ellipsometry.

2.5 Change in mass during electric charge using QCM

For this experiment, besides the above 0.02-mol L^{-1} PEG-diamine solution (pH 11), 2-mol L^{-1} NH_3 + 0.3-mol L^{-1} NaCl solution and 0.3-mol L^{-1} NaCl solution, adjusted to pH 11, were employed. In QCM, Ti electrode on quartz was immersed in the above solutions for 10 min, followed by the charging from OCP to $-1.7\text{ V}_{\text{SCE}}$, with a sweep rate of $2 \times 10^{-3}\text{ V s}^{-1}$, and the Δf of Ti-QCM electrode at room temperature was monitored.

2.6 Cathodic polarization

For this experiment, 0.02-mol L^{-1} PEG-diamine + 0.3-mol L^{-1} NaCl solutions, 0.01-mol L^{-1} PEG-diamine + 0.3-mol L^{-1} NaCl solution, 0.02-mol L^{-1} NH_3 + 0.3-mol L^{-1} NaCl solution, and 0.3-mol L^{-1} NaCl solution, adjusted to pH 11, were employed. The same Ti disk, as described above, was fixed in a polytetrafluoroethylene holder. The exposed area contacting the electrolyte was 38.5 mm^2 . Ti disk was immersed in the above solutions for 10 min, followed by charging from OCP to $-1.7\text{ V}_{\text{SCE}}$, which was the range in which data were obtained stably with a sweep rate of $2 \times 10^{-3}\text{ V s}^{-1}$, as a significant outbreak of gas did not occur from the electrodes. The current response was measured using a potentiostat (HAB-501A, Hokuto Denko, Tokyo, Japan).

Fig. 4 Time transient of the frequency shift after the injection of each PEG-diamine solution measured by QCM. The decreasing frequency shift indicates the increase in PEG-diamine molecules adsorbed onto the Ti surface.

Fig. 5 Thickness of the chemically adsorbed PEG layer on a Ti surface determined by ellipsometry. The bars represent statistically significant differences ($p < 0.01$).

3. Results

3.1 Adsorption behavior of PEG-diamine

The time transient of the Δf after the injection of each solution measured by QCM is shown in Fig. 4. The decreasing Δf indicates the increase in mass and/or fluctuation of the adsorbed molecule. Immediately after injection of the PEG-diamine solution, the mass abruptly increased and instantly decreased, followed by a gradual increase. In this experiment, Δf represents the mass change due to the adsorption and desorption of PEG-diamine molecules from the Ti surface. More PEG-diamine molecules were adsorbed by Ti in pH 11 than in pH 3. In addition, Δf for Unmodified-PEG and PEG-diamine were almost identical.

The thickness of the remaining PEG-diamine layer on Ti-QCM electrode immersed in pH 11 solution was much larger than that in pH 3 solution, as shown in Fig. 5. In the case of Unmodified-PEG without termination by amine, the thickness of the adsorbed layer at pH 11 was almost identical to that at pH 3.

3.2 Adsorption behavior of PEG-diamine

The mass shift (Δm) converted from Δf of Ti-QCM

Fig. 6 Mass shift of the PEG-diamine layer on a Ti surface by QCM measurement. (A) $-3 V$ vs. SCE potential applied after PEG-diamine injection, (B) $-1 V$ vs. SCE potential applied after PEG-diamine injection, and (C) $-1 V$ vs. SCE potential applied before PEG-diamine injection.

electrode before and after injection of the concentrated PEG-diamine solution and charging $-3 V_{\text{SCE}}$ is shown in Fig. 6(A). Just after the injection of the PEG-diamine solution, the mass abruptly increased, indicating the immediate physical and chemical adsorption of PEG-diamine onto Ti-QCM electrode. After charging $-3 V_{\text{SCE}}$, hydrogen evolution from the electrode occurred, and H_2 bubbles disturbed the data acquisition. After the termination of electric charging, the mass is measured to be larger than that before electric charging. More PEG-diamine molecules were adsorbed onto the electrode by the cathodic charge.

The Δm before and after injection of the concentrated PEG-diamine solution during the charge of $-1 V_{\text{SCE}}$ is shown in Fig. 6(B). Just after the injection of the PEG-diamine solution, the mass abruptly increased, indicating that immediately physical and chemical adsorption of PEG-diamine to Ti-QCM electrode occurs. Even after charging $-1 V_{\text{SCE}}$, Δm was stable but gradually decreased. This indicates that the PEG-diamine molecules were attracted to Ti-QCM electrode and condensed on the surface without H_2 bubbling. The cathodic charge of $-1 V_{\text{SCE}}$ did not adsorb any extra PEG-diamine molecules because of its weak attractive force. After the termination of electric charging, the

mass slightly increased owing to the relaxation from the attractive force to Ti-QCM electrode.

The Δm of Ti-QCM electrode cathodically charged $-1 V_{\text{SCE}}$, and the injection of the concentrated PEG-diamine solution is shown in Fig. 6(C). When the Ti-QCM electrode was cathodically charged, no mass change was observed, because PEG-diamine molecule was absent. Just after injection of the PEG-diamine solution, the mass abruptly increased, indicating the immediate physical and chemical adsorption of PEG-diamine to Ti-QCM electrode. After the termination of electric charging, the mass slightly increased owing to the relaxation from the attractive force to Ti-QCM electrode.

3.3 Change in the thickness of immobilized layer of PEG-diamine using an ellipsometer

The change in the thickness of the PEG-diamine layer on Ti electrode at 5, 10, 15, and 20 min after electrodeposition measured *in situ* by the ellipsometer is shown in Fig. 7. The thickness at 5 min after electrodeposition could not be measured. The thickness increased with electrodeposition time. More electrodeposited PEG-diamine molecules corresponded to longer cathodic potential charging time. After electrodeposition, the thickness increased once at 5 min, followed by a gradual decrease.

The change in the thickness of the PEG-diamine layer by the anodic potential charge is shown in Fig. 8. After electrodeposition of PEG-diamine molecules to Ti electrode with $-3 V_{\text{SCE}}$ for 5 min four times and the termination of electric charging, the thickness of the molecular layer increased once and then gradually decreased, as mentioned above. After 30 min remaining, an anodic potential $+0.1 V_{\text{SCE}}$ higher than that of OPC was charged for 5 min, and left for 20 min. Despite repeating this process, the thickness did not change by charging the anodic potential.

3.4 Change in mass during electric charge

The change in the Δm of Ti-QCM electrode in the PEG-diamine solution when the potential decreased from OCP to $-1.7 V_{\text{SCE}}$ and increased to $-0.5 V_{\text{SCE}}$ is shown in Fig. 9(A). Fluctuation on the cathodic polarization curve was caused by H_2 gas bubbling by hydrogen evolution, decreasing once

Fig. 7 Change in the thickness of the PEG-diamine layer on Ti surface after electrodeposition for 1, 5, 10, 15, and 20 min determined by ellipsometry *in situ* measurement.

Fig. 8 Thickness shift of the PEG-diamine layer on a Ti surface after 20-min electrodeposition and application of +0.1 V vs. SCE.

Fig. 9 Mass shift applied potential between open circuit potential and -1.7 V vs. SCE in (A) PEG-diamine + NaCl solution, (B) in $\text{NH}_3(\text{aq})$ + NaCl solution, and (C) NaCl solution measured by QCM.

on the anodic polarization curve at -1.3 V_{SCE}. The mass gradually increased with cathodic polarization from OCP to -1.4 V_{SCE}, increasing more significantly with cathodic charge from -1.4 to -1.7 V_{SCE} and continuously increased with anodic polarization from -1.7 to -0.5 V_{SCE}.

The change in the Δm of Ti-QCM electrode in the NH_3 + NaCl solution when the potential decreased from OCP to -1.7 V_{SCE} and increased to -0.3 V_{SCE} is shown in Fig. 9(B). The mass decreased with cathodic polarization

Fig. 10 Cyclic voltammetry curve of Ti in (A) 0.02-mol L^{-1} NH_3 + 0.3-mol L^{-1} NaCl solution, (B) 0.02-mol L^{-1} PEG-diamine + 0.3-mol L^{-1} NaCl solution, (C) 0.01-mol L^{-1} PEG-diamine + 0.3-mol L^{-1} NaCl solution, and (D) 0.3-mol L^{-1} NaCl solution, adjusted to pH 11.

from OCP to *ca.* -1.4 V_{SCE} and increased to -1.7 V_{SCE}. The mass also increased after anodic polarization until -1.4 V_{SCE} and decreased until -0.3 V_{SCE}.

The change in the Δm of Ti-QCM electrode in NaCl solution when the potential decreased from OCP to -1.7 V_{SCE} and increased to -0.4 V_{SCE} is shown in Fig. 9(C). The mass decreased with cathodic charge from OCP to *ca.* -1.7 V_{SCE} and continuously decreased to -0.4 V_{SCE} on anodic polarization.

3.5 Cathodic polarization

Polarization curves by cyclic voltammetry from OCP to -1.7 V_{SCE} represented by an actual amount of current are shown in Fig. 10. From OCP to -1.3 V_{SCE}, all curves overlap. From approximately -1.3 V_{SCE}, the current density in solution with NH_3 molecules increased and reached the highest current density value among the four types of solutions at -1.7 V_{SCE} (Fig. 10(A)). The current density in two types of solutions with PEG-diamine and NaCl solution without either PEG-diamine or NH_3 molecules increased from approximately -1.5 V_{SCE}. These current density values were lower than those in NH_3 solution. Comparing current density values in these three solutions at -1.7 V_{SCE}, 0.02-mol L^{-1} PEG-diamine was the highest (Fig. 10(B)) and the second highest was 0.01-mol L^{-1} PEG-diamine (Fig. 10(C)), whereas NaCl solution without either PEG-diamine or NH_3 molecules was the lowest (Fig. 10(D)).

Cathodic polarization curves of Ti in the PEG-diamine solution and NaCl solution without PEG-diamine molecules from OCP to -1.7 V_{SCE}, represented by an actual amount of current density, are shown in Fig. 11(A). From OCP to -1.4 V_{SCE}, both curves overlap. From -1.4 to -1.6 V_{SCE}, the current density in solution with PEG-diamine molecules was smaller than that without molecules. Meanwhile, from -1.6 to -1.7 V_{SCE}, the current density in solution with PEG-diamine molecules was larger than that without molecules. From -1.4 to -1.6 V_{SCE}, PEG-diamine molecules were physically and chemically adsorbed, working as a barrier, and the resulting electric resistance increased, inducing a decrease in the current in the PEG-diamine solution. From -1.6 to

Fig. 11 Cyclic voltammetry curve of 2 mass% PEG-diamine + 0.3-mol L⁻¹ NaCl, 0.3-mol L⁻¹ NaCl electrolyte, and 0.3-mol L⁻¹ NaCl electrolyte (A) from open circuit potential to -1.7 V vs. SCE, (B) from -1.7 V vs. SCE to open circuit potential, and (C) three types of electrolyte, 1 mass% PEG-diamine + 0.3-mol L⁻¹ NaCl, 2 mass% PEG-diamine + 0.3-mol L⁻¹ NaCl, and 0.3-mol L⁻¹ NaCl from OCP to -1.7 V vs. SCE.

-1.7 V_{SCE}, the electrode reaction relating to PEG-diamine molecules generated a larger cathodic current in the solution with PEG-diamine molecules than that without PEG-diamine.

Meanwhile, polarization curves of Ti in the PEG-diamine solution and NaCl solution without PEG-diamine molecules from -1.7 V_{SCE} to OCP, represented by an actual amount of current density, are shown in Fig. 11(B). From -1.7 to -1.4 V_{SCE}, the current density in solution with PEG-diamine molecules was larger than that without PEG-diamine. Therefore, anodic polarization induced a different Ti surface state from the charging cathodic current. PEG-diamine molecules are clearly and consecutively influenced by the electrode reaction.

4. Discussion

As indicated by Fig. 4, more PEG-diamine molecules were adsorbed by Ti in pH 11 than in pH 3. In addition, the Δf in Unmodified-PEG and PEG-diamine were almost the same. The adsorbed molecule mass in pH 11 was estimated as 4.1 $\mu\text{g cm}^{-2}$ corresponding to approximately 25 molecules per nm². The concentration of active hydroxyl groups on Ti is 12 nm⁻².²⁵ Therefore, no adsorbed molecules existed on

the first layer of the Ti substrate but rather on the second and over layers, whose bonding force was possibly weak. Meanwhile, the adsorbed amount was relatively small in pH 3. The point of zero charge of TiO₂, covering Ti, is 6.7 in the case of anatase and 5.2 for rutile.²⁶ Therefore, the Ti surface is positively charged at pH 3 and negatively charged at pH 11. PEG-diamine molecules are positively charged in solution. Meanwhile, despite the lack of electric charge on Unmodified-PEG, the electrostatic force for adsorption between these molecules and Ti surface was much larger at pH 11 than at pH 3. The surface electric charge of Ti substrate directly influences the adsorption of PEG-diamine and Unmodified-PEG molecules. In the case of Unmodified-PEG without termination with amine, the thickness of the adsorbed layer at pH 11 was almost the same as that at pH 3 (Fig. 5). This indicates that Unmodified-PEG does not have a sufficient amount of electric charge to remain on the Ti surface under rinsing in deionized water, and the adsorption is not influenced by pH or surface electric charge of Ti. Meanwhile, the thickness of Unmodified-PEG was much smaller than that of PEG-diamine at pH 11, whereas the Δf in the case of the QCM of Unmodified-PEG and that of PEG-diamine were the almost same. PEG-diamine molecules were both physically and chemically adsorbed onto Ti, because the molecule was positively charged, and the Ti surface was negatively charged. Although Unmodified-PEG molecules undergo polarization due to hydroxyl groups, its effect is very small. These molecules were only physically adsorbed onto Ti surface. Therefore, before ellipsometry was performed, more Unmodified-PEG molecules were removed during rinsing, whereas more PEG-diamine molecules remained. The thickness of Unmodified-PEG was eventually much smaller than that of PEG-diamine at pH 11.

As observed in Fig. 6, the immediate physical and chemical adsorption of PEG-diamine to Ti-QCM electrode occurred just after the immersion of Ti in PEG-diamine solution; further, more PEG-diamine molecules were adsorbed onto the electrode by the cathodic charge.

By measuring the change in the thickness of the immobilized layer of PEG-diamine using an ellipsometer (Fig. 7), it was revealed that PEG-diamine molecules were physically and chemically adsorbed by Ti electrode, attracted to the surface, and condensed on the surface. More PEG-diamine molecules were attracted to the surface during electrodeposition. After the termination of electrodeposition, bound molecules were at once relieved and swelled, inducing the apparent increase in the thickness. Thereafter, physically adsorbed molecules in the outside of PEG-diamine layer were gradually separated from the surface, and the apparent thickness decreased with time for convergence to a certain value. PEG-diamine molecules once electrodeposited were not detached from the Ti electrode by the charging anodic potential (Fig. 8). In other words, the bonding of PEG-diamine molecules to the Ti surface by electrodeposition was strong and did not detach by anodic potential charge.

In Fig. 9(A), PEG-diamine molecules immobilized by electrodeposition with a cathodic charge to -1.7 V_{SCE} were condensed on the Ti-QCM electrode surface. This condensation appeared as the increase in mass, and this tendency

clearly appeared from -1.4 to -1.7 V_{SCE}. This apparent increase in mass continued, even after the commencement of anodic polarization because immobilized and condensed PEG-diamine molecules swelled with the decrease in cathodic potential. In the case of NH₃, immobilization of NH₃ occurred from -1.4 V_{SCE} in cathodic polarization along with that of PEG-diamine. In anodic polarization, the immobilization continued until -1.4 V_{SCE} and disappeared over -1.4 V_{SCE} (Fig. 9(B)). Because NH₃ is the minimum unit of amine and does not swell, unlike PEG-diamine molecule, the influence of the swelling of NH₃ molecules was very small. The increase in the net mass with electro-deposition occurred in the potential range between -1.4 V_{SCE} and -1.7 V_{SCE}. However, there was no clear increase in the mass in the QCM measurement in NaCl solution without PEG-diamine and NH₃ (Fig. 9(C)). This result supports the increase in mass with the immobilization of PEG-diamine and NH₃ molecules.

In Fig. 10, the current density was influenced by the molecules in the electrolyte. The existence of molecules with amino group in the solution caused an increase in the current density. In relation to the two different solutions of PEG-diamine, higher concentration of these molecules corresponded to a higher current density. This indicates that the amino group received electrons from the cathodic electrode.

Here, we attempt to estimate the quantity of electricity that increased by existence of the PEG-diamine molecules in the electrolyte of cyclic voltammetry. In the ranges from -1.6 to -1.7 V_{SCE} and -1.7 to -1.4 V_{SCE}, the current density measured in the solution with PEG-diamine molecules was larger than that without PEG-diamine molecules. The cyclic voltammetry curves were approximated by an exponential function as

$$J = ae^{bE} \quad (1)$$

where J is current density (A cm⁻²), E is potential (V), e is Napier's constant, and a and b are constants. Meanwhile, potential E in eq. (1) is related to time t as

$$E = ct \quad (2)$$

where c is sweep ratio (V sec⁻¹) equal to 2×10^{-3} V s⁻¹. Therefore,

$$J = ae^{bct}. \quad (3)$$

The amount of electricity per unit area Q (C cm⁻²) consumed by the cathodic electrode reaction in the range from E_1 (time: t_1) to E_2 (time: t_2) is as follows:

$$Q = \int_{t_1}^{t_2} J dt = \int_{\frac{E_1}{c}}^{\frac{E_2}{c}} ae^{bct} dt = \frac{a}{bc} \left[e^{\frac{bcE_2}{c}} - e^{\frac{bcE_1}{c}} \right]. \quad (4)$$

Subscripts "PEG1" and "PEG2" refer to the potential range from -1.6 to -1.7 V_{SCE} and -1.7 to -1.4 V_{SCE} in the solution with PEG-diamine molecules, respectively. Current densities J_{PEG1} and J_{PEG2} were approximated as follows:

$$J_{\text{PEG1}} = -8.43 \times 10^{-18} \cdot e^{-19.2E} \quad (R^2 = 0.999) \quad (5-1)$$

$$J_{\text{PEG2}} = -1.65 \times 10^{-12} \cdot e^{-12.0E} \quad (R^2 = 0.997) \quad (5-2)$$

where R is the correlation coefficient.

The total quantity of electricity per unit area Q_{PEG} in the solution with PEG-diamine molecules in the potential ranges

from -1.6 to -1.7 V_{SCE} and -1.7 to -1.4 V_{SCE} was calculated as follows:

$$\begin{aligned} Q_{\text{PEG}} &= \int_{\frac{-1.6}{2 \times 10^{-3}}}^{\frac{-1.7}{2 \times 10^{-3}}} (-8.43 \times 10^{-18}) e^{-19.2 \times 2 \times 10^{-3} t} dt \\ &\quad + \int_{\frac{-1.7}{2 \times 10^{-3}}}^{\frac{-1.4}{2 \times 10^{-3}}} (-1.65 \times 10^{-12}) e^{-12.0 \times 2 \times 10^{-3} t} dt \\ &= (-2.70 \times 10^{-2}) + (-4.88 \times 10^{-2}) \\ &= -7.58 \times 10^{-2} (\text{C cm}^{-2}). \end{aligned} \quad (6)$$

Subscripts "NO1" and "NO2" refer to the potential ranges from -1.6 to -1.7 V_{SCE} and -1.7 to -1.4 V_{SCE} in the solution without PEG-diamine molecules, respectively. Current densities J_{NO1} and J_{NO2} were approximated as

$$J_{\text{NO1}} = -1.13 \times 10^{-13} \cdot e^{-13.3E} \quad (R^2 = 0.999) \quad (7-1)$$

$$J_{\text{NO2}} = -1.41 \times 10^{-11} \cdot e^{-10.4E} \quad (R^2 = 0.987) \quad (7-2)$$

The total quantity of electricity per unit area Q_{NO} in the solution without PEG-diamine molecules in the potential ranges from -1.6 to -1.7 V_{SCE} and -1.7 to -1.4 V_{SCE} was calculated as follows:

$$\begin{aligned} Q_{\text{NO}} &= \int_{\frac{-1.6}{2 \times 10^{-3}}}^{\frac{-1.7}{2 \times 10^{-3}}} (-1.13 \times 10^{-13}) e^{-13.3 \times 2 \times 10^{-3} t} dt \\ &\quad + \int_{\frac{-1.7}{2 \times 10^{-3}}}^{\frac{-1.4}{2 \times 10^{-3}}} (-1.41 \times 10^{-11}) e^{-10.4 \times 2 \times 10^{-3} t} dt \\ &= (-2.09 \times 10^{-3}) + (-2.96 \times 10^{-2}) \\ &= -5.05 \times 10^{-2} (\text{C cm}^{-2}). \end{aligned} \quad (8)$$

Therefore, the electrical quantity ΔQ , which increased with the existence of PEG-diamine molecules in solution, is calculated as follows:

$$\begin{aligned} \Delta Q &= Q_{\text{PEG}} - Q_{\text{NO}} = -7.58 \times 10^{-2} - (-5.05 \times 10^{-2}) \\ &= -2.53 \times 10^{-2} (\text{C cm}^{-2}). \end{aligned} \quad (9)$$

This ΔQ was converted to the number of electrons per nm², N , which was consumed by the electrode reaction related to PEG-diamine molecules,

$$N = 1.58 \times 10^3 (\text{nm}^{-2}). \quad (10)$$

Meanwhile, it is known that the concentration of hydroxyl groups on the surface oxide layer of Ti is *ca.* 12 nm^{-2.25}. Therefore, most of the electrons are considered to be consumed in the electrode reactions in addition to the reaction between these hydroxyl groups and PEG-diamine molecules.

The ionization equilibrium equation of PEG-diamine is as follows:

Because, the pH of 2 mass% PEG-diamine solution was 11.3, its electrolytic dissociation constant was estimated as 1.94×10^{-7} , with 5.0% of PEG-diamine ionized. Considering that the molecules of PEG-diamine are attracted to the surface of the cathode electrode, the reaction in which PEG-diamine molecules received electrons may proceed as follows:

Fig. 12 Immobilization mechanism of PEG-diamine molecule to titanium surface.

In this reaction, PEG-diamine molecules catalytically enhanced hydrogen evolution.²⁷⁾

In this electrodeposition process, because Ti disk was immersed into the solution with PEG-diamine molecules to measure OCP before electrodeposition, it is considered that amines randomly exist as NH_3^+ in the PEG molecule groups (Fig. 12(A)). As soon as $-3 \text{ V}_{\text{SCE}}$ is applied, the layer of PEG-diamine molecules condensed on the Ti surface, hydrogen was generated, and the pH increased near the Ti surface (Fig. 12(B)). Ionizing PEG-diamine molecules lost electric charge at one instance, and the electrostatic force between PEG-diamine molecule and hydroxyl group on the Ti surface oxide became weak (Fig. 12(C)-I). PEG-diamine molecules are slightly separated from the Ti surface. These molecules ionize again because of decreasing pH (Fig. 12(C)-II) and immobilize on the Ti surface again (Fig. 12(C)-III). In this manner, PEG-diamine molecules do not tightly remain on the Ti surface under electrodeposition but shake around the Ti surface while undergoing repeated ionization and un-ionization. Because of these motions of PEG-diamine molecules near the Ti surface, the structure of the PEG-diamine layer is assumed to rearrange to a U-shape structure (Fig. 12(D)).

5. Conclusion

Surface electric charge directly influenced the adsorption of PEG molecule in which both terminals of PEG were terminated with NH_2 (PEG-diamine) and Unmodified-PEG

molecules. PEG-diamine molecules were attracted to the Ti-QCM electrode and condensed on the surface. After the termination of electric charging, the relaxation from the attractive force to the Ti-QCM electrode occurred. Bonding of the PEG-diamine molecules with the Ti surface by electrodeposition was strong and did not detach by anodic potential charge. PEG-diamine was immediately adsorbed onto the Ti surface by electrostatic force with a weak force in a random manner, and rearranged and condensed on the Ti surface with electrochemical reactions between the molecules and Ti surface by cathodic electric charge. In this manner, PEG-diamine molecules do not tightly remain on the Ti surface under electrodeposition but shake around the Ti surface while undergoing repeated ionization and un-ionization. The elucidation of this electrodeposition mechanism will help reveal the electrodeposition phenomenon of other biofunctional molecules to metal surfaces.

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