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Effect of pH on the interaction between zwitterions and titanium oxide

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ABSTRACT

The isoelectric points (IEPs) of two zwitterions, glycine and both-terminals-terminated poly(ethylene glycol) (NH_2–PEG–COOH), were determined from the titration curves, and the thicknesses of zwitterion layers immobilized on titanium (Ti) with immersion and electrodeposition at various pH based on IEPs were evaluated with ellipsometry to investigate the effect of pH and the immobilization technique on the interactions between the zwitterions and the Ti surface. From the titration curves, pK_1, pK_2, and the IEP of glycine were determined as 2.8, 8.9, and 5.9, respectively, and pK_1, pK_2, and the IEP of NH_2–PEG–COOH were determined as 2.1, 11.7, and 6.9, respectively. At a certain specific pH, ^+H_3N–CH_2–COO^- or ^+H_3N–PEG–COO^- was formed by hydrolysis of glycine or NH_2–PEG–COOH. In addition, the Ti surface was negatively charged at this pH. As a result, for immersion, the electrostatic reactivity between terminal groups of zwitterions and hydroxyl groups on the Ti surface was the highest and the thickness of the immobilized layer was significantly the largest at pH 12. For electrodeposition, glycine, with its lower molecular weight, was more easily attracted to the Ti surface than NH_2–PEG–COOH, which has a higher molecular weight, while the thickness of the immobilized layer was the greatest at pH 12 in both zwitterions.

1. Introduction

When a metal is implanted in the human body, the initial reaction between the metal surface and the biological environment is protein adsorption onto the surface. The protein adsorption in this initial stage governs the adhesion of cells and bacteria and even the tissue compatibility of the metal. Platelet aggregation on an implanted material is activated by adsorbed proteins, and a thrombus is formed [1]. The initial bacterial adhesion, followed by a biofilm formation, is also considered to be related to protein adsorption [2]. Thus, protein adsorption onto the surface must be inhibited at the initial stage according to the purpose of the materials. On the other hand, cell growth and differentiation on a material are necessary events for hard and soft tissue compatibility of the material [3]; they are generally controlled by the adsorption of protein onto the material.

Poly(L-lysine)–*grafted*–poly(ethylene glycol) (PLL–*g*–PEG) immobilization onto metals has been extensively studied and is highly effective in reducing the adsorption of blood serum, blood plasma, and single proteins such as fibrinogen and albumin [4,5]. Immobilization of the cell-adhesive peptide Arg–Gly–Asp (RGD) onto PLL–*g*–PEG on metals has recently been studied as a way to induce the specific attachment of fibroblasts and osteoblasts [6]. The advantage of this immobilization is the enhancement of the attachment of fibroblasts and osteoblasts by the RGD motif, but the inhibition of bacterial adhesion as a result of the PEG part continues. Thus, two opposing functions on metals, the attachment of fibroblasts and osteoblasts with RGD and the inhibition of bacterial adhesion with PEG, are achieved by mediating zwitterions.

Zwitterions with a –COOH group and a $-NH_2$ group, such as amino acids, are generally effective in mediating molecules. One terminal group is useful to bond biofunctional molecules such as RGD. At a certain pH, known as the isoelectric point (IEP), the $-NH_2$ group is positively charged and the –COOH group is negatively charged. The exact IEP values are specific to different amino acids. On the other hand, the other terminal group is required to bind stably with a surface oxide on a metal. The surface oxide film of a metal is usually covered by hydroxyl groups. The hydroxyl groups are positively or negatively charged according to the pH of the surrounding solution. The surface charge is apparently zero at a certain pH, which is known as the point of zero charge (pzc). Therefore, a zwitterion-mediated surface for the immobilization of biofunctional molecules such as RGD is created with consideration of the interaction between the charged zwitterion and the charged metal surface in a solution. However, little information on this interaction has been available. In addition, the immobilization technique with PLL–*g*–PEG includes multistage techniques, such as

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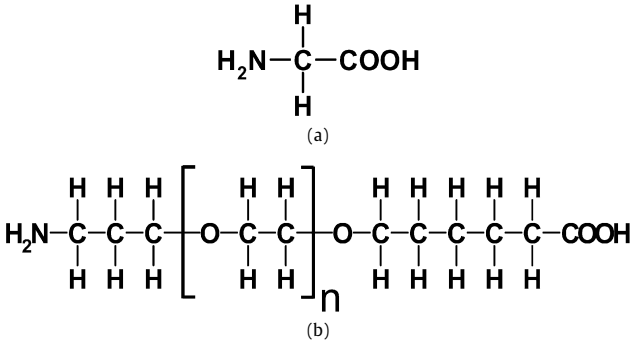

Fig. 1. Chemical structures of glycine and zwitterionic NH_2–PEG–COOH for immobilization on Ti with immersion and electrodeposition.

synthesis, polymerization, and immersion, and the immobilization strength of PLL–*g*–PEG on the metals is unclear.

Therefore, a simple immobilization technique with consideration of the IEP and pzc is necessary. In our current studies, we reported that commercially available PEG terminated with the –NH_2 group at both terminals (NH_2–PEG–NH_2) immobilized onto titanium (Ti) offered several biofunctions, such as the inhibition of protein adsorption and platelet adhesion [7]. The manner of NH_2–PEG–NH_2 immobilization onto Ti was characterized by X-ray photoelectron spectroscopy and glow-discharge optical emission spectroscopy [8]. In the first layer of NH_2–PEG–NH_2 immobilized with electrodeposition, more terminated amines exist at the interface between the NH_2–PEG–NH_2 layer and Ti oxide (TiO_2) and combine with TiO_2 as an NHO ionic bond formed between NH_3^+ and O^-, while more amines randomly exist as NH_3^+ in the NH_2–PEG–NH_2 layer immobilized by immersion. Immobilization of NH_2–PEG–NH_2 is directly relevant to an active hydroxyl group on TiO_2; in fact, the immobilized amount increased with increased the concentration of active hydroxyl groups [9].

The objective of this study was to determine the IEPs from the titration curve of zwitterions and to analyze the interactions between the charged zwitterions and charged hydroxyl groups on Ti at various pHs based on IEPs. The thicknesses of the zwitterion layers immobilized with immersion and electrodeposition at various pHs were determined with ellipsometry, and the effect of pH and the effect of the immobilization technique on the interactions between zwitterions and the Ti surface was investigated.

2. Experimental

2.1. Determination of IEPs and the dissociation constants from titration curves

We employed glycine (G7126, MW = 75.07, Sigma–Aldrich Japan, Japan) and both-terminals-terminated PEG with amine and carboxyl groups (NH_2–PEG–COOH, SUNBRIGHT PA-020HC, MW = 2162, NOF Corporation, Japan). The chemical structures of glycine and NH_2–PEG–COOH are shown in Fig. 1. Glycine and NH_2–PEG–COOH were dissolved in a 0.5 mol L^{-1} NaCl solution with a concentration of 1 mmol L^{-1} in the case of electrodeposition, while they were dissolved in distilled water without NaCl in the case of immersion. The original pHs of glycine and NH_2–PEG–COOH solutions were 5.9 and 4.1, respectively. First, these solutions were adjusted to pH 1 with the addition of 1 mol L^{-1} HCl. Their pK_1*s*, pK_2*s*, and IEPs were determined from the titration curves of the glycine and NH_2–PEG–COOH solutions obtained by titrating 1 mol L^{-1} NaOH from pH 1 to 12.

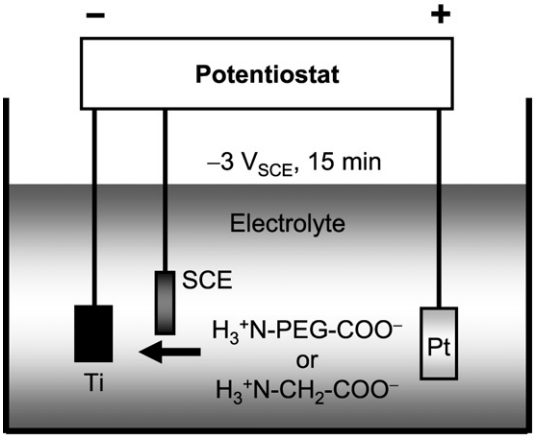

Fig. 2. Schematic illustration of electrodeposition. During cathodic polarization, ionized glycine or NH_2–PEG–COOH migrated to the cathode (Ti).

2.2. Immobilization of glycine and NH_2–PEG–COOH

Commercially pure Ti disks 8 mm in diameter and 1–2 mm in thickness were obtained from rods (Rare Metallic Co., Ltd., Japan). The Ti disks were mirror-polished with SiC paper with 320 and 600 grid, a 9-μm diamond suspension, and a 0.04-μm colloidal silica suspension. The Ti disks were cleaned from macroscopic contamination by ultrasonication in acetone for 15 min and dried with a stream of nitrogen (99.9%). Glycine and NH_2–PEG–COOH were immobilized on the Ti with immersion and electrodeposition in glycine solutions with pH 0, 3, 6, 9, and 12 and NH_2–PEG–COOH solutions with pH 2, 4, 7, 10, and 12, respectively. Those pH values were selected on the basis of the IEP determined with the titration. In the case of immersion, the Ti was immersed in the above solution without NaCl at 310 K for 24 h. In the case of electrodeposition, the Ti was fixed in a polytetrafluoroethylene holder that was insulated from the electrolyte with the exception of an open window (28.3 mm^2). The open circuit potential (OCP) of Ti against a saturated calomel electrode (SCE) before electrodeposition was measured. Thereafter, the cathodic potential was applied from the OCP to -3 V_{SCE} and maintained at this potential at 310 K for 15 min. During cathodic polarization, glycine or NH_2–PEG–COOH migrated to the cathode (Ti), where it was immobilized as shown in Fig. 2. After immersion and electrodeposition, each specimen was rinsed in distilled water and dried with a stream of nitrogen (99.9%).

2.3. Thickness and surface topography of glycine and the NH_2–PEG–COOH immobilized layer

The thicknesses of glycine and the NH_2–PEG–COOH layer immobilized on Ti with electrodeposition and immersion were determined with ellipsometry (DVA-36Ls, Mizojiri Optical Co., Ltd., Japan) in air. The light source was a He–Ne laser with a wavelength of 632.8 nm, and the incident angle to the surface of the specimen was 70°. The thickness was calculated using a refraction index (n) and an extinction coefficient (k) obtained from an original Ti surface without glycine or the NH_2–PEG–COOH layer: the n and k of Ti were 2.39 and 3.07, respectively.

In addition, the NH_2–PEG–COOH layer immobilized on Ti with electrodeposition was imaged in the dynamic force mode with scanning probe microscopy (SPM, SPI3700, SII NanoTechnology, Inc., Japan) using silicon cantilevers (spring constant, 16 N m^{-1}; resonance frequency, 137 kHz; SI-DF20 K-A102001604, SII NanoTechnology, Inc., Japan) in air. The imaging size was 1×1 μm.