Bioinspired phospholipid polymer for improvement of biofouling on titanium alloy substrate

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Inspired by mussel adhesion mechanism, a new bioinspired polymer containing phospholipid polar groups for ultra-low biofouling was designed. The polymer (named as PMDP) was composed of both 2-methacryloyloxyethyl phosphorylcholine (MPC) unit and 3,4-dihydroxyphenyl group for surface anchoring. The PMDP coating with 20 nm in thickness could be formed on titanium (Ti) alloy substrate simply by 10s dip coating without drying process. Even when the Ti alloy substrate with PMDP coating was immersed in the aqueous medium for a week, no change in the thickness was observed, that is, the PMDP adhered very stably. Adsorption of protein occurred on the Ti alloy substrate; however, it could not be observed on the PMDP coated Ti alloy substrate. Thus, the PMDP coating was effective to suppress biofouling. It is concluded that the surface treatment process with PMDP is quite simple, quick and reliable, which has great potential for improvement of biofouling on the Ti alloy substrate as implant devices.

Key words: surface modification, phospholipid polymer, 3,4-dihydroxyphenyl groups, titanium alloy substrate, reduced biofouling

1. INTRODUCTION

Titanium (Ti) alloys have many desirable properties, such as relatively low modulus, good fatigue strength, formability, machinability, and corrosion resistance. Accordingly, they are widely used in biomedical devices and components since the late 1970s, especially in cardiaic and cardiovascular applications (e.g. prosthetic heart valves, protective cases in pacemakers, artificial hearts, stents, and circulatory devices) [1]. However, the Ti alloy substrates induce biological responses, such as thrombus formation and tissue reaction, which are initiated from protein adsorption and deformation [2]. As a result, anticoagulant therapy is necessary to minimize the risk of thromboembolic complications. Therefore, surface modification of the Ti alloy substrate is indispensable to improve its biocompatibility.

Inhibiting the protein adsorption is a major strategy for improvement of biocompatibility. Protein adsorption is the first essential event followed by the biological responses, such as acute thrombus formation, inflammation, and later fibrous encapsulation, bacterial adhesion, inflection [3]. It is generally believed that reducing protein adsorption can significantly attenuate these adverse responses. Poly(ethylene glycol) (PEG) systems have been extensively studied as biofouling-resistant materials [4]. However, since the PEG based systems are susceptible to degradation by spontaneous oxidation under physiological condition, these systems lacks long-term stability, which reduces their effectivity as surface coating [5].

Another promising and effective method is to prepare the artificial cell membrane surface using phospholipid polymers. The represented polymers have been synthesized using 2-methacryloyloxyethyl phosphorylcholine (MPC) [6], which bear phosphorylcholine groups (the main component of outer cell membrane) in the side chain. The MPC polymers are widely applied for surface modification of implantable medical devices and artificial organs [7-10].

There are many reports on surface immobilization of the MPC polymers on Ti alloy substrate. However, these methods have many limitations for widespread practical use. The layer-by-layer assembly (LBL) has complex multistep procedures [11], and self-assembled monolayer (SAMs) needs surface-specific interaction [12], and surface-initiated atom transfer radical polymerization (ATRP) requires unstable polymerization conditions [13-15]. For practical applications, it is desirable to use a simpler and more convenient method to immobilize the MPC polymer onto Ti alloy surface.

Recently, for making convenient adhesion of organic compounds to metal substrate, mussel-inspired chemistry has been widely investigated [16]. The mussels are experts at rapid and permanent adhesion to varieties of wet surfaces ranging from inorganic materials and organic materials. Such adhesive properties rely on the exhaustive repeated 3,4-dihydroxy-L-phenylalanine (DOPA) motif found in the foot protein of mussels [17]. Although the exact mechanism of adhesion is not fully understood, it has been widely speculated the 3,4-dihydroxyphenyl group of DOPA is responsible for adhesion [18,19]. Lee reported that oxidation of DOPA motif in the foot proteins dramatically reduces the strength of the adhesion to metals [20]. This mussel inspired chemistry can be applicable for surface modification with polymers. When the polymer with 3,4-dihydroxyphenyl groups is in contact with the metal substrate, the thin
polymer film can spontaneously deposit on surface. The functionalization of the polymers provides new characteristics of the metal substrate. Actually, it has been reported that the PEG with 3,4-dihydroxyphenyl groups was used to modify TiO₂ surface in the pH range from 6.0 to 7.4 to reduce protein adsorption on the surface [21].

In this study, we synthesized water-soluble bioinspired polymers (PMDP), which have both phosphorylcholine groups of the MPC unit and 3,4-dihydroxyphenyl groups. The surface modification on the Ti alloy substrate was carried out using aqueous medium of the polymer. Surface characteristics and stability of the coated polymer layer were examined and the role of 3,4-dihydroxyphenyl groups for adhesion was also discussed. Furthermore, the interaction between PMDP and proteins were investigated.

2. EXPERIMENTAL SECTION

2.1 Materials

Water-soluble MPC polymers composed of MPC units and methacrylic acid unit were obtained from NOF Co. (Japan). The compositions of the MPC units were 30 unit mol% (PMA3) and 50 unit mol% (PMA5). The number average molecular weight (Mn) of PMA3 and PMA5 were 2.7 x 10⁵ and 3.2 x 10⁵, respectively. Dopamine hydrochloride was purchased from Sigma-Aldrich (USA). 1-Ethyl-3-(3-dimethylamino propyl) carbodiimide, hydrochloride (WSC) was purchased from Dojindo (Japan). The Ti alloy substrate was cut into 10 mm x 10 mm pieces, and polished with #2000 and #3000 polish paper, then rinsed in acetone and ethanol in air, the substrates were cleaned using an oxygen plasma apparatus (PR500 plasma reactor, Yamato Metals, Ltd. (Japan). The Ti alloy substrate was immersed in water to equilibrate 10 mm x 10 mm pieces, and polished with #2000 and #3000 polish paper, then rinsed in acetone and ethanol using sonication for 15 min, respectively. After drying in air, the substrates were cleaned using an oxygen plasma apparatus (PR500 plasma reactor, Yamato Science, Japan) for 10 min before using. As a protein, bovine serum albumin (BSA, Sigma-Aldrich) was used without further purification.

2.2 Synthesis of phospholipid polymer

The water-soluble phospholipid polymer with 3,4-dihydroxyphenyl groups (PMDP) was synthesized by the condensation reaction between PMA and dopamine hydrochloride. The reaction scheme is shown in Fig. 1. The WSC and dopamine hydrochloride were dissolved in 4 mL PMA aqueous solution (5.0 wt%) and 96 mL pH 6.0 buffered solution (potassium dihydrogen phosphate and sodium hydroxide) was added. The reaction was taken at room temperature for 24 h under Ar gas atmosphere to prevent oxidation of 3,4-dihydroxyphenyl groups. The molar ratio of [dopamine hydrochloride]/[COOH] was 2.0. After the reaction, the polymer solution was ultrafiltered by ultrafiltration membranes (Millipore Co., USA; molecular size cut off: 3.0 x 10⁴) until no further release of unreacted dopamine through the membrane, which was confirmed by ultraviolett (UV, V-560, Jasco Co., Japan) adsorption. The polymer solution was freeze-dried. The PMDP prepared from the PMA3 were named as PMDP3 and that from the PMA5 as PMDP5, respectively. The chemical structure of these polymers was confirmed with both UV and FTIR spectroscopy (FT/IR-615, Jasco Co., Japan) for 32 scans over the range from 650 to 4000 cm⁻¹ at a resolution of 4.0 cm⁻¹.

2.3 Surface modification on Ti alloy substrate with PMDP

The coating of Ti alloy substrate was performed by simply dipping of Ti alloy substrate in 2 mg/mL PMDP aqueous solution at room temperature. The surface of the substrate was analyzed to confirm the coating polymer layer on the substrate by FT-IR spectroscopy with attenuated total reflection (FT-IR/ATR, Jasco Co., Japan), and the surface morphology was observed by atomic force microscopy (AFM, Nilon Veeco, Japan), and the polymer adhesion process was followed by quartz crystal microbalance with dissipation (QCM-D, Q-Sense, Sweden).

2.4 Surface characterization and stability evaluation of the coating polymer layer

After coating, the Ti alloy substrates were immersed in water at room temperature for at least 2 days to evaluate the stability of the coating polymer layer. The hydrophilicity of the Ti alloy substrates before and after immersion in the PMDP solution were evaluated with a contact angel goniometer (CA-W, Kyowa Co. Ltd., Japan). The captive-bubble method was used to determine the static contact angle (SCA). Each Ti alloy substrate was immersed in water to equilibrate and then fixed horizontally on a metal plate. A small air bubble was attached to the surface of the Ti alloy substrates. The measurement was repeated 5 times for each substrate and the average was calculated. The thickness of the PMDP layer formed on the substrates was measured with an ellipsometer (J.A. Woollam Co., Inc., Japan). The surface elemental analysis was carried out with an X-ray photoelectron spectroscope (XPS, AXIS-HSI165, Kratos/Shimadzu Co., Japan).

To examine the effects of oxidation of the 3,4-dihydroxyphenyl groups in the PMDP, the PMDP aqueous solution was kept in air to oxidize spontaneously. After one month, the solution was freeze-dried and the chemical structure of the polymer was analyzed by both UV and FTIR spectroscopies. The polymer was dissolved in water and the solution was used for coating of the Ti alloy substrate and the stability of the coating polymer layer was evaluated by ellipsometry.

2.5 Measurement of amount of protein adsorbed on the Ti alloy substrate

The amount of BSA adsorbed on PMDP3 coated surface was quantified by QCM-D. At first, Ti coated
QCM sensor was equipped to the QCM-D. After flowing 2.0 mg/mL of PMDP3 aqueous solution through the QCM cell, the sensor was exposed to a phosphate buffered saline (PBS; pH 7.4) solution until a stable baseline of the QCM signals was obtained. Then, 1.0 mg/mL of BSA in PBS was flowed for 10 min to replace the BSA solution and wash away the weakly adsorbed BSA from the surface. The QCM signals were monitored during the procedure. All of the measurements were performed at 37 °C and repeated at least three times.

3. RESULTS AND DISCUSSION

3.1 Characterization of PMDP

The IR spectra of the two PMDP are shown in Fig. 2 with those starting materials, PMA and dopamine hydrochloride. The IR spectra of PMDP3 and PMDP5 were similar. The 3,4-dihydroxyphenyl group in PMDP could be verified by the appearance of absorbance peak at 1553 cm⁻¹, which is attributed to aromatic ring of dopamine hydrochloride. In addition, the existence of the ester carbonyl group of the methacrylate units in PMDP could be proved by the appearance of absorbance peak at 1715 cm⁻¹. As shown in Fig. 3, the introduction of the 3,4-dihydroxyphenyl group in PMDP was also confirmed by UV spectra. The adsorption at 280 nm corresponding to the 3,4-dihydroxyphenyl groups was observed. From the absorbance, it could be calculated that the amount of 3,4-dihydroxyphenyl group in the PMDP is 4.0 mol % in PMDP3 and 2.0 mol% in PMDP5, respectively.

3.2 Surface modification on Ti alloy substrate with PMDP

The Ti alloy substrate was immersed into an aqueous solution of PMDP with various coating periods. After 10 sec immersion in PMDP solution, the Ti alloy substrates were picked up and dried under vacuum for observation with AFM. The AFM images shown in Fig.4. Even though the root mean square (RMS) value of the surface roughness of original Ti alloy substrate was 1.0 nm, that of the PMDP3 coated Ti alloy substrate was only 0.5 nm, which indicated a smoother surface could be obtained by polymer coating. The amount of polymer on the Ti alloy substrate was measured with QCM-D. The results showed that only 35 ng/cm² of the PMA deposited on the substrate; on the other hand, 354 ng/cm² of the PMDP3 adhered on the substrate. These results demonstrated that the PMDP could be covered and adhered on the Ti alloy substrate immediately from its aqueous solution and provided uniform coating layer by simply dipping procedure. FT-IR spectra also supported the existence of the PMDP at the Ti alloy substrate from the peaks of 3,4-dihydroxyphenyl group and ester carbonyl group after coating procedure (Data not shown).

The evaluation of the surface hydrophilicity was conducted by the contact angle measurement. The MPC polymer is water-soluble, which means the polymer is quite hydrophilic. The contact angle was 54 deg on the original Ti alloy substrate as shown in Fig. 5. After treated with the PMDP3 solution, the contact angle decreased dramatically and reached 0 deg. This value was maintained even after the substrate was immersed in water for 2 days. This result suggested that the PMDP still remained on the substrate. On the other hand, in the case of PMA and PMDP5, the same as the original Ti alloy substrate) after two days immersion. These polymers may be detached from the substrate. As shown in Fig. 6, stability of the PMDP3 coating was confirmed by the thickness change during washing process using ellipsometry. Under both coating time periods (10s and 24h), the thickness of the PMDP3 coating layer was not changed. The possible explanation of the stability is selective affinity of the 3,4-dihydroxyphenyl groups to the Ti alloy substrate. Thus, reduced content of 3,4-dihydroxyphenyl groups due to oxidation may lead to the instability of polymer layer. The PMDP aqueous solution was oxidized by air. Both UV and FTIR spectroscopies followed the oxidation. In the UV spectrum after oxidation, absorbance at 280 nm attributed to aromatic ring was decreased. Alternately, the IR absorbance at 2852 cm⁻¹ appeared. This is attributed to ketone groups. From the above results, it was confirmed that the 3,4-dihydroxyphenyl groups were converted to quinine groups. The oxidized PMDP solution was used as a coating solution. As the Fig. 7 illustrated, the thickness of the coating polymer layer changed with washing period and the thickness decreased within 2 days;
therefore, the oxidation reaction weaken adhesion force of the polymer on the Ti alloy substrate. From these results, the 3,4-dihydroxyphenyl groups in the PMDP played an important role for making stable coating.

3.3 Protein adsorption resistance of Ti alloy substrate treated with the PMDP

Protein adsorption resistance is one of the most important properties for biomedical materials. The effects of the coating with PMDP3 were evaluated by the BSA solution. The BSA is the most high concentration protein in the blood plasma. According to the QCM signals, 530 ng/cm² of BSA adsorbed on the original Ti alloy substrate; whereas after treated with the PMDP3, the QCM signal due to BSA adsorption could not be detected. Thus, the protein adsorption resistibility may be improved by this coating.

4. CONCLUSION

A uniform PMDP3 coating could be performed on the Ti alloy substrate simply by only 10 s dipping process in the aqueous solution without further treatments. The PMDP coating was stable due to anchoring of the 3,4-dihydroxyphenyl groups on the Ti alloy substrate. The reduction of protein adsorption on the surface treated with the PMDP may induce a significant suppression of biological responses. Thus, finally, we concluded that the simple and reliable surface treatment on the Ti alloy substrate was achieved using the PMDP and it will provide high-performance cardiovascular implantable medical devices.

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