polyethylene (PE) film surfaces [19]. They carried out the protein adsorption experiments under a similar condition as we used in our experiments. Their results indicated that, the adsorbed amount of BSA on the acrylic acid (AA) grafted PE surface with a ζ-potential of −27.3 mV was 0.78 μg cm^{-2}, an amount which is much higher than that on the PMBSSi surface with a similar ζ-potential; and the adsorbed amount of BSA on the *N,N*-dimethylaminopropyl acrylamide (DMAPAA) grafted PE surface with a ζ-potential of 17.0 mV was 4.76 μg cm^{-2}, an amount which is significantly higher than that on the PMBASi surface with a higher ζ-potential of 26.1 mV. This comparison clearly reveals that despite the charged moieties involved, the MPC moieties on the copolymer still played their role effectively in suppression of protein adsorption. In addition, the adsorbed protein amounts on both the cationic PMBASi surface and the anionic PMBSSi surface were in a similar low level as those on the neutral PMSi surface, with no statistical difference significance. These results indicate that the incorporation of charged moieties would not significantly influence the protein resistant property of an MPC copolymer. Based on previous studies on the effects of ionic strength on protein adsorption, we have made a hypothesis that the function of MPC moieties in the suppression of protein adsorption, rather than the electrostatic character resulting from the charged moieties, dominated the interactions between the protein and the charged MPC copolymer surfaces [11]. The results of this study test, support, and expand the hypothesis.

Considering that the MPC mole fractions in both PMBASi and PMBSSi were only about half of that in PMSi, the fact of no big difference in the protein adsorption on the neutral PMSi surface in comparison with on the charged PMBASi or PMBSSi surfaces implies that, an about 50% mole fraction of the MPC moiety in a copolymer is sufficient to construct a protein adsorption resistant surface and additional more fraction seems not to bring more reduction in the protein adsorption. In addition, as the charged PMBASi and PMBSSi contain hydrophobic moieties (i.e. BMA) but the neutral PMSi dose not, the similar level of protein adsorption meanwhile suggests that the introduction of the hydrophobic moieties in an MPC copolymer surface may not result in an increase in the protein adsorption if the MPC moiety is predominant in the copolymer composition. This should be associated with the fact that both the PMBASi and the PMBSSi surfaces had high mobility as elucidated by the DCA analysis (Fig. 6 and Table 3). The high mobility facilitates the surface reorientation when the surface is in contact with an aqueous medium, and the hydrophobic components (for example, BMA in both PMBASi and PMBSSi) are buried in the inner of the polymer layer as the hydrophilic components such as MPC moieties migrate to the outmost surface to minimize the surface energy [27,28]. The MPC moieties are predominant at the surface and thereby dominate the surface properties, especially such as the interactions with proteins when in contact with a protein solution.

Although the adsorbed protein amounts on three coated surfaces had no statistical difference significance with each other, quantitatively they still present a subtle but systematic dependence on the surface charge. When ranking the amounts of protein adsorbed on the different coated surfaces, in the cases of negatively charged pepsin and BSA (Fig. 7a and b), the order is PMBASi (cationic) > PMSi (neutral) > PMBSSi (anionic), while in the cases of positively charged RNase A and LYZ (Fig. 7c and d), the order is PMBASi (cationic) < PMSi (neutral) < PMBSSi (anionic). This can be correlated to the possible electrostatic interactions between the charged proteins and the charged surfaces [17]. However, as discussed above, the possible effect from electrostatic interactions would be too small and too limited to influence the overall situation.

3.4. Cell adhesion

At the molecular level, it is generally believed that the rapid adsorption of proteins to the surface is the initial stage of cell adhesion [29]. In view of the high capability of the three MPC copolymers to suppress protein adsorption, we supposed that they would have corresponding capability to resist the cell adhesion. Mouse fibroblast L929 cells, which are typical adherent cells and are widely used in various biological studies and evaluations as model cells, were employed to evaluate the cell adhesion on the sample surfaces. Fig. 8 shows the microscopic images of sample surfaces on which L929 cells were cultured for one day and four days. In the case of uncoated surface, L929 cells gradually adhered,

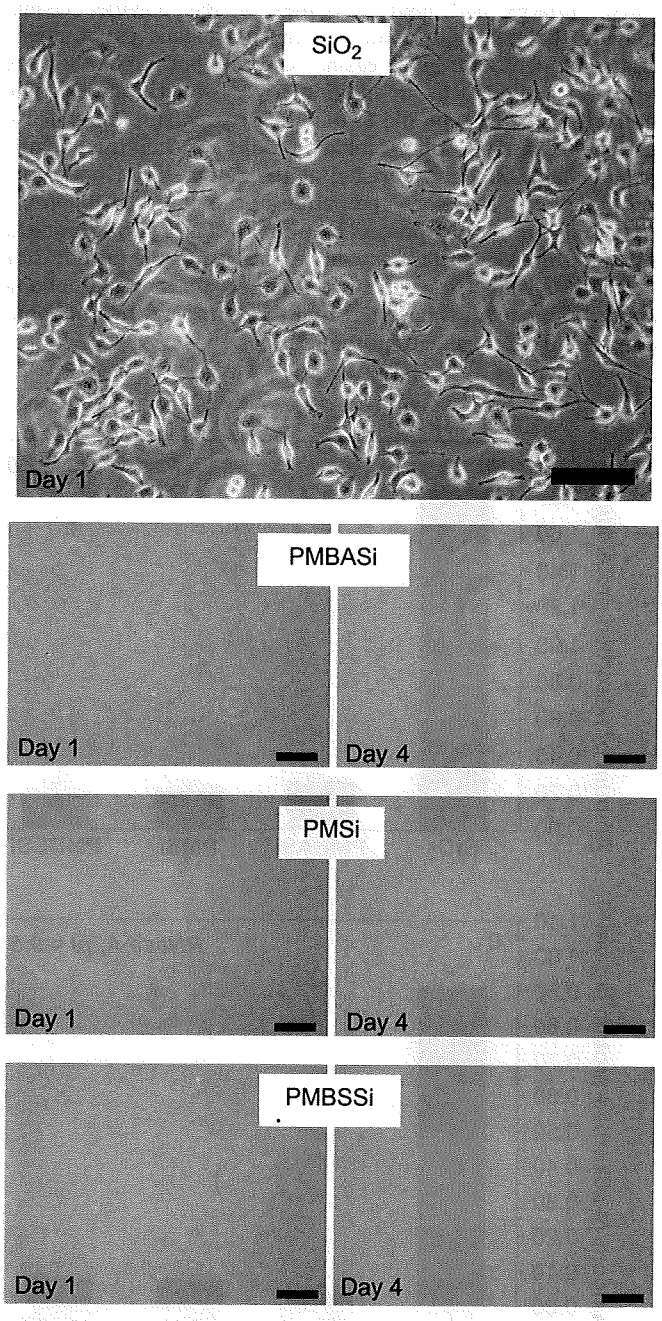

Fig. 8. Microscopic images of different sample surfaces on which L929 cells were cultured for one day and four days. MPC copolymers coated SiO_2 surfaces exhibited no cell adhesion, while uncoated SiO_2 exhibited serious cell adhesion. Scale bar represents 100 μm.

spread, and flattened on the surface after seeded, and then formed confluence after cultured for one day (Fig. 8), and finally densely covered the surface four days later (data not shown). In contrast, no cell adhesion was observed on PMBASi, PMSi, and PMBSSi surfaces after cultured for one day and even for four days (Fig. 8). This indicates that like the neutral PMSi, both the cationic PMBASi and the anionic PMBSSi possess the high capability to suppress cell adhesion due to the MPC moieties at the surface. Ikada et al. reported that cells could adhere and proliferate on the charged monomer grafted PE surfaces with a ζ-potential larger than 12 mV [19]. Before we performed the evaluation, we had previously speculated that the cationic PMBASi surface might induce cell adhesion because of the possibly increased electrostatic attraction between the positive charged surface of the coating and the negatively charged surface of the cell. Evidently, the evaluation result overturned the speculation, and it seems that the charged moieties in PMBASi and PMBSSi would not cause the effect in inducing cell adhesion. This further supports our aforementioned hypothesis that the electrostatic character resulting from the charged moieties of the copolymers would not dominate the surface behaviors of bioadsorptions.

4. Conclusions

The behaviors of protein adsorption and cell adhesion on three model MPC copolymer surfaces with different surface charges were comparatively investigated. Results imply that the introduction of charged moieties, either the cationic or the anionic, in an MPC copolymer, would not increase the risk in inducing both the protein adsorption and cell adhesion. This tests, supports, and expands our previous hypothesis that the function of MPC moieties in the suppression of protein adsorption, rather than the electrostatic character resulting from the charged moieties, would dominate the interactions between proteins/cells and MPC copolymer surfaces. These electrically charged MPC copolymer surfaces can be applied to those biological applications simultaneously requiring non-biofouling properties and electrical properties.

Acknowledgement

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Appendix

Figures with essential colour discrimination. Certain figures in this article, in particular Figures 2, 3, 5 and 8, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.biomaterials.2009.06.005.

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Nanoscale evaluation of lubricity on well-defined polymer brush surfaces using QCM-D and AFM

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ABSTRACT

For preparing a "highly lubricated biointerface", which has both excellent lubricity and biocompatibility, we investigated the factors responsible for resistance to friction during polymer grafting. We prepared poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), poly(2-hydroxyethyl methacrylate) (PHEMA), and poly(methyl methacrylate) (PMMA) brush layers with high graft density and well-controlled thickness using atom transfer radical polymerization (ATRP). We measured the water absorptivity in the polymer brush layers and the viscoelasticity of the polymer-hydrated layers using a quartz crystal microbalance with dissipation monitoring (QCM-D) measurements. The PMPC brush layer had the highest water absorptivity, while the PMPC-hydrated layer had the highest fluidity. The friction properties of the polymer brush layers were determined in air, water, and toluene by atomic force microscopy (AFM). The friction on each polymer brush decreased only when a good solvent was chosen for each polymer. In conclusion, the brush layer possessing high water absorptivity and fluidity in water contributes to reduce friction. PMPC grafting is an effective and promising method for obtaining highly lubricated biointerfaces.

1. Introduction

In recent years, there has been increasing interest for the surface modification of biomaterials in order to improve their surface properties. All biomaterial surfaces require the ability to suppress biological reactions when they are in contact with living organisms, and we call such contact surfaces as "biointerfaces". Lubricity is also essential for biomaterials such as artificial joints, blood pump bearings, endoscope surfaces, and catheters. The loosening of artificial joints caused by wear between the articulating surfaces is the most serious problem that limits their life and clinical success [1–3]. Biomimetic molecular design of materials is one of the promising approaches to prepare biointerfaces. The cell membrane inspired surface based on phosphorylcholine-group-bearing polymers has shown an excellent resistance to protein adsorption and cell adhesion [4–13]. And also they showed prevention of cell response when they were implanted into tissues. These polymers include 2-methacryloyloxyethyl phosphorylcholine (MPC) units [14]. Poly(MPC) (PMPC) has the ability to resist protein adsorption and also stabilizes functional proteins such as enzymes and antibodies, even when the proteins are immobilized artificially on the surface [15,16]. PMPC is also expected to improve the lubricity of material surfaces since the same phospholipid polar groups are present on the surface of the human articular cartilage. In fact, it has been reported that PMPC grafting onto the polyethylene liner of an artificial hip joint clearly reduces the wear between the articulating surfaces occurring in the long run [17,18]. In this manner, PMPC grafting onto a material surface has already been proved to be an effective method for improving surface lubricity and biocompatibility. PMPC is known to possess a high free water fraction around the chain, which is one of the factors responsible for reducing protein adsorption [15,19]. In this study, we focused on the hydration of grafted polymers and carried out an in-depth investigation of the factors providing both lubricity and biocompatibility on a nanoscale.

We prepared high-density polymer brush layers on a silicon (Si) wafer. Recently, living polymerization techniques have been extensively investigated in order to grow high-density polymer brushes with a controlled length and narrow molecular weight distribution. Atom transfer radical polymerization (ATRP) is one of the best surface-initiated living radical polymerization methods because of its versatility in the choice of monomer types, tolerance to impurities, and mild reaction conditions [20,21]. In this research, we prepared nanoscaled PMPC, poly(2-hydroxyethyl methacrylate)

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(PHEMA), and poly(methyl methacrylate) (PMMA) brush layers by ATRP. We investigated the effects of the hydration of the polymer brush layers on resistance to friction on a nanoscale using a quartz crystal microbalance with dissipation monitoring (QCM-D) and an atomic force microscope (AFM). QCM-D allows the simultaneous measurements of mass and viscoelasticity on a material surface through changes in the frequency (F) and energy dissipation (D) in a noninvasive manner. The sensitivity of measurements in the ng/cm^2 range in liquid phase enables us to clarify the dynamic behavior of the polymer brush layer on a nanoscale [22,23]. Using the QCM-D technique, we evaluated the water absorptivity in the polymer brush layers and the viscoelasticity of the polymer-hydrated layers in water. Subsequently, many AFM or lateral force microscope (LFM) studies have been devoted to the understanding of the influence of thin film structure on friction [24–28]. By evaluating the combined results of the QCM-D and AFM measurements, we identified the key factors responsible for obtaining a highly lubricated biointerface.

2. Experimental section

2.1. Materials

MPC was synthesized and purified by the previously reported method [14]. The Si wafers were purchased from Matsushita Electric Industrial Co. (Osaka, Japan). The surfaces of the Si wafers were covered with approximately 100-nm-thick SiO_2 layers. HEMA and MMA were purchased from Kanto Chemical Co. (Tokyo, Japan) and used as received. Copper (I) bromide (CuBr), 2,2′-dipyridyl, and ethyl-2-bromoisobutyrate were purchased from Sigma–Aldrich Co. (St. Louis, USA) and used as received. All the other reagents and solvents were commercially available in extra-pure grade and were used as purchased. Oxygen and argon gases used were of high-purity grade.

2.2. Preparation of the polymer brush layers on the Si wafers

The SiO_2-coated Si wafers were cut into 1.0 cm × 2.0 cm chips, rinsed sufficiently with ethanol and acetone, and etched by oxygen plasma for 20 min (300 W, 100 mL/min gas flow, PR500, Yamato Scientific Co. Ltd., Tokyo, Japan). We used a monochlorosilane, 3-(2-bromoisobutyryl)propyl dimethylchlorosilane (BDCS) as the surface initiator for obtaining a homogeneous monolayer of the initiator on the Si wafers. We synthesized BDCS by the previously described method [29]. The cleaned substrates were immersed in a 5 mmol/L toluene solution of BDCS for 24 h. The wafers were removed from the solution, rinsed with methanol, and dried in an argon stream before being used for graft polymerization. The graft polymerization of MPC, HEMA, and MMA on the Si wafers was performed using ATRP. MPC was dissolved in 10 mL of dehydrated methanol, and HEMA and MMA were dissolved in 10 mL of a mixed solvent comprising 4 parts of methanol and 1 part of water. We used dehydrated methanol as the solvent for MPC since it underwent rapid, uncontrolled polymerization in water [30]. Copper (I) bromide (20 mg, 0.135 mmol) and 2,2′-bipyridyl (43 mg, 0.27 mmol) were added to the solutions of MPC, HEMA, and MMA with stirring under argon at room temperature. The molar ratio of the monomer to the free initiator, [Monomer]/[Initiator], was changed in order to change the polymerization degree, by which we controlled the thickness of the polymer brush layers. After the solution was stirred for 30 min under an argon atmosphere, the BDCS-immobilized Si wafers were immersed into the solution, and simultaneously, ethyl-2-bromoisobutyrate (20 μL, 0.135 mmol) was added as the free initiator. The polymerization was carried out at room temperature with stirring under an argon atmosphere. The Si wafers were removed from the polymerization mixture after the desired time period required for the monomer to completely convert into the corresponding polymer. Subsequently, they were placed in a Soxhlet apparatus, extracted with methanol for 20 h, and dried in vacuo at room temperature. The scheme for the preparation of the PMPC, PHEMA, and PMMA brush layers is shown in Fig. 1. The rates of conversion to the free polymer were confirmed by ^{1}H NMR (JEOL JNM-NR30, Tokyo, Japan). We prepared PMPC with polymerization degrees of 20, 40, 50, 60, 80, 100, 150, and 200 (henceforth each sample will be referred to as PMPC20, PMPC50, ..., PMPC200), and PHEMA and PMMA with polymerization degrees of 50, 100, and 150 (PHEMA/PMMA50, PHEMA/PMMA100, PHEMA/PMMA150). The number after abbreviation represents polymerization degree, that is, PMPC20 is PMPC graft chain consisting of 20 monomer units.

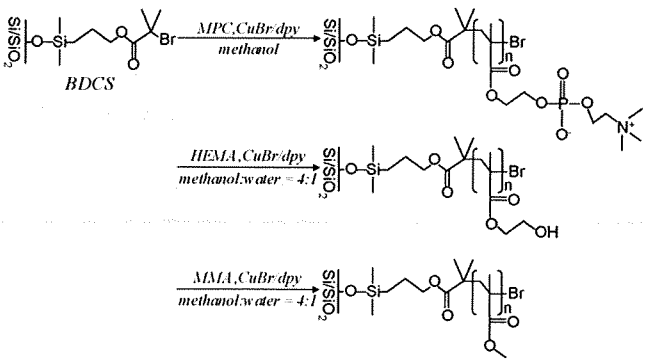

Fig. 1. Synthetic scheme for the fabrication of the PMPC, PHEMA, and PMMA brush layers on a Si wafer surface.

2.3. Surface characterization

The surface elemental composition was determined using X-ray photoelectron spectroscopy (XPS) (AXIS-Hsi, Shimadzu/Kratos, Kyoto, Japan) with a magnesium anode non-monochromatic source. All the samples were completely dried in vacuo before use. High-resolution scans for C_{1s}, O_{1s}, N_{1s}, and P_{2p} were performed at takeoff angles of 90°. All the binding energies were referred to the C_{1s} peak at 285.0 eV. The static water contact angles were measured using a goniometer (CA-W, Kyowa Interface Science Co., Tokyo, Japan) at room temperature. The samples were completely dried in vacuo before the measurements. Water droplets of 6 μL were contacted onto the substrates and the contact angles at 10 s were directly measured by photographic images. The data were collected at three positions on each sample. The thickness of the PMPC, PHEMA and PMMA brush layers on the Si wafers under dry conditions was determined by ellipsometry (DVA-36L3, Mizojiri Optical Co., Tokyo, Japan). Irradiation with a He–Ne laser (632.8 nm) was performed at an incident angle of 70°. The refractive indices (n_r) of Si, PMPC, PHEMA, and PMMA were applied 1.623, 1.488, 1.500, and 1.500, and the extinction coefficients (k_e) were 1.604, 0.000, 0.000, and 0.000, respectively [31]. All the measurements were recorded in air at room temperature. The data were collected at nine locations for each sample. The surface morphologies of the PMPC, PHEMA, and PMMA brush layers were observed with a Nanoscope IIIa AFM (Nihon Veeco, Tokyo, Japan) operated in tapping mode. The measurements were performed under ambient conditions using a standard cantilever at a scan rate of 1.0 Hz. Immediately prior to the measurements, the samples were rinsed by sonication in ethanol and dried in an air stream. The root-mean-square (RMS) surface roughness was calculated from the roughness profiles.

2.4. Measurements of the amount of hydration water and the viscoelasticity of the polymer-hydrated layers

QCM-D is a technique used for measuring the mass of the material/molecules attached to the surface of the quartz crystal via changes in the resonant frequency, ΔF, while simultaneously obtaining information about the viscoelasticity of the layer by measuring the energy dissipation factor, D. The F-shift of the QCM-D is due to the change in the total coupled mass, including that of the water coupled to the layer. In aqueous solvents, the adsorbed film may contain a considerably large amount of water, which is sensed as a mass uptake by the QCM. By measuring the energy dissipation, it becomes possible to judge if the adsorbed film is rigid or elastic which is not possible by merely observing the frequency response. The measurements of F and D were performed with a commercial QCM-D (Q-Sense, Gothenburg, Sweden). The sensor crystals used in the measurements were 5-MHz AT-cut sputter-coated SiO_2 crystals (Q-Sense, Gothenburg, Sweden). The PMPC, PHEMA, and PMMA brush layers were prepared on the sensor crystals via the same ATRP method; [Monomer]/[Initiator] was 100/1 for all the samples. All the measurements were recorded at four different resonant frequencies (5, 15, 25, and 35 MHz). The temperature in the QCM-D liquid chamber was stabilized to 24.5 °C. Q-Sense software was used to acquire the experimental data. We measured the resonant frequencies and dissipation of both the unmodified sensor and the polymer-grafted sensor in air and water. The frequency difference measured between the unmodified sensor and the polymer-grafted sensor in air was defined as ΔF_{air}, and that in water was defined as ΔF_{water}. In a similar manner, the dissipation difference measured between the unmodified sensor and the polymer-grafted sensor in air was defined as ΔD_{air}, and that in water was defined as ΔD_{water}. $\Delta F_{water}/\Delta F_{air}$ is the hydration water ratio, which indicates the mass change due to presence of the hydration water. $\Delta D_{water}/\Delta D_{air}$ is the energy dissipation ratio, which indicates the viscoelastic change due to the hydration. The viscoelasticity of a polymer brush layer includes both the mobility of the polymer chain and fluidity of the hydration water layer.

2.5. Interfacial friction measurements

A Nanoscope IIIa AFM was used to measure the friction force. The experiments were performed in contact mode in air, water, and toluene with several times. Commercially available 200-μm-long V-shaped Si_3N_4 cantilevers (NP-S, Veeco NanoProbe Tips) with an announced force contact of 0.12 N/m were used. The surface friction force data were acquired by scanning in the Trace (T) and Retrace (R) directions with the slow scan axis disabled. The Trace/Retrace pair of scan lines provided a friction loop, and one-half of the separation between the trace and retrace frictions (TMR) was a measure of the friction force. The length of a scan was maintained at 2.0 μm and the scan rate at 2.0 Hz, yielding a sliding velocity of 8.0 μm/s. The applied load N [nN] was varied by changing the vertical deflection of the cantilever. The friction coefficient μ was calculated using Amontons' law ($F = \mu N$).

We converted the experimental normal (V_N) and lateral (V_L) deflection signals expressed in terms of voltage into normal (F_N) and lateral (F_L) forces in Newton by

$$F_N = K_N S_N V_N$$
$$F_L = K_L S_L V_L$$

where K_N and K_L are the normal and lateral force constants [N/m]; and S_N and S_L, the normal and lateral force optical deflection sensitivities [nm/V]. The abovementioned force contact of 0.12 N/m was used as the normal force contact K_N. The lateral force contact K_L was calculated by [32]

$$K_L = \frac{2K_N l^2}{3h^2(1+\upsilon)}$$

where l is the cantilever length [m]; h, the cantilever height [m]; and υ, Poisson's ratio. The S_N values can be obtained from the f–d curve in AFM; S_L is related to S_N by a relation proposed by Liu and Evans [33]. Using these factors, we could convert the voltage signals obtained from AFM into force values. The applied load was also required to be calculated. The applied load N exerted by the cantilever can be given by [34]

$$N = (D - N_0)S_N K_N$$

where D is the value of the deflection setpoint [V]; and N_0, the value of the deflection signal [V] when the cantilever is in its free state. N_0 was determined by the f–d curve measurements (Fig. 2). To obtain the values of S_N and N_0, we measured the f–d curve immediately after every friction force measurement in order to exclude the possible errors induced by the systematic drift.

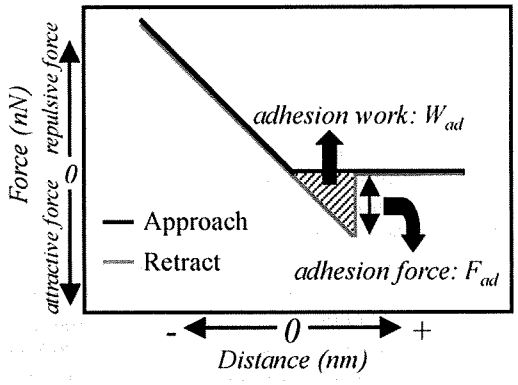

Fig. 2. Schematic illustration of the representative force–distance (f–d) curve measured by AFM and the method for defining F_{ad} and W_{ad}.

3. Results and discussion

3.1. Synthesis of the polymer brush layers via ATRP

We prepared well-controlled PMPC, PHEMA, and PMMA brush layers using ATRP. In order to control the thickness of the polymer graft layers, we controlled the polymerization degree by changing [Monomer]/[Initiator]. From ^{1}H NMR spectrum of the free polymer polymerized in an aqueous medium, we confirmed that the monomer completely converted to the desired polymer under the given conditions.

3.2. Surface characterization

The grafting of PMPC, PHEMA, and PMMA on Si wafers was confirmed using XPS (Fig. 3). The peaks in the carbon atom region (C_{1s}) at 286.5 and 289.0 eV in all the samples indicated the ether bond and ester bond in the methacrylate group, respectively. In the PMPC-grafted substrates, peaks in the nitrogen atom region (N_{1s}) at 403.0 eV attributed to the ammonium group and those in the phosphorus atom region (P_{2p}) at 133.0 eV attributed to the phosphate group were detected. These peaks were specific to the phosphorylcholine group in the PMPC unit.

The relationships of the static water contact angle and the dry thickness with [Monomer]/[Initiator] are shown in Fig. 4. The data in Fig. 4 were measured with high reproducibility and had little difference among the measured positions. The static water contact angles on the PMPC-grafted surfaces ranged from 10° to

25°. The PMPC grafting considerably increased the hydrophilicity, and a slight introduction of the PMPC chains enhanced the wettability. The contact angles on the PHEMA-grafted surfaces were approximately 40°. The hydrophilicity of the PHEMA-grafted surface was also increased. On the other hand, the contact angles on the PMMA-grafted surface were approximately 60°, similar to those on the unmodified Si. The PMMA-grafted surfaces were as hydrophobic as the unmodified Si. The thicknesses of the PMPC, PHEMA, and PMMA brush layers were 4–10, 2–7, and 2–5 nm, respectively. The thicknesses of all the polymer brushes increased with [Monomer]/[Initiator]. We prepared nanostructured polymer brush layers and controlled their thickness by changing the molar ratio of the monomer to the free initiator in the polymerization solution. The graft density σ was calculated using the dry thickness of each polymer brush layer from the equation

$$\sigma = \frac{h\rho N_A}{M_n}$$

where h is the layer thickness [nm] determined by ellipsometry; ρ, the density of each dry polymer layer (1.30 g/cm^3 for PMPC [35], 1.15 g/cm^3 for PHEMA [36], and 1.20 g/cm^3 for PMMA [37]); N_A, Avogadro's number; and M_n, the number-average molecular weight of the polymer chains on the surface. M_n was determined by measuring the molecular weight of the free polymer because these molecular weights had similar values [20]. We calculated M_n from the formula

$$M_n = \mathrm{DP} \times M_0 \times \frac{C}{100}$$

where DP is the polymerization degree determined by [Monomer]/[Initiator]; M_0, the molecular weight of each monomer; and C, the rate of conversion to the polymer determined by ^{1}H NMR measurements. The conversion rate was 100% for all the samples. The graft densities of the PMPC, PHEMA, and PMMA chains were calculated to be 0.17, 0.26, and 0.19 chains/nm^2, respectively. The graft densities of each polymer chain were relatively low compared with those in other literatures [36,37,40]. This would result from the low density of the bromoisobutyryl group at the BDCS-immobilized surface. The BDCS would not be self-assembled at the surface because it has relatively short length of the methylene chains. However, a polymer-grafted layer with a graft density of more than 0.10 chains/nm^2 forms a high-density brush structure [38,39]. It was confirmed that the polymer graft layers prepared via ATRP formed "brush" layers.

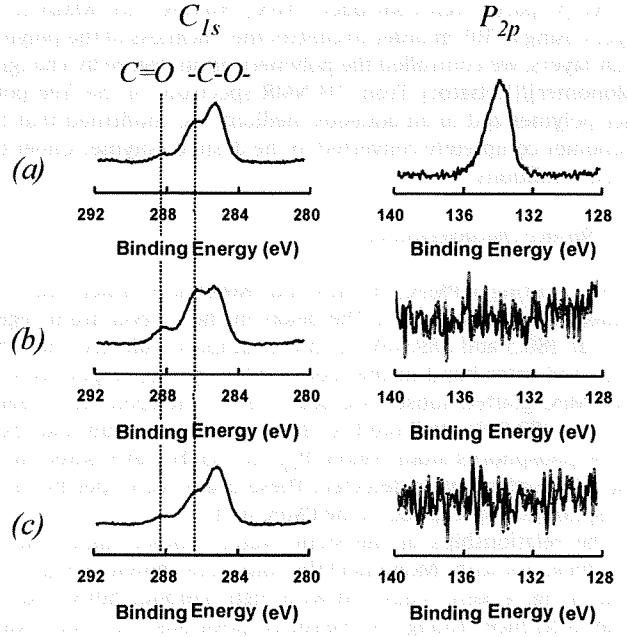

Fig. 3. The XPS spectra of (a) PMPC, (b) PHEMA, and (c) PMMA-grafted surface.

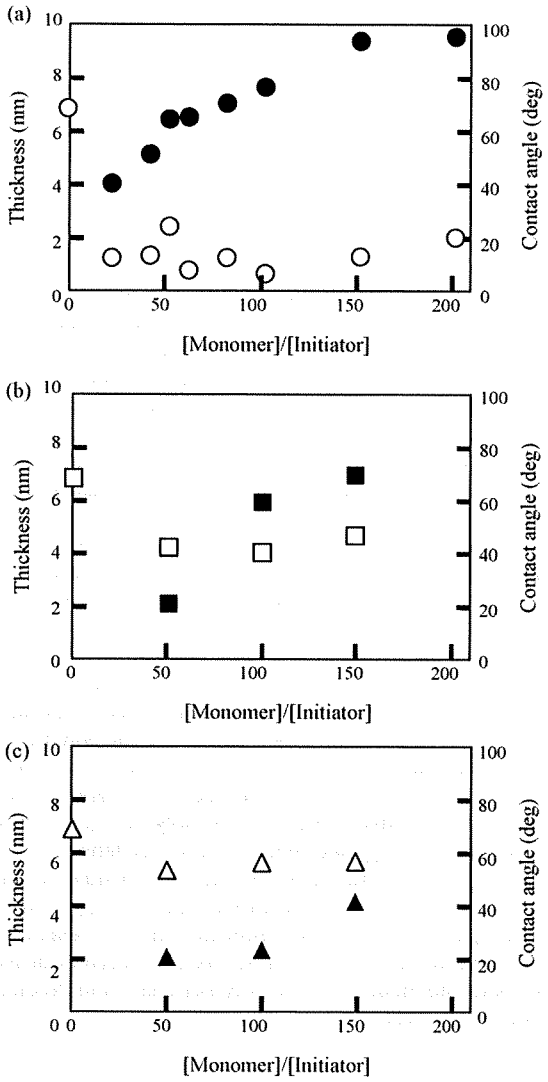

Fig. 4. Relationship between the molar ratio of the monomer to the free initiator and the static water contact angle (open plots) and the thicknesses (closed plots) of (a) the PMPC, (b) PHEMA, and (c) PMMA brush layers.

The brush conformation of the polymer graft layer examined using an AFM in air is shown in Fig. 5. Although the surface of the unmodified Si was nearly flat, the brush-like structure of each polymer graft layer was observed. The RMS surface roughness of all the samples was 0.4–0.8 nm. Compared to previous reports [40], these RMS values were very small, indicating that the polymer brush layers prepared by ATRP were considerably homogeneous with high graft densities.

3.3. Water absorptivity of the polymer brush layers and the viscoelasticity of the polymer-hydrated layers

Fig. 6 shows the hydration water ratio in each polymer brush layer, $\Delta F_{water}/\Delta F_{air}$, and the energy dissipation ratio of each polymer-hydrated layer, $\Delta D_{water}/\Delta D_{air}$. The hydration water ratio for the PMPC brush layer was the highest and decreased in the order PMPC > PHEMA > PMMA. These results indicated that most of the water molecules were coupled to the PMPC brush layer. The results of the hydration water ratio measurements accorded with those

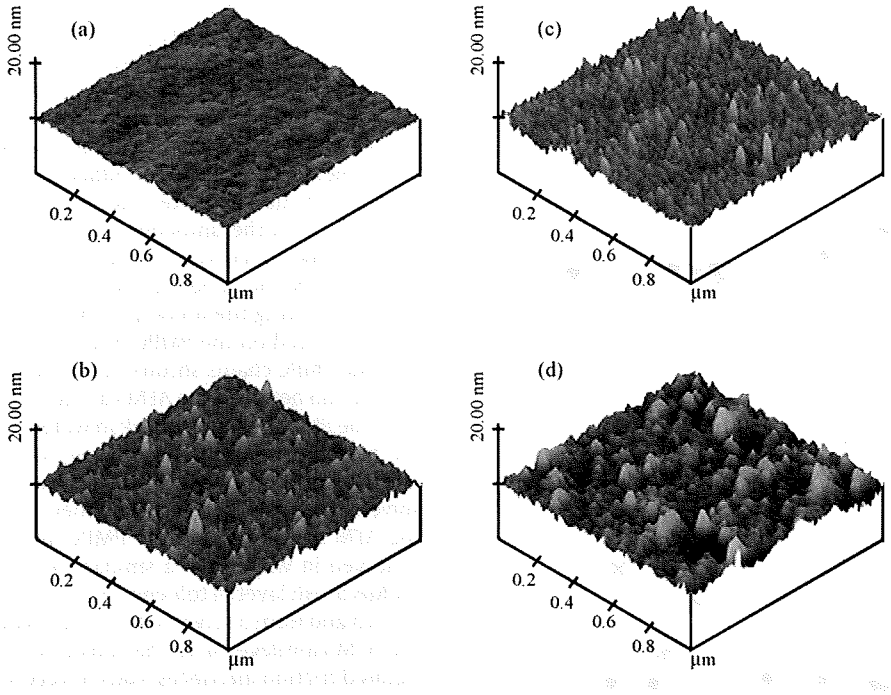

Fig. 5. AFM 3D images of (a) unmodified Si, (b) PMPC100, (c) PHEMA100, and (d) PMMA100 in air.

of the water contact angle measurements. From the values of the energy dissipation ratio, it was apparent that the PMPC-hydrated layer had the maximum fluidity in water; the PHEMA-hydrated layer had the second highest fluidity, while the PMMA-hydrated layer was the most rigid. These results indicate that the hydrophilic PMPC chains and PHEMA chains stretched in water and had greater mobility than the PMMA chains. The $\Delta D_{water}/\Delta D_{air}$ of the PMMA brush layers was negative because the PMMA chains shrink in water and the PMMA-hydrated layer is less fluid than the water layer on the unmodified SiO_2 sensor, whose surface is hydrophilic due to the presence of exposed –OH groups. Moreover, the PMPC-hydrated layer had a high energy dissipation ratio because it possessed a higher free water fraction than that of PHEMA or PMMA [19]. Generally the fluidity of free water is considerably higher than that of binding water. The results of the viscoelasticity measurements of the polymer brush layers well accorded with the previous report on the amount of free water around PMPC, PHEMA, and PMMA [19].

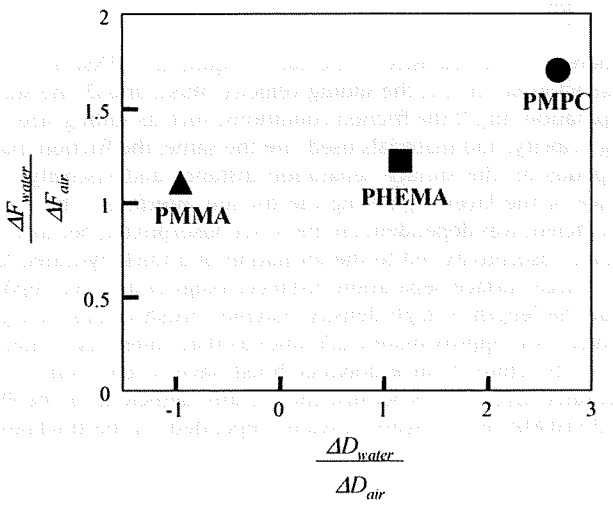

Fig. 6. The hydration water ratio in the polymer brush layer, $\Delta F_{water}/\Delta F_{air}$, and the energy dissipation ratio of the polymer-hydrated layer in water, $\Delta D_{water}/\Delta D_{air}$, measured by QCM-D.

3.4. Friction properties of the polymer brush layers and lubrication mechanism

Kobayashi and Takahara studied the lubrication of PMPC brush surface from viewpoint of macroscopic friction measurements [41]. They observed very low friction on the PMPC brush surface due to high hydrophilicity of the PMPC chains. Very recently, Klein et al. reported the lubrication of PMPC brush surface at physiological pressure and observed extremely low friction coefficient on the surface [42]. This is also due to higher hydration properties of PMPC chains. We investigated the nanoscale interfacial friction forces on the unmodified Si, PMPC, PHEMA, and PMMA brush layers using an AFM in contact mode. The representative values of the friction coefficients calculated as a function of the normal load are shown in Fig. 7. In air (Fig. 7 (a)), the friction coefficients of the PMPC brush layers were the same as those of the unmodified Si, and were characteristically high under a load less than 20 nN. When the load was above 20 nN, the friction coefficients of all the samples in air were stabilized at approximately 0.2. In contrast, in water (Fig. 7 (b)), the friction coefficients of the PMPC brush layers considerably decreased, and were below 0.08 when the load was below 20 nN. Then, the friction coefficients of the PMPC brush layers gradually increased with the normal load, and those of PMPC50, PMPC100, and PMPC150 were stabilized at approximately 0.09, 0.06, and 0.03, respectively. The friction coefficients of the PMPC brush layers in water decreased with an increase in their thicknesses of the PMPC brush layer. Fig. 7 (c) shows the friction coefficient of the PHEMA brush layers in water. When the load was below 20 nN, the friction coefficients of PHEMA50 were above 0.12, while those of PHEMA100 and PHEMA150 were below 0.12. As the normal load increased, the friction coefficients of all the PHEMA brush layers approached to approximately 0.10. Fig. 7 (d) shows the friction coefficients of the PMMA brush layers in water and those of PMMA100 in toluene. The friction coefficients of all the PMMA brush layers in water were high (above 0.2) when the

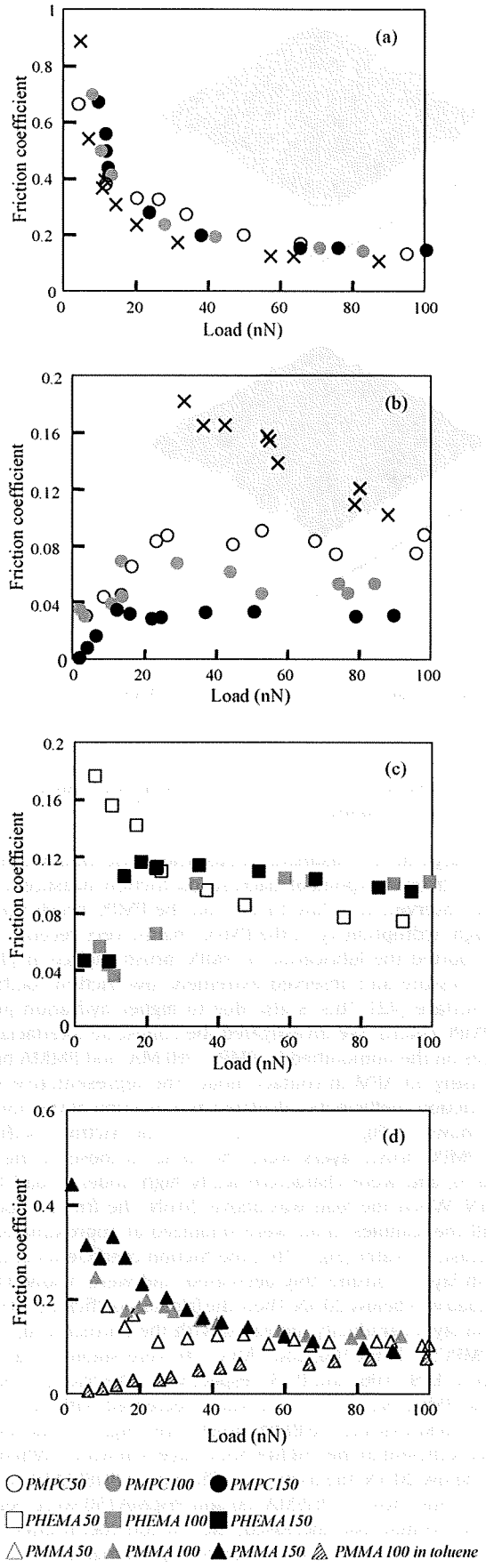

Fig. 7. The friction properties of the polymer brush layers measured by AFM: (a) the friction coefficients of the unmodified Si, PMPC50, PMPC100, and PMPC150 measured in air; (b) the friction coefficients of the unmodified Si, PMPC50, PMPC100, and PMPC150 measured in water; (c) the friction coefficients of the PHEMA50, PHEMA100, and PHEMA150 measured in water; (d) the friction coefficients of the PMMA50, PMMA100, and PMMA150 measured in water and PMMA100 measured in toluene.

load was below 20 nN. Subsequently, these values were gradually stabilized at approximately 0.15. In toluene, however, the friction coefficients of PMMA100 were clearly reduced. The friction coefficients of PMMA100 in toluene were below 0.04 when the load was below 20 nN, and gradually increased with the normal load.

The high friction coefficients for the load below 20 nN in these experiments were due to the adhesion force between the AFM cantilever and the substrate. The adhesion force between the AFM cantilever and the unmodified Si surface was 10–30 nN, as measured by the *f–d* curves, and acted as the load. When the load was below 20 nN, the adhesion force significantly influenced the load, thus magnifying the friction coefficients. The same adhesion force was measured on the PMPC brush layers in air. It was considered that the PMPC chains shrunk in air and were unable to prevent the interaction between the AFM cantilever and the substrate. In addition, the PMMA chains shrunk in water, and the AFM cantilever slid on the solid surface because water was a poor solvent for PMMA. The friction of the polymer brush layer only decreased when a good solvent was used for each polymer. No adhesion force between the AFM cantilever and the PMPC and PHEMA brush layers was observed in water, and a similar effect was also detected on the PMMA brush layer in toluene. In a good solvent, the polymer chains stretch and form a solvated layer that prevents the direct contact of the AFM cantilever with the substrate. Satisfying the condition of isolated friction interfaces leads to very low friction. As the normal load increased, the AFM cantilever penetrated the brush layer, and its interaction with the substrate gradually increased.

Correlating these friction properties with the results of the QCM-D measurements, it was found that both the hydration water ratio and energy dissipation ratio of the polymer-hydrated layer in water were strongly related to the friction resistance (Fig. 8). This result is consistent with the general friction theory. A usual friction phenomenon between solid surfaces is explained by the Bowden–Tabor theory [43]. In this theory, real surfaces in contact would only meet at the small areas at the peaks of their inevitable surface roughness, known as adhesion areas, where the pressure would be high and friction would be generated. In this case, friction mainly depends on the shear forces occurring at the adhesion areas, and the friction coefficient calculated by Amontons' law becomes 0.2–1.0. On the other hand, when a gas or liquid layer separates the friction interfaces and the adhesion area is absent, the friction clearly decreases. This condition is termed as hydrodynamic lubrication, and friction force F is given by [43]

$$F = \frac{\eta A v}{D}$$

where η is the viscosity of the gas or liquid layer [Ns/m^2]; A, the contact area [m^2]; v, the sliding velocity [m/s]; and D, the surface separation [m]. If the friction conditions, such as sliding size, sliding velocity, and materials used, are the same, the friction mainly depends on the surface separation distance and viscosity resistance of the layer separating the friction interfaces. The friction coefficient was dependent on the water absorptivity, because high water absorptivity led to the formation of a thick hydrated layer and large surface separation distance. Tsujii et al. have reported that the length of high-density polymer brush chains in a good solvent was approximately 2.5 times as that under dry conditions [38]. Therefore, a thick polymer brush layer could form a thick hydrated layer. This is because the friction coefficients of the PMPC and PHEMA brush layers in water depended on the thickness of

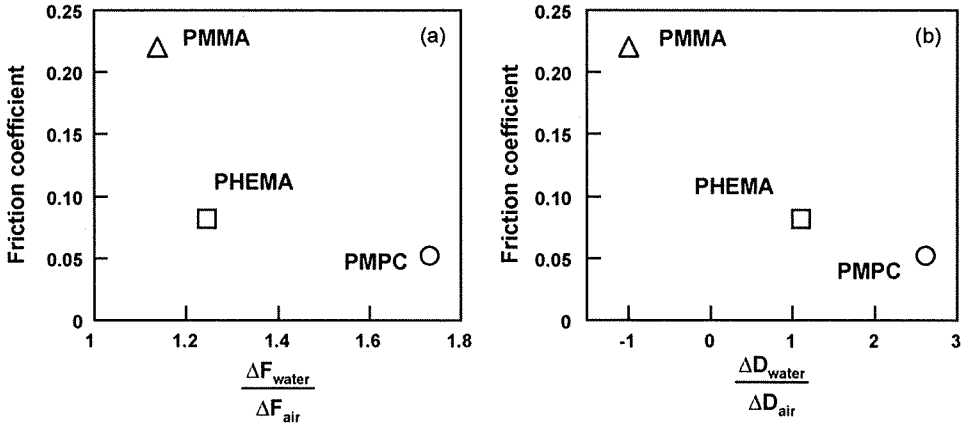

Fig. 8. Relationship (a) between the hydration water ratio, $\Delta F_{water}/\Delta F_{air}$, and the friction coefficient, and (b) between the energy dissipation ratio of the polymer-hydrated layers, $\Delta D_{water}/\Delta D_{air}$, and the friction coefficient.

the brush layers. The grafting method is significant in effecting the surface separation because a polymer layer binds to a substrate through a chemical reaction. Several previous studies have revealed that physically adsorbed polymer chains do not provide adequate wear characteristics because the physisorbed layers are observed to quickly wear away under repeated sliding cycles [44,45]. We also have reported that the polypropylene surface grafted with PMPC via a photoinduced graft polymerization method showed a good resistance against high pressure and repeated sliding cycles [46]. The dissipation ratio of the polymer-hydrated layer was related to the viscosity resistance of the layer. The high fluidity of the PMPC-hydrated layer led to low viscosity resistance. These results accorded with those mentioned in previous reports. Galliano et al. have reported lower friction coefficients for crosslinked polydimethylsiloxane (PDMS) networks with large mesh sizes; they contended that this resulted from the presence of a greater number of free and pendant chains at the interface of the large-mesh-size networks as compared to those in more tightly crosslinked networks possessing smaller mesh sizes [47]. Similar results have been reported by Gong et al. in their study of the interfacial friction of hydrogels [48,49]. They found that gels having brush-like dangling chains on their surface could manifest friction forces that were 1–2 orders of magnitude lower than those of gels without these dangling chains. These two reports indicated that the surface separation was caused by crosslinked PDMS networks or gels, and that the fluidity of the layer was caused by free and pendant chains or brush-like dangling chains.

4. Conclusions

In order to investigate the key factors responsible for the improvement of the lubricity of material surfaces, we prepared three kinds of well-controlled polymer brush layers with different monomer units and characterized them at a nanoordered level using a QCM-D and an AFM. The friction resistance was strongly correlated to the water absorptivity and fluidity of the polymer-hydrated layer. Increasing the surface separation distance with polymer-hydrated layer is the first key factor for obtaining a highly lubricated biointerface, and the high fluidity of the polymer-hydrated layer is the second key factor. PMPC grafting is a very effective and promising method for achieving both the abovementioned factors.

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Surface modification of a titanium alloy with a phospholipid polymer prepared by a plasma-induced grafting technique to improve surface thromboresistance

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ABSTRACT

To improve the thromboresistance of a titanium alloy ($TiAl_6V_4$) surface which is currently utilized in several ventricular assist devices (VADs), a plasma-induced graft polymerization of 2-methacryloyloxyethyl phosphorylcholine (MPC) was carried out and poly(MPC) (PMPC) chains were covalently attached onto a $TiAl_6V_4$ surface by a plasma induced technique. Cleaned $TiAl_6V_4$ surfaces were pretreated with H_2O-vapor-plasma and silanated with 3-methacryloylpropyltrimethoxysilane (MPS). Next, a plasma-induced graft polymerization with MPC was performed after the surfaces were pretreated with Ar plasma. Surface compositions were verified by X-ray photoelectron spectroscopy (XPS). In vitro blood biocompatibility was evaluated by contacting the modified surfaces with ovine blood under continuous mixing. Bulk phase platelet activation was quantified by flow cytometric analysis, and surfaces were observed with scanning electron microscopy after blood contact. XPS data demonstrated successful modification of the $TiAl_6V_4$ surfaces with PMPC as evidenced by increased N and P on modified surfaces. Platelet deposition was markedly reduced on the PMPC grafted surfaces and platelet activation in blood that contacted the PMPC-grafted samples was significantly reduced relative to the unmodified $TiAl_6V_4$ and polystyrene control surfaces. Durability studies under continuously mixed water suggested no change in surface modification over a 1-month period. This modification strategy shows promise for further investigation as a means to reduce the thromboembolic risk associated with the metallic blood-contacting surfaces of VADs and other cardiovascular devices under development.

1. Introduction

Suboptimal blood compatibility in many cardiovascular devices puts patients at increased risk for thromboembolism, often necessitating the use of chronic anti-coagulation and its accompanying increased risk for bleeding. The composition of the biomaterial surface, the nature of the blood flowing across the device surfaces and the bias of the patient's blood toward hemostatic reactions all combine to define thrombotic and thromboembolic risk. Thus much work has focused on utilizing computational fluid dynamics to improve flow characteristics over biomaterial surfaces in circulatory support devices [1] and similarly there has been great interest in developing chemical modifications for blood contacting surfaces [2].

Our recent interest has been in developing a rotary blood pump for pediatric applications in which aggressive anticoagulation may be problematic and biocompatibility is thus of primary concern. For design and machinability considerations the titanium alloy $TiAl_6V_4$ makes up the blood contacting surfaces of this pump as well as several other rotary blood pumps in clinical use and in pre-clinical development [1,3–9]. Although titanium and its alloys have exhibited generally acceptable biocompatibility in a variety of settings, its surface modification remains of interest for blood contact since platelet deposition still can occur in vitro and thrombosis and thromboembolism can occur in these devices in vivo [10–12].

Biomaterial surface modification by plasma based techniques has frequently been applied due to the high efficiency of this

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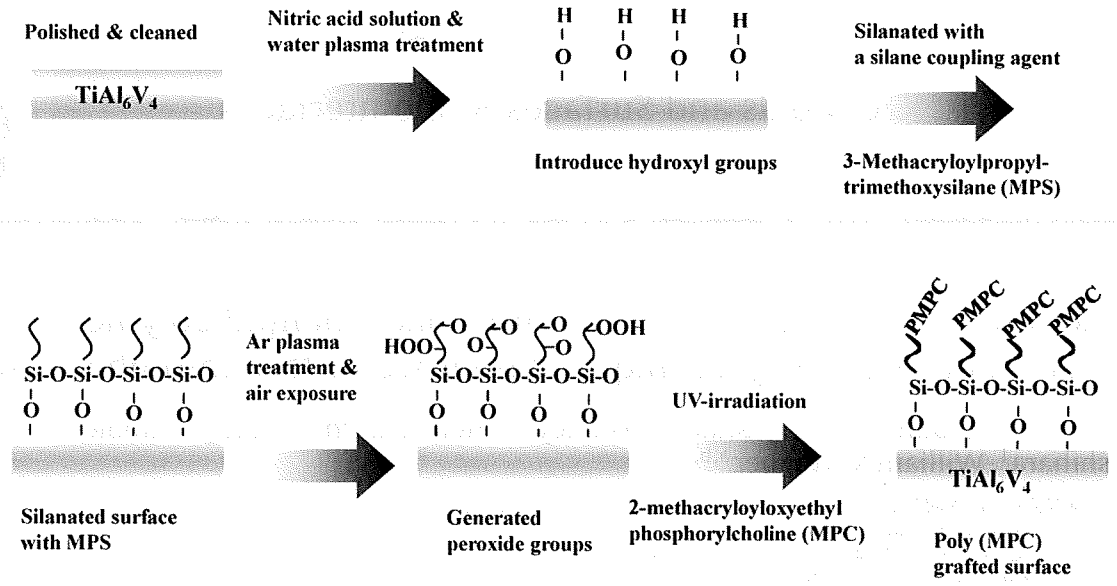

Fig. 1. Scheme of surface functionalization and modification of a $TiAl_6V_4$ surface with PMPC.

approach for a variety of substrates and geometry [13]. Active functional groups can be introduced by utilizing a specific atmosphere such as water, ammonia or argon (Ar) which allows further modification by covalent attachment of other polymers or biomolecules on the substrate [14–17]. Plasma induced graft polymerization after Ar plasma pretreatment is attractive for its high efficiency and others have previously reported on the effects of plasma treatment time, plasma power and storage time on surface modifications with this method [18,19]. Polytetrafluoroethylene, silicon, and stainless steel surfaces have successfully been modified by Ar plasma induced grafting copolymerization with a poly (ethylene glycol) containing macromonomer to improve their surface blood compatibility [20–22].

Synthetic phospholipid polymers (phosphorylcholine (PC) group-bearing polymers) have been extensively studied due to their excellent biological and blood compatibility [23]. One of the most representative phospholipid polymers, 2-methacryloyloxyethylphosphorylcholine (MPC) polymer has received considerable interest for medical applications [24–26]. Many researchers have shown previously that surface modification with the MPC polymers or the introduction of PC groups by blending, coating and graft modification techniques are effective in improving blood compatibility by resisting non-specific protein adsorption and platelet activation and adhesion on polymer biomaterial surfaces [27–31]. The MPC polymers have also been applied to some metallic biomaterial surfaces including ventricular assist devices (VADs) and demonstrated apparent improvements in blood compatibility [32–35]. However, to date most of the surface modification techniques with the MPC polymers involve physical adsorption onto metallic surfaces. We recently reported on the MPC polymer covalent immobilization approach for $TiAl_6V_4$ that showed promise in improving blood biocompatibility [36]. While effective, concerns with this technique were the lability of the amide bond linking the copolymer to the surface and the extent of surface coverage that might be achieved.

Our objective in this study was to introduce a new, potentially more robust method of polymerizing MPC off of a modified $TiAl_6V_4$ surface using a plasma-induced graft technique that might offer durability under high shear and for extended blood contacting periods. The modified $TiAl_6V_4$ surface was characterized in terms of its surface composition and acute platelet deposition and activation after contact with ovine blood in vitro.

2. Materials and methods

2.1. Materials

A titanium alloy ($TiAl_6V_4$) sheet was obtained from California Metal & Supply Inc., Gardena, CA. MPC was obtained from NOF Corp. (Tokyo, Japan), which was synthesized as previously described [24]. 3-Methacryloylpropyltrimethoxysilane (MPS, Aldrich, USA) was used as a silane coupling agent. Heparin (Pharmacia & Upjohn Co., Ann Arbor, MI) was used for blood anticoagulation.

2.2. Surface pretreatment and silanization of a $TiAl_6V_4$

The $TiAl_6V_4$ sheet was polished with 3.0, 1.0, 0.25, and 0.1 μ diamond pastes in sequence (Electron Microscopy Sciences, Washington, PA) and cleaned ultrasonically three times for 5 min each with ethanol and acetone. The surfaces were then passivated with a 35% nitric acid solution for 1 h and rinsed with distilled water. Next, the $TiAl_6V_4$ surfaces were pretreated under H_2O plasma with radio frequency glow discharge (RFGD, MARCH GCM250, March Instrument Inc, CA). The RFGD power applied was 25 W at a frequency of 13.65 MHz. The titanium surface was subjected to RFGD for 5 min supplying H_2O vapor at a vacuum pressure of 0.4 Torr. The H_2O plasma pre-treated $TiAl_6V_4$ surfaces were silanated by immersion in an MPS solution for 3 h in a 90 °C oil bath. The MPS solution consisted of 2% MPS in ethanol that was hydrolyzed by adding water and stirring for 1 h. The pH of the MPS solution was adjusted to approximately 3–4 by adding 0.1 M HCl. The silanated samples were dried at 110 °C for 1 h, then rinsed repeatedly with ethanol and water, and stirred in deionized water for 1 h to remove adsorbed MPS. Samples treated in this manner were referred to as Ti-MPS (Fig. 1).

2.3. Plasma-induced surface graft modification with MPC

The silanated $TiAl_6V_4$ samples were treated with Ar plasma by using RFGD (25 W, 20 s, 0.6 Torr), and then the surface was exposed to the atmosphere for 10 min to create surface peroxide groups. The $TiAl_6V_4$ sample was then immersed in MPC solution (0.5 mM) which was placed in a transparent polystyrene round-bottom tube (BD Bioscience, San Jose, CA) The monomer solution was passed through argon gas for 1 min and 0.005 mM

riboflavin (Sigma–Aldrich, St. Louis, MO) was added to eliminate any oxygen [14]. Then, graft modification onto the $TiAl_6V_4$ surface was carried out under a high intensity UV lamp for 24 h. This decomposed the surface peroxide groups to free radicals and poly(MPC) (PMPC) was grafted from the surface. The modified samples were rinsed three times with ethanol and water and stirred in deionized water for 24 h to remove physically adsorbed PMPC. Samples treated in this manner were referred to as Ti–MPS–PMPC (Fig. 1).

2.4. Surface characterization

The surface composition of the titanium samples was analyzed by X-ray photoelectron spectroscopy (XPS) using a surface science instruments S-probe spectrometer and a take-off angle of photoelectron was 55°. The Service Physics ESCAVB Graphics Viewer program was used to determine peak area, calculate the elemental compositions from peak areas, and peak fit the high-resolution spectra. The surface composition on a given sample was averaged from three composition spots for each sample. The mean value for three different samples was determined.

The static contact angle of water on the surfaces of unmodified and modified titanium samples was measured at room temperature using a contact angle goniometer (VCA optima, AST Product Inc., Billerica, MA) by placing 1 μL of double distilled water on the surfaces. The droplet was imaged using a video camera coupled to a light microscope, and the contact angle was determined on the screen of a monitor employing imaging software. Five measurements were made on each sample to obtain the contact angle of the sample. The contact angle was also measured weekly in several of the modified samples that underwent continuous stirring under deionized water for 1 month to test the long term stability of the surface modification. XPS was also performed on the surfaces after 1 month of water contact.

2.5. Blood collection and blood contact test

Whole ovine blood was collected by jugular venipuncture directly into a syringe containing heparin (3.0 or 6.0 U/mL for 1 and 2.5 h blood contacting experiments, respectively) using an 18 gauge 1 ½″ needle, after discarding the first 3 mL. NIH guidelines for the care and use of laboratory animals were observed. Modified titanium and unmodified samples were placed into Vacutainer® blood collection tubes without additives (BD Biosciences, Franklin Lakes, NJ) filled with heparinized ovine blood and incubated for a specified time at 37 °C on a hematology mixer (Fisher Scientific, Pittsburgh, PA).

2.6. Observation and quantification of platelet deposition and activation

The $TiAl_6V_4$ surfaces were observed by scanning electron microscopy (SEM; JSM-6330F, JEOL USA, Inc., Peabody, MA) after 2.5 h contact with heparinized blood (6.0 U/mL). The $TiAl_6V_4$ surfaces were also observed with epi-fluorescence microscopy (ZEISS, Carl Zeiss, Inc. Thornwood, NY) after contact for 1 h with heparinized blood (3.0 U/mL) that was treated with quinacrine dihydrochloride (10 μM final concentration, Sigma) to fluorescently label the platelets. The number of platelets for each sample was also estimated by a lactate dehydrogenase (LDH) assay [37] with an LDH Cytotoxicity Detection Kit (Clonetech Laboratories Inc., CA). In this assay, the surfaces were rinsed thoroughly with 50 mL PBS following 2.5 h contact with blood (6.0 U/mL heparin) and then immersed in 1 mL of 2% Triton X-100 solution (Sigma) for 20 min to lyse deposited platelets. Calibration of spectrophotometer absorbance results to platelet numbers was accomplished using a calibration curve generated from known dilutions of ovine platelet rich plasma in the lysing solution.

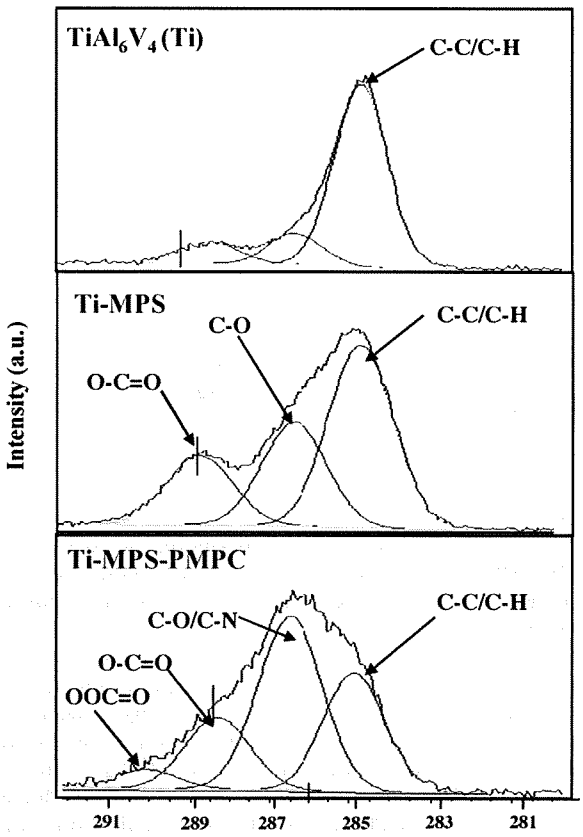

Fig. 2. XPS high resolution (C1s) spectra of the modified and unmodified $TiAl_6V_4$ samples.

The percentage of activated ovine platelets in the bulk phase of the blood during surface contact was determined using flow cytometric quantification of Annexin V binding as recently described [38]. Blood samples (10 μL) were taken during test surface contact experiments described above for LDH measurement of platelet deposition after 2 h. Activation levels from five independent samples were averaged for each surface type.

2.7. Statistical analyses

Data are presented as means with standard deviation. Statistical significance between sample groups was determined using ANOVA followed by post-hoc Newman–Keuls testing and accepted at $p < 0.05$.

3. Results

The high-resolution spectra from XPS are shown in Fig. 2. The C1s data was calibrated to the hydrocarbon peak (C–C/C–H) at 285.0 eV. The MPS modified $TiAl_6V_4$ surface (Ti–MPS) showed an increase in the peak at 286.6 eV and 288.5 eV which is likely due to C–O and O–C=O type species. The PMPC modified $TiAl_6V_4$ surface showed a further increase in the peak at 286.6 eV by the addition of the C–N type species that are attributed to the MPC units. Furthermore, the Ti–MPS–PMPC also has a peak at 290.5 eV which is likely due to peroxide OOC=O type species that is attributed to the Ar plasma treatment. The surface atomic compositions of $TiAl_6V_4$ samples are shown in Table 1. The oxygen composition of the pre-treated $TiAl_6V_4$ surfaces (Ti–H_2O plasma) rose significantly in comparison with the Ti which was not pre-treated ($p < 0.05$). The MPS mod-

Table 1
Atomic percentage by X-ray photoelectron spectroscopy.

	C	O	Ti	Al	Si	N	P
$TiAl_6V_4$ (Ti)	42.0 (±8.0)	41.1 (±5.2)	9.5 (±1.1)	4.3 (±3.1)	1.0 (±1.0)	1.0 (±0.5)	0.1 (±0.2)
Ti (H_2O plasma)	24.1 (±6.6)	50.8 (±0.4)	10.5 (±3.3)	2.3 (±1.1)	1.3 (±1.4)	0.8 (±0.3)	0.0 (±0.0)
Ti–MPS	49.4 (±8.9)	34.3 (±5.4)	1.3 (±1.3)	1.2 (±1.1)	13.9 (±4.8)	0.2 (±0.4)	0.0 (±0.0)
Ti–MPS–PMPC	42.8 (±12.6)	40.0 (±7.8)	1.4 (±1.6)	0.0 (±0.0)	14.1 (±3.8)	1.8 (±0.3)*	1.0 (±0.2)*

$N = 7$, ± standard deviation for Ti. $N = 3$, ± standard deviation for Ti (H_2O plasma). $N = 5$, ± standard deviation for Ti–MPS. $N = 4$, ± standard deviation for Ti–MPS–PMPC.
* $p < 0.05$ vs. other surfaces.

Table 2
Surface tension on the unmodified and modified titanium samples.

	$TiAl_6V_4$ (Ti)	Ti (H_2O plasma)	Ti–MPS	Ti–MPS–PMPC
Contact angle (°)	58.3 (±6.2)	34.7 (±4.4)	87.6 (±6.4)	18.1 (±4.0)*

$n = 3$, ± standard deviation.
* $p < 0.05$ vs. other surfaces.

ified surface showed an increase in Si which was attributed to the presence of MPS ($p < 0.05$). Furthermore, the XPS data from Ti–MPS–PMPC provide evidence for the successful modification with PMPC by reflecting increased nitrogen (N) and phosphorus (P) ($p < 0.05$).

The surface tension on the modified and unmodified titanium samples was shown in Table 2. The contact angle on $TiAl_6V_4$ surfaces was decreased from 58 ± 6° to 35 ± 4° after H_2O plasma treatment. The Ti–MPS increased in surface tension to 88 ± 6° due to modification with hydrophobic MPS on the $TiAl_6V_4$ surface. However, the surface tension of the PMPC grafted surfaces decreased substantially (Ti–MPS–PMPC = 18 ± 4°) by modification with the hydrophilic PMPC on the surface in comparison with all of the other surfaces ($p < 0.05$). The surface contact angle on the PMPC grafted surface (Ti–MPS–PMPC) measured every 7 days during a 1-month period of mixing with water did not significantly change (Fig. 3). Additionally, the surface composition of several of the modified samples was also measured after mixing for 1 month in water. The XPS analysis results also showed no significant difference in the surface composition of phosphorus before (*P*: 1.1 ± 0.1%) and after (*P*: 1.0 ± 0.1%) the water contacting experiment.

The modified and unmodified $TiAl_6V_4$ surfaces after contact with anticoagulated ovine blood for 1 h at 37 °C were observed with an epi-fluorescence microscope (Fig. 4). Fluorescent platelets are seen to be adhered and aggregated on the surface. The unmodified titanium had relatively high numbers of deposited platelets, whereas the PMPC modified surfaces (Ti–MPS–PMPC) showed few adherent platelets. Platelet adhesion and morphology was also observed with SEM after contact with ovine blood for 2.5 h at 37 °C. The SEM images on the surfaces of the positive control polystyrene, unmodified and modified titanium samples are seen in Fig. 5. The polystyrene control surface (Fig. 5A) supported heavy platelet deposition with most of the deposited platelets exhibiting extended pseudopodia. The unmodified titanium surface (Fig. 5B) had a moderate amount of deposited platelets on its surface, with the number of platelets appearing to be less than for the polystyrene surface. The silanated titanium surface (Ti–MPS, Fig. 5C) also showed a moderate amount of deposited platelets, and these platelets exhibited extended pseudopodia. The quantity of adhered platelets on the Ti–MPS surface appeared to be slightly more than for the unmodified titanium (Fig. 5B). Platelet deposition was decreased dramatically on the Ti–MPS–PMPC surfaces, and the platelets that were adhered generally retained their discoid morphology with some pseudopodia extension (Fig. 5D). It is worth noting that it was difficult to identify adhered platelets on the Ti–MPS–PMPC surface. The number of deposited platelets as quantified by the lactate dehydrogenase (LDH) assay after blood contact (Fig. 6) was significantly less for Ti–MPS–PMPC surfaces than for all of the other surfaces ($p < 0.01$). Flow cytometric quantification of bulk phase platelet activation using the Annexin V assay (Fig. 7) was also significantly lower in blood contacting Ti–MPS–PMPC surfaces than for unmodified titanium and polystyrene samples ($p < 0.05$).

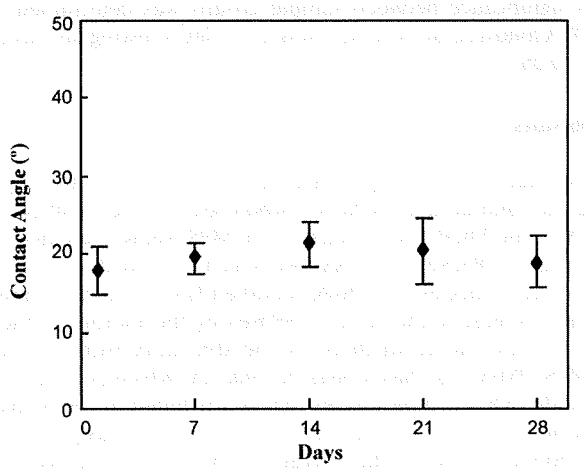

Fig. 3. Contact angle measurements of the PMPC modified $TiAl_6V_4$ surface (Ti–MPS–PMPC) after continuous mixing under deionized water.

4. Discussion

Improvement of surface blood compatibility is of keen interest to the cardiovascular device community given the morbidity and mortality associated with device-related thrombotic complications and with the anticoagulation applied to minimize these risks. In the case of VADs, biocompatibility concerns are a major reason why these devices are underutilized in heart failure patients [3,39–41]. PC groups and PC group-bearing polymer modified surfaces have previously demonstrated marked improvement in blood compatibility when compared to unmodified surfaces [24–31]. Moreover studies involving self-assembled monolayers (SAM) with well defined and highly ordered and oriented PC groups have shown that this type of surface is one of the most promising for anti-fouling since it strongly resists non-specific protein adsorption and cell adhesion [42–44]. There are several successful studies where a PC group-bearing polymer was physically adsorbed onto the metallic surfaces in vascular stent and VAD applications [9,33,34]. In the VAD studies the blood contacting surface containing physically adsorbed MPC polymer was shown to be superior in terms of blood compatibility when compared to the same device with a blood contacting surface modified with a diamond like carbon coating [9,34]. Despite the improved hemocompatibility in these studies, there was con-

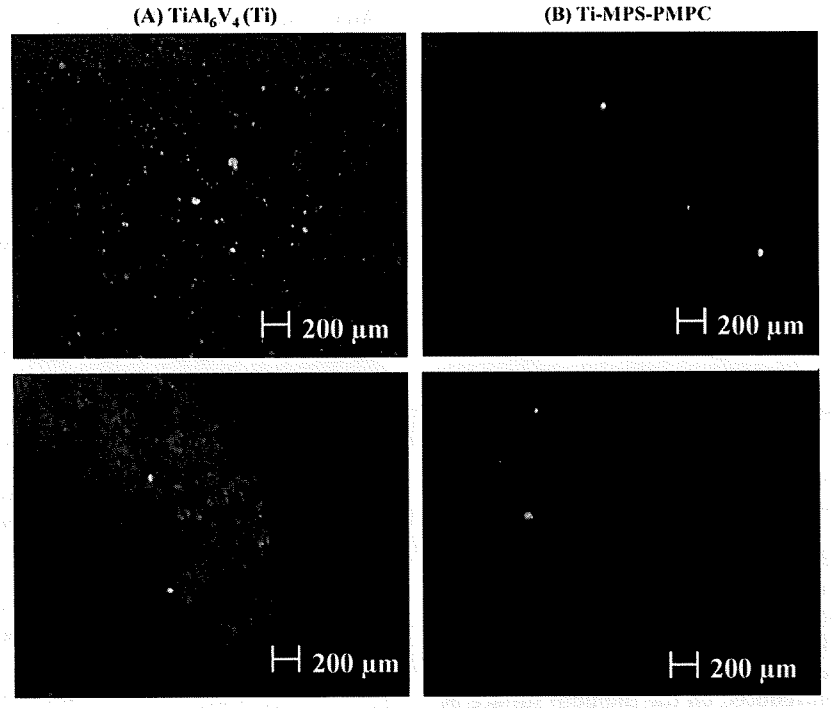

Fig. 4. Fluorescent micrograph images of unmodified and modified $TiAl_6V_4$ samples after contact with minimally anticoagulated (3.0 U/mL heparin) ovine blood for 60 min at 37 °C.

cern about the long term stability of the surface PMPC. Furthermore, in the report of Kihara et al, the physically adsorbed MPC polymer was demonstrated to elute over time during VAD operation [34].

In spite of widespread examination of grafting PC groups onto polymer or silica surfaces, there are few reports where a PC group-bearing polymer has been grafted onto metallic surfaces, and even fewer that have subsequently evaluated the hemocompatibility of the resulting surfaces [36,45,46]. In this study, we showed that a $TiAl_6V_4$ surface could be successfully modified with the MPC moiety by a plasma-induced grafting method after the $TiAl_6V_4$ surface was silanized, and then Ar plasma treated. Our XPS results demonstrated successful modification of the $TiAl_6V_4$ surface with PMPC and the surface showed significant decreases in platelet deposition and activation in comparison with unmodified surfaces. The higher platelet deposition observed on the control Ti–MPS surface relative to the Ti surface could generally be attributed to an increase in surface hydrophobicity as reflected by the higher contact angle of this surface relative to the unmodified Ti alloy. Such an increase in hydrophobicity may serve as a driving force for increased protein adsorption and in this process result in more fibrinogen adsorption in a state that would support platelet adhesion on the surface.

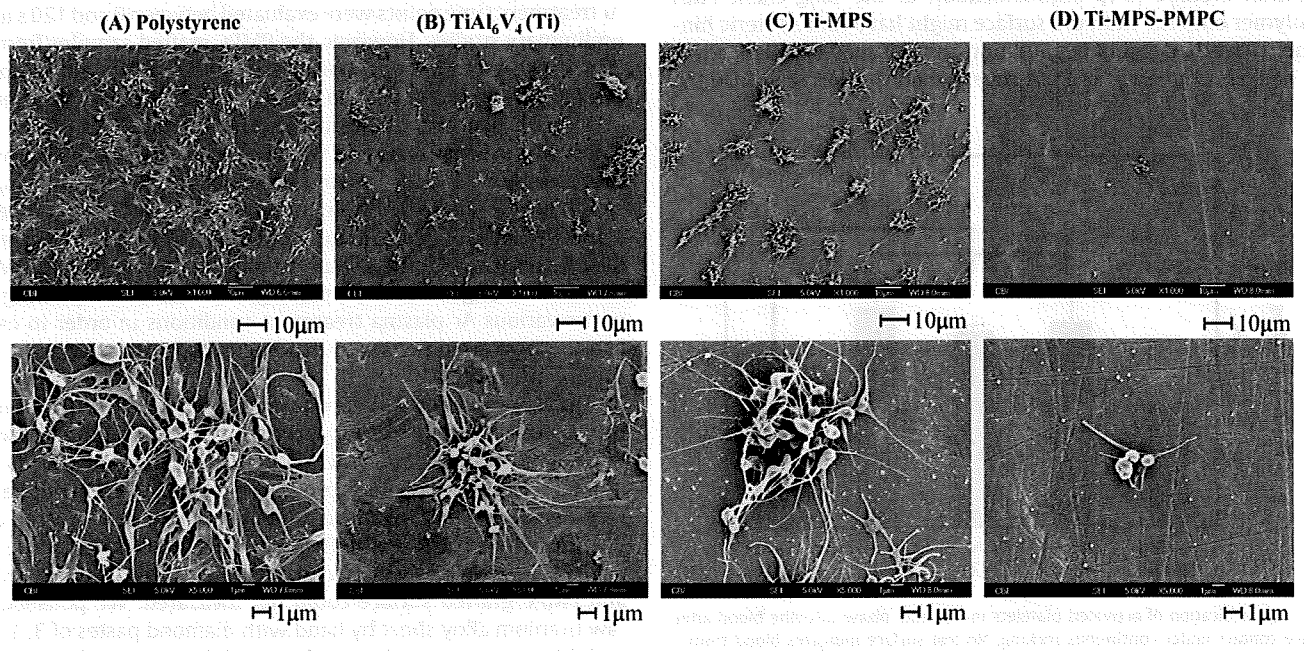

Fig. 5. SEM micrographs of polystyrene, unmodified and modified $TiAl_6V_4$ samples after contact with ovine blood (heparin 6U/mL) for 2.5 h at 37 °C. (A) polystyrene (B) Ti (C) Ti–MPS (D) Ti–MPS–PMPC.

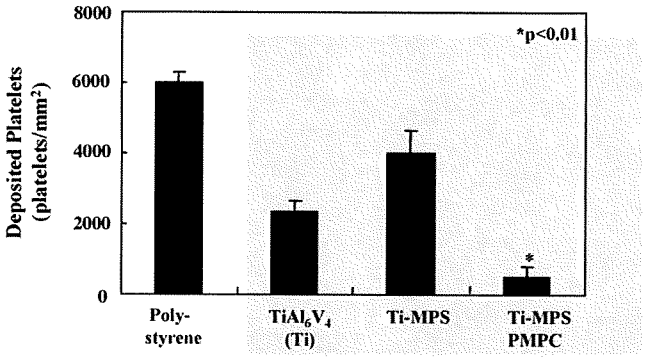

Fig. 6. Platelet deposition onto surfaces after contact with ovine blood for 2.5 h as determined by lactate dehydrogenase (LDH) assay ($n=3$).

For the PMPC-grafted surface the contact angle and XPS results after a month of mixed water contact were not significantly different from our initial surface tension and XPS evaluation signifying the stability of the grafted PMPC and its sustained hydrophilicity. We previously reported on reduced thrombogenicity after immobilizing an MPC polymer poly(MPC-co-methacryl acid) (PMA) onto titanium surfaces. This surface modification strategy involved a condensation reaction between the carboxyl groups of the PMA and amino groups which were introduced on the titanium surface by a silane coupling agent [34]. However despite its covalent attachment, the long term stability of the PMA immobilized surface is a concern. The cause for concern is that over time the amide bonds between PMA and the titanium surface may be hydrolyzed under continuous blood contact. The PMPC grafting in this report is not linked to the titanium surface via an amide bond, potentially providing an advantage over the PMA immobilized surface in terms of longer-term stability under similar circumstances. Another advantage over the PMA immobilization strategy is that the PMPC grafting technique uses Ar plasma treatment which could be applied to other metallic surfaces, and also on many medically-relevant polymeric surfaces including polytetrafluoroethylene (PTFE).

The PMPC grafting method of this report might allow for more control of PMPC surface coverage and offer improved uniformity of surface PC groups when compared with the previous PMA immobilization method [34]. Immobilization of the long-chain PMA copolymer onto the titanium surface might have caused steric hindrance to further copolymer addition and this may have led to low and non-uniform areas of PMA coverage on the surface. Since the PMPC grafting method employs the addition of MPC rather than the long chain PMA copolymer there is less concern about steric hindrance. Less steric hindrance might lead to higher coverage of the MPC unit on the modified surface provided the surface has sufficient radical formation sites. However it is worth noting that in this grafting method it may be difficult to prepare a surface with 100% MPC unit coverage. This method also has some limitation in terms of the level of control achievable with respect to chain length of the modified PMPC. To obtain better control of the PMPC chain length on the surface, an alternative surface modification method such as surface initiated atom transfer radical polymerization could be considered. If a method were developed to prepare a covalently attached PMPC chain self assembled monolayer on a $TiAl_6V_4$ surface, it would ensure high coverage and uniformity of the PC group and might be even more advantageous in the manufacture of biocompatible blood contacting device surfaces.

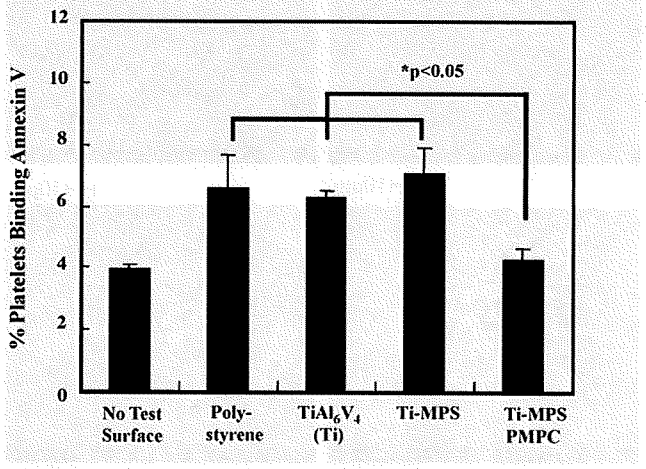

Fig. 7. Quantification of activated platelets in the bulk phase of ovine blood after surface contact under continuous rocking. No test surface indicates blood from a rocked tube into which no test surface was placed. Platelet activation was quantified by flow cytometric measurement of Annexin V binding onto platelets ($n=3$).

In preliminary studies, we chose MPS as a pre-modification agent since it possesses a double bond in its structure and could provide a radical formation site in UV-initiated graft polymerization of MPC. This approach alone, without a UV catalyst, had minimal success (data not shown), so Ar plasma was employed to modify the Ti–MPS surface with the hypothesis that Ar plasma treatment would provide a greater number of radical formation sites, as well as peroxide, on the surface than with UV irradiation alone [18]. The application of both an Ar plasma pre-treatment followed by UV irradiation in the presence of a catalyst such as benzophenone [30,46] on the Ti–MPS surface might have led to even further surface grafting, however this was not investigated.

The graft modification approach using Ar plasma treatment with the PMPC on $TiAl_6V_4$ described in this study might be improved with additional study into ways to further control the surface coverage of the grafted PMPC polymer and therefore maximize the anti-thrombogenic properties of the surface. Several factors that should be considered to improve the surface modification process include control of the water plasma in the MPS silanization process, treatment time under Ar plasma and RFGD power settings. The latter two factors are important in this modification technique since they affect the amount of peroxide groups that are able to become radical formation sites by the decomposition of the peroxide groups [12]. In this study, the Ar plasma treatment time was set to 20 s under 25 W of plasma power at a pressure of 0.6 Torr. Several other Ar treatment time points were evaluated between 0 and 120 s in our preliminary studies. However, the PMPC grafted samples from the 20 s Ar plasma treatment showed the lowest surface contact angle ($18.2° \pm 4.1$) and were used in our subsequent studies. The amount of peroxide concentration generated on the $TiAl_6V_4$ surfaces after the 20 s Ar plasma treatment was $3.4(\pm 1.6) \times 10^{-8}$ mol/cm^2, which was assessed with the plasma treated titanium samples in this study by a peroxide determination method using a 2,2-diphenyl-1-pricylhydrazyl (DPPH) solution [18,19]. However, further study may be necessary to better understand the relationship between the amount of generated peroxide groups and PMPC grafting rate under various Ar plasma treatment conditions in order to establish the best conditions and maximize PMPC incorporation onto the $TiAl_6V_4$ surface.

The MPS modified surface was prepared under hydrolytic condition which allows the methoxy groups in MPS to change to hydroxyl groups and activates the reaction between TiOH and MPS as well as MPS self interaction, thus producing bulk deposition on the surface. Furthermore, the surface roughness might be increased after PMPC grafting, because, the PMPC grafted layer might not be uniform [46]. However the surface roughness or thickness on the MPS and PMPC grafted surface could not measured. We polished the raw titanium alloy sheet by hand with diamond pastes of 3, 1, 0.25 and 0.1 μm size, thus the roughness of the original titanium surface was of a scale that additional texture added from the surface

modification was not likely to be detectable. We chose to use this surface polishing technique since this is the process used industrially to prepare the titanium alloy surfaces before use in blood pump assembly. The surface roughness of the unmodified $TiAl_6V_4$ sample and bulk layer deposition of MPS may have contributed to deviations observed in the atomic composition (Table 1) and contact angle (Table 2) data on the unmodified and modified surfaces. Further study is necessary to measure the surface roughness and thickness of the grafting layer depending on the reaction conditions with a highly polished $TiAl_6V_4$ sample. Fluorescence microscopy observation after staining the PMPC [46], atomic force microscopy and ellipsometry analysis data could be helpful to measure the thickness and roughness of PMPC grafting layer.

In this study, UV irradiation was used to decompose the generated peroxide group and initiate the graft polymerization of MPC. However, the peroxide decomposition also occurs by heating [12,13] and polymerization of MPC might be carried out at 70 °C without UV irradiation if UV irradiation is not appropriate due to complex device geometry. Another limitation to the study is that the blood biocompatibility tests were performed using an in vitro system with ovine blood and under flow conditions that, though mixed, do not replicate any specific application. A next step might be to apply this modification to the interior surfaces of a rotary VAD and assess platelet deposition onto these surfaces in a mock circulatory loop under appropriately high shear blood flow. Comparison studies with other types of modified surfaces such as poly(ethylene glycol)-based or other types of zwitterionic moieties might be of interest to compare the efficacy of this plasma induced grafting technique.

5. Conclusions

$TiAl_6V_4$ surfaces were successfully modified with PMPC chains by a plasma-induced grafting technique following surface silanization and Ar plasma treatment. The PMPC modified $TiAl_6V_4$ surfaces showed significantly decreased platelet deposition and bulk phase platelet activation in vitro relative to the unmodified Ti samples. This PMPC grafting on $TiAl_6V_4$ surfaces shows promise for further investigation as a means to reduce the thromboembolic risk associated with the blood-contacting surfaces of cardiovascular devices. In the setting of a rotary blood pump design, such a coating may allow reduction in anticoagulation levels. Further pre-clinical evaluation is warranted to investigate this potential.

Acknowledgements

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Superlubricious surface mimicking articular cartilage by grafting poly(2-methacryloyloxyethyl phosphorylcholine) on orthopaedic metal bearings

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Abstract: Aseptic loosening of the artificial hip joint with osteolysis due to the wear particles from polyethylene cup has remained as a serious issue. To reduce this wear and develop a novel artificial hip joint system, we produced a superlubricious metal-bearing material: for this, we grafted a 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer onto the surface of a cobalt–chromium–molybdenum (Co-Cr-Mo) alloy. For ensuring long-term benefit retention of poly(MPC) on the Co-Cr-Mo alloy for application as a novel artificial hip joint system, several issues must be considered: strong bonding between poly(MPC) and Co-Cr-Mo surface, high mobility of free end groups of the poly(MPC) layer, and high density of the introduced poly(MPC). Considering these issues, we introduced a 3-methacryloxypropyl trimethoxysilane (MPSi) intermediate layer and a photoinduced graft polymerization technique to create a strong covalent bond between the Co-Cr-Mo substrate and the poly(MPC) chain via the MPSi layer. The thickness and density of the poly(MPC) layer on the surface increased with the MPC concentration and photoirradiation time. The grafted poly(MPC) layer successfully provided super-lubricity to the Co-Cr-Mo surface. The poly(MPC)-grafted crosslinked polyethylene/poly(MPC)-grafted Co-Cr-Mo or cartilage/poly(MPC)-grafted Co-Cr-Mo bearing interface mimicking natural joints showed an extremely low friction coefficient of 0.01, which is as low as that of natural cartilage interface. A superlubricious metal-bearing surface would enable the development of a novel biocompatible artificial hip joint system-artificial femoral head for partial hemiarthroplasty and metal-on-polymer/metal type for total hip arthroplasty. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 91A: 730–741, 2009

Key words: joint replacement; metal surface treatment; photopolymerization; phosphorylcholine; hydrophilicity

INTRODUCTION

Every year, the number and prevalence of primary and revision hip and knee joint replacements increases substantially worldwide.[1] As a result, the quality of artificial joints is becoming increasingly important. Most patients who receive an artificial joint experience dramatic pain relief and rapid improvement in both their daily activities and quality of life. The most widely used bearing couple in artificial hip joint systems is a combination of an ultrahigh molecular weight polyethylene (UHMWPE) acetabular component and a metal femoral component. Cobalt–chromium–molybdenum (Co-Cr-Mo) alloy is one of the most widely used metal bearing materials in artificial joint systems. The Co-Cr-Mo alloy has good mechanical properties, castability, corrosion resistance, and wear resistance, whereas stainless steel and titanium alloys have a disadvantage with regard to corrosion resistance and wear resistance, respectively.

In total hip arthroplasty (THA), osteolysis caused by the wear particles from UHMWPE has been recognized as a serious issue.[2–4] Efforts to decrease

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these particles have focused on bearing material improvement and the use of combinations other than metal-on-UHMWPE.[5–7] Recently, a metal-on-metal type artificial hip joint system consisting of Co-Cr-Mo acetabular and femoral components has been studied.[8] The advantages of the Co-Cr-Mo/Co-Cr-Mo bearings are that they do not generate UHMWPE wear debris and they exhibit decreased wear when compared with Co-Cr-Mo/UHMWPE bearings.[9,10] However, even in Co-Cr-Mo/Co-Cr-Mo bearings, aseptic loosening induced by wear particles and metallosis remains as serious issue in revision surgeries.[11,12] In addition to metallosis, electrochemical corrosion and carcinogenesis occurring due to the dissemination of wear particles to the other parts of the body have been reported.[13]

On the other hand, improvements in the bearing materials and surface modifications of the Co-Cr-Mo alloy have been attempted, in order to reduce such wear particles.[14,15] Surface coating may reduce the wear without compromising the bulk mechanical properties of the implant materials. Various "hardening treatments" of metal bearing surfaces, such as diamond-like carbon coating, titanium nitride coating, and ion implantation have also been attempted.[16,17] Although these surface modifications may improve THA survivorship, the limited THA longevity imposes restrictions for its application to younger patients. Consequently, the possibility of replacing the femoral head alone, whether solid or articular surface replacement, remains an attractive feature of such implants during revision surgeries of THA. However, the Co-Cr-Mo alloy or the hardening-treated Co-Cr-Mo alloy may induce damage to cartilaginous tissue.

In contrast, the previous study reported that highly lubricious hydrogel polymer used as an artificial cartilage did not damage cartilaginous tissue.[18] We have recently developed a highly lubricious artificial hip joint system by a "mild treatment" with soft materials. In this treatment, poly(2-methacryloyloxyethyl phosphorylcholine) (MPC) was grafted onto the surface of CLPE (CLPE-*g*-MPC).[19–21] MPC is a methacrylate with a phospholipid polar group in a side chain, and it has both good solubility in polar solvents including water and polymerization ability by conventional radical polymerization.[22] Many MPC polymers have been widely investigated as biomaterials.[23–27] As a result, various medical devices have already been developed using MPC polymers, and they are being used clinically. The efficacy of MPC polymers as biomaterials has been well verified.[28–30]

In general, there are two methods for modifying the polymer surface. The first method involves surface absorption or reaction with small molecules,[31,32] and the second is grafting polymeric molecules onto the substrate through covalent bonding.[33] Most frequently, grafting polymerization is performed using either of the following methods: (1) surface-initiated graft polymerization, termed as the "grafting from" method, in which monomers are polymerized from initiators or comonomers and (2) adsorption of the polymer to the substrate, termed as the "grafting to" method (i.e., dipping, crosslinking, and ready-made polymers with reactive end groups reacting with the functional groups of the substrate).[34,35] In our previous study, the Co-Cr-Mo-*g*-MPC prepared by the adsorption of the polymer to the Co-Cr-Mo substrate, termed as the "grafting to" method, was not uniform, and the CLPE-*g*-MPC/Co-Cr-Mo-*g*-MPC bearing couples showed high friction.[36] These results were probably ascribed to a low density of the poly (MPC) on Co-Cr-Mo prepared by the "grafting to" method. To solve the issue in this study, we developed a superlubricious surface with nanometer-scale poly(MPC) modification and was accomplished by using a photo-induced radical polymerization technique that was similar to the one used in the "grafting from" method. The "grafting from" method has an advantage over the "grafting to" method in that it synthesizes a high-density polymer brush.

To ensure *in vivo* long-term retention of this poly(MPC) graft on the Co-Cr-Mo alloy, it is necessary to create strong covalent bonding between the Co-Cr-Mo alloy substrate and the poly(MPC) graft chain. Organosilanes have already been known as surface coupling agents to enhance bonding between a metal or a metal oxide surface and an organic resin such as dental resin, and they can strongly bind metals to resins in dental implants.[37] Organic silanes or silane coupling agents comprise at least a hydrolyzable alkoxysilyl or chlorosilyl group and an organofunctional group.[38] The agents are effective to introduce organofunctional groups into the siloxane network polymer. The organofunctional group in the silane could be useful to improve bonding with the organic overlayer. 3-Methacryloxypropyl trimethoxysilane (MPSi) is a simple surface coupling agent consisting of three methoxysilane groups, a propyl chain, and a functional methacrylate, and the structure of its main chain is equivalent to that of MPC.

In this study, based on the hypothesis that the "grafting from" method has advantages over the "grafting to" method in that it can synthesize a uniformly and controllable polymer layer, a superlubricious metal bearing material in which the poly(MPC) with biocompatibility and hydrophilicity was grafted onto the surface of the Co-Cr-Mo alloy (Co-Cr-Mo-*g*-MPC) has been introduced for developing a novel artificial hip joint system, that is, artificial femoral head and metal-on-metal (Co-Cr-Mo/Co-Cr-Mo) type for THA. The surface structure and preliminary tribological properties of Co-Cr-Mo-*g*-MPC were also investigated.

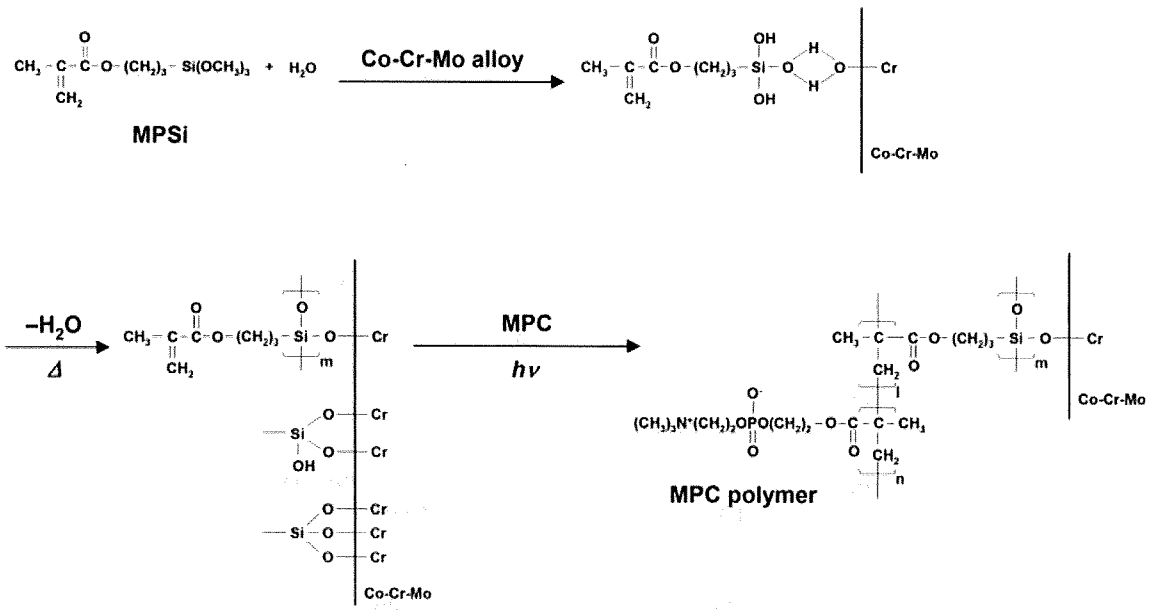

Figure 1. Chemical reaction on Co-Cr-Mo during polymerization of MPC.

MATERIALS AND METHODS

Chemicals

MPC was synthesized industrially by using the method developed by Ishihara et al.,[22] and it was supplied by NOF Corp. (Tokyo, Japan). MPSi was purchased from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan). Succinic acid and ethanol were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). 2-Hydroxy-l-[4-(hydroxyethoxy)phenyl]-2-methyl-lpropanone (DAROCUR® 2959; D2959) was purchased from Ciba Specialty Chemicals Holding Inc. (Basel, Switzerland). D2959 is a highly efficient radical photoinitiator for ultraviolet (UV) curing of the systems containing unsaturated monomers and prepolymers, and it is particularly well known as a cytocompatible UV photoinitiator with UV intensities of <6 mW/cm^2 that can perform polymerization for up to 10 min with a UV light of 365 nm.[39]

Co-Cr-Mo alloy substrate and pretreatments

The Co-Cr-Mo alloy was supplied by Yoneda Advanced Casting Co., Ltd (Takaoka, Japan). This alloy was manufactured according to the ASTM F75 standard specification for Co-28Cr-6Mo alloy.[40] The Co-Cr-Mo samples were polished so that the average surface roughness ranged between 0.01 and 0.02 μm.

The polished Co-Cr-Mo samples were washed with acetone and then immersed in 35 vol % nitric acid at room temperature for 35 min according to the ASTM F86-04 standard.[36,41] This treatment results in passivation by surface oxidation, and it could lead to the dissolution of certain foreign materials that may remain from the previous procedure. Moreover, a previous study reported that the surface of as-polished Co-Cr-Mo alloy might lack the Cr content that the bulk possesses, and that surface etching by nitric acid treatment would have produced a Cr-rich surface layer.[36] We therefore treated the surface with nitric acid with the aim of increasing the Cr concentration by "resurfacing."

After the nitric acid treatment, the Co-Cr-Mo samples were irradiated with O_2 plasma at a 500-W high-frequency output and 150-mL/min O_2 gas flow for 5 min by using an O_2 plasma etcher (PR500, Yamato Scientific Co., Ltd., Tokyo, Japan). The O_2 plasma treatment increased the thickness of the surface oxide layer.[42]

MPSi silanization and MPC graft polymerization

The synthesis of Co-Cr-Mo-*g*-MPC is schematically illustrated in Figure 1. The pretreated Co-Cr-Mo samples were immersed in an ethanol solution containing 5 mass % MPSi, 1 mass % succinic acid, and 0.1 mass % D2959 at room temperature for 12 h for silanization of the trimethoxysilane group. In this study, D2959 was used as a photoinitiator for surface-initiated polymerization so as to be included in the MPSi layer. Generally, for surface-initiated polymerization, such an initiator covalently bonded to the substrate to yield a "grafting from" polymerization is usually used. They were then annealed at 70°C for 3 h in air for dehydration. The MPC was dissolved in degassed pure water to attain concentrations ranging from 0.25 to 1.00 mol/L. Subsequently, the MPSi (containing D2959)-coated Co-Cr-Mo samples were immersed in aqueous MPC solutions. Photoinduced graft polymerization on the Co-Cr-Mo surface was performed using ultraviolet irradiation (UVL-400HA ultra-high pressure mercury lamp; Riko-Kagaku Sangyo Co., Ltd., Funabashi, Japan) with an intensity of 5 mW/cm^2 at 60°C for 23–180 min; a filter (Model D-35; Toshiba Corp., Tokyo, Japan) was used restrict the passage of ultraviolet light to wavelengths of 350 ± 50 nm. After the polymerization, the Co-Cr-Mo-*g*-MPC samples were removed from the solution, washed with pure water and