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Nanoscale Structured Phospholipid Polymer Brush for Biointerface

Kazuhiko Kitano[1], Ryosuke Matsuno[2,3], Tomohiro Konno[2,3],
Madoka Takai[2,3], and Kazuhiko Ishihara[1,2,3]
[1]Department of Bioengineering, [2]Department of Materials Engineering, [3]Center for NanoBio Integration (CNBI),
The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-8656, Japan,
Fax: +81-3-5841-8647, e-mail: kitano@mpc.t.u-tokyo.ac.jp

To prepare the biomaterial surface having both lubricity and biocompatibility, we aimed to prove the mechanism of the resistance of friction and protein adsorption with grafting polymer. We prepared poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) grafted layer using an atom transfer radical polymerization (ATRP) method, which had the advantage of controlling surface structures on nanoscale. From the results of surface characterization, it was confirmed that the thickness of the PMPC grafted layer was 4-10 nm and the conformation of the PMPC grafted layer was brushlike. We investigated the friction properties in air and in water with an atomic force microscopy (AFM). The friction coefficients of the PMPC brush layers were decreased dramatically in water and the resistance of friction depended on the thickness of the PMPC brush layer. We also investigated the protein adhesion properties by measuring the force-distance curves using the AFM cantilever immobilized with a bovine serum albumin (BSA). The adhesion force between the BSA and the PMPC brush layers were markedly reduced and the resistance of the BSA adhesion depended on the thickness of the PMPC brush layer. For resisting both friction and protein adsorption in water, it was a key factor to keep the thick hydrated layer made by the elongated hydrophilic PMPC brush chains.
Key words: phospholipid polymer, polymer brush, atom transfer radical polymerization, atomic force microscopy, lubricity

1. INTRODUCTION

In recent years there has been increasing interest in surface modification with polymers to improve a solid surface properties for biomaterials. Lubricity is one of the essential properties for biomaterials such as artificial joints, blood pump bearings, and catheters. As for artificial joints, the loosening caused by wear between the articulating surfaces is the most serious problem limiting their survival and clinical success. We aimed for obtaining both lubricity and biocompatibility for biomaterial surfaces. We used poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) as a surface modifier, which is well known for biocompatible polymer whose side chain is composed of phosphorylcholine resembling phospholipid of cell membrane[1-4]. The polymers with MPC units onto the surface of medical devices have already been shown to suppress biological reactions when they are in contact with living organisms. Using the fundamental research results, PMPC are now clinically used on the surfaces of intravascular stents, guide wires, soft contact lenses, and artificial heart[5-7]. Surface grafting of PMPC is excellent method to obtain the biocompatibility[8-10]. We expect that the PMPC grafting also improves lubricity of a solid surface because there are the same phospholipid polar groups on the surface of the human articular cartilage. It has been reported that the PMPC grafting onto the polyethylene liner of the artificial hip joint clearly reduced wear between the articulating surfaces for long term[11,12]. However why the PMPC grafting improves surface lubricity or biocompatibility has not been clear yet. In this study, in order to investigate the surface properties of the PMPC grafted surface, we prepared the nanoscale structured PMPC grafted layer using an atom transfer radical polymerization (ATRP) method, which was famous for preparing well-controlled polymer grafted layer[13,14]. We mainly studied two surface properties on nanoscale. The first is about the friction properties. We measured the friction force of the PMPC grafted surfaces with an atomic force microscopy (AFM). The second is about the protein adhesion properties. We obtained force-distance (*f-d*) curves with a protein immobilized AFM cantilever, and calculated adhesion force of the protein on the PMPC grafted surfaces.

2. EXPERIMENTS

2.1 Surface preparation

2.1.1 Surface-initiator immobilization

SiO_2 coated silicon wafers (Si) were cut into 1.0 cm x 2.0 cm, rinsed sufficiently with acetone and ethanol and treated with oxygen plasma. To prepare the homogeneous monolayer of the initiator on the silicon wafers, monochlorosilane, 3-(2-bromoisobutyryl)-propyl dimethylchlorosilane (BDCS), was used as the surface initiator. We synthesized BDCS as previously described[15]. 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 used for the graft polymerization.

2.2.2 Graft polymerization of MPC

The graft polymerization of MPC on the silicon wafers was performed using an ATRP method. MPC was dissolved in 10mL of dehydrated methanol. Copper bromide (I) (20 mg, 0.135 mmol) and 2,2'-dipyridyl (43 mg, 0.27 mmol) were added with stirring under argon at room temperature. The amount of MPC was changed

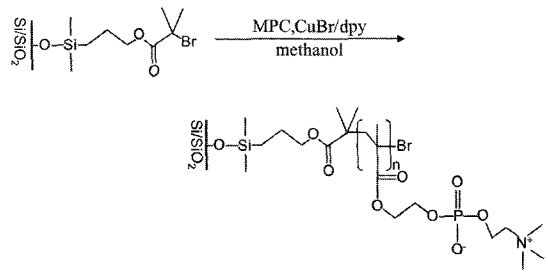

Fig.1 Preparation of the PMPC brush layers on silicon wafer via ATRP.

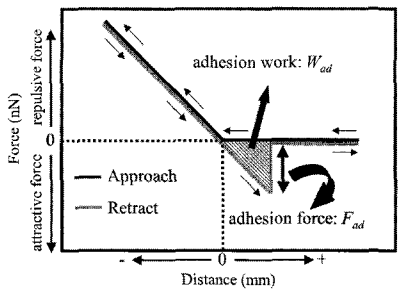

Fig.2 Schematic illustration of a typical *f-d* curve.

variedly in order to control the thickness of the PMPC brush layers. After the solution was stirred for 30 min under an argon gas atmosphere, the BDCS immobilized silicon wafers were immersed into the solution and at the same time ethyl 2-bromoisobutyrate (20 μL, 0.135 mmol) was added as a sacrificial initiator. The polymerization was carried out at room temperature with stirring under an argon gas atmosphere. The silicon wafers were removed from the polymerization mixture after the desired time period. Subsequently, they were extracted with a Soxleht apparatus in methanol for 20h and dried in vacuo at room temperature. The scheme of the reaction is shown in Fig.1.

2.3 Surface characterization

The surface chemical composition was determined by X-ray photoelectron spectroscopy (XPS). Survey scans (0-1100 eV) were performed to identify the C, N, O, and P elements. A take off angle of the photoelectrons was 90°. All binding energies were referenced the C_{1s} peak at 285 eV. The static water contact angles were measured using a goniometer at room temperature. Water droplets of 6 μL were contacted onto the substrates and the contact angles at 10 sec were directly measured by photographic images. The data was collected at 3 positions on each sample. The thickness of the PMPC brush layers in air was measured by ellipsometry. The surface morphology of the PMPC brush layers was observed with an AFM in air. Images were captured in a 1 μm x 1 μm area.

2.4 Interfacial friction measurements

A Nanoscope IIIa AFM (Digital Instruments) was used to characterize interfacial friction properties. Experiments were performed in contact mode in air and in water. V-shaped Si_3N_4 cantilevers with an announced force contact of 0.12 N/m were used. Surface friction data were acquired by scanning in the Trace and Retrace directions by disabling the slow scan axis. The friction voltage signals were corrected and converted to units of force by the previously proposed method[16]. For investigating the friction-load relationship, the scan size was maintained at 2.0 μm and the scan rate at 2.0 Hz, giving a sliding velocity of 8 μm/s. The applied load was varied by changing the vertical deflection of the cantilever. The load was calculated with a method reported previously.[17]. To calculate the load, we measured the *f-d* curve right after every friction imaging. The friction versus sliding velocity measurements was carried out between the sliding velocity of 0.4 μm/s and 488 μm/s. A scan size of 2 μm was used for the measurements.

2.5 Investigation of the protein adhesion properties

2.5.1 Bovine serum albumin (BSA) immobilization onto the AFM cantilever

The BSA-immobilized cantilever was prepared as follows. The oxygen-plasma treated Si_3N_4 cantilever was reacted with an ethanol solution of 3-aminopropyltriethoxysilane (APTES) for 2 h at room temperature, and then rinsed with water and ethanol. The surface silanized with APTES was reacted with a 5 % solution of glutaraldehyde in phosphate buffered saline (PBS) for 3 h, and then rinsed with PBS, followed by immersing in 3 mg/mL BSA in PBS at 37 °C for 3 h. The cantilever was then rinsed with PBS.

2.5.2 Measurements of the *f-d* curves

We measured the *f-d* curves in PBS (pH = 7.4) using the BSA-immobilized AFM cantilever and obtained the adhesion properties between the BSA and the PMPC brush layers. Fig.2 shows a typical *f-d* curve for an AFM cantilever contacted with a solid surface. The maximum normal deflection of the retracting curve was defined as adhesion force, F_{ad}, and the area framed by approaching curve and retracting curve was defined as adhesion work, W_{ad}. We used these two parameters for comparing adhesion properties. More than two *f-d* curves were obtained at one location through repeated cantilever approach/retract cycles, and the measurements were also repeated at more than five locations on each sample.

3. RESULTS AND DISUCUSSIONS

3.1 Surface characterization

The grafting of PMPC on the silicon wafers was confirmed using XPS. The peaks in the carbon atom region (C_{1s}) at 286 eV and 289 eV indicated the ether bond and the ester bond, respectively, and those in the nitrogen atom region (N_{1s}) at 403 eV and phosphorus atom region (P_{2p}) at 133 eV were specific to the phosphorylcholine group in the MPC unit. The results of the contact angle and the dry thickness are shown in Fig.3. The static water contact angles on the PMPC brush layers were about 10-25°, which was 20-30 % of those on the unmodified Si. The PMPC grafting greatly increased hydrophilicity, and a very little introduction of the PMPC chains made dramatic effects on the wettability by water. The thickness of the PMPC brush layers was increased with an increase in polymerization degree. We controlled the thickness of the PMPC brush layers by changing the amount of the MPC monomer in the polymerization solution. The dry thickness of the PMPC brush layers was used to estimate the graft density σ by,

$$\sigma = h\rho N_A / M_n$$

where h is the layer thickness determined by ellipsometry, ρ is the density of dry polymer layer

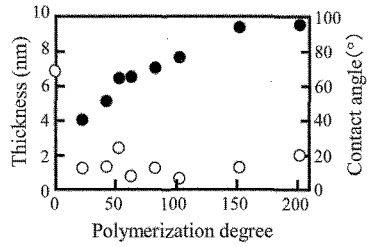

Fig.3 The dry thickness (●) and the static water contact angle (○) of the PMPC brush layers.

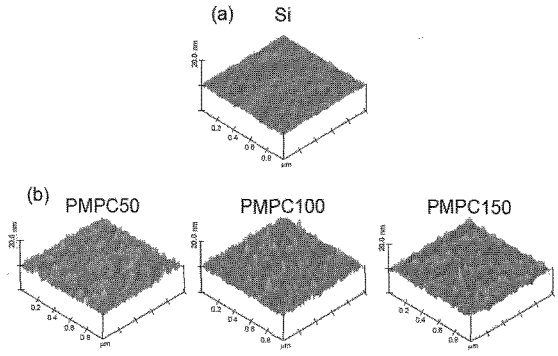

Fig.4 The AFM images of (a) the unmodified Si, (b) PMPC 50, PMPC 100, and PMPC 150.

(1.30g/cm^3 for PMPC[13]), N_A is Avogadro's number, and M_n is the number-average molecular weight of a polymer chain grafted on surface. M_n was determined by measuring the molecular weight of a free polymer because previous reports described that the molecular weight of a polymer chain grafted on surface was the same as that of a free polymer[18]. As a result, the average of the graft density of the PMPC brush layers was 0.17 chains/nm^2. It was said that polymer-grafted layer which had more than 0.10 chains/nm^2 graft density became high dense brush conformation[19]. We confirmed that the PMPC grafted layer prepared via ATRP became "brush" layer. We also confirmed the brush conformation of the PMPC grafted layers with an AFM in dry condition. The AFM images are shown in Fig.4. Compared with the unmodified Si, brush structure of the PMPC 50, 100, and 150 (the numbers, 50, 100, and 150, were the polymerization degree) was observed. The root-mean-square (RMS) surface roughness of all samples was about 0.5 nm, which indicated that the PMPC brush layers prepared by ATRP were very homogeneous.

3.2 Friction properties

Interfacial friction forces for the unmodified Si, PMPC 50, PMPC 100, and PMPC 150 measured as a function of normal load in air (a) and in water (b) are shown in Fig.5. In air, the friction coefficients of the PMPC brush layers were the same value as those of the unmodified Si, and showed the same behavior, which characteristically showed the high friction coefficients under lower load. These results were due to the adhesion force between the AFM cantilever and the substrate acting as normal load. The adhesion force between the AFM cantilever and the unmodified Si was 10-30 nN, measured by the *f-d* curves. Under lower load, the adhesion force had relatively a large effect on normal load and the friction coefficients became very high. The same value of the adhesion force was measured by the *f-d* curve measurements about the PMPC brush layers in

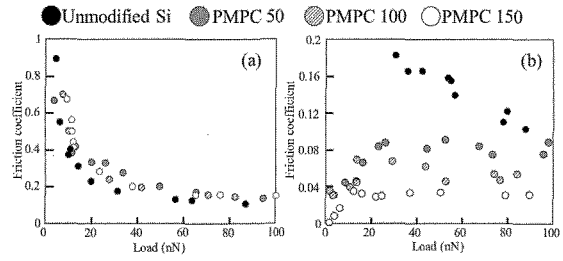

Fig.5 The friction coefficients as a function of normal load in (a) air and in (b) water.

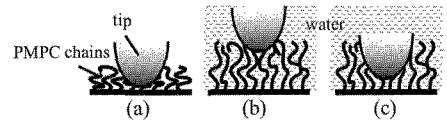

Fig.6 Schematic illustration of the sliding interface (a) in air, (b) in water under lower load, and (c) in water under higher load.

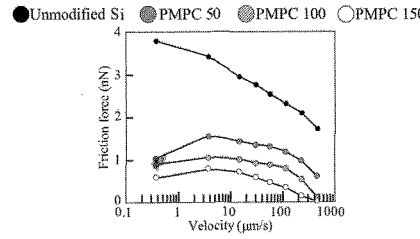

Fig.7 The friction force as a function of sliding velocity in water.

air. It was indicated that the PMPC chains were compressed in air and had no effect on interaction between the AFM cantilever and the silicon substrate (Fig.6 (a)). On the other hand, in water, the friction coefficients of the PMPC brush layers greatly decreased. Under lower load, the friction coefficients of the PMPC brush layers were especially low, and gently increased as normal load increased. These results indicated that the adhesion force between the AFM cantilever and the substrate did not occur on the PMPC brush layers in water because the hydrophilic PMPC chains elongated in water, took in a lot of water, and made the hydrated layer (Fig.6 (b)). The PMPC hydrated layer prevented from the direct contact between the AFM cantilever and the substrate, and achieved highly lubricity. As normal load increased, the AFM cantilever penetrated into the layer (Fig.6 (c)), and the interaction against the substrate gradually occurred. This was the reason why the friction coefficients increased as normal load increased. Seen from the thickness dependency, the friction coefficients of the PMPC brush layers decreased with an increase in the thickness of the PMPC brush layer because the thicker brush layer made the thicker hydrated layer. Satisfying the condition of noncontact friction interfaces leads to very low friction.

The friction forces measured as a function of a sliding velocity in water are shown Fig.7. The friction force of the unmodified Si decreased monotonically with an increase in the sliding velocity. On the other hand, there was maximum value in the friction force-velocity curve about the PMPC brush layers. These phenomena were also found in gel friction reported by Gong *et al.*[20]. By

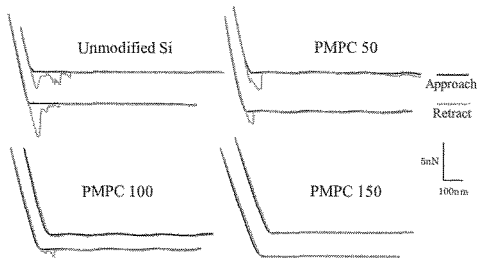

Fig.8 The representative *f-d* curves measured with the BSA- immobilized cantilever.

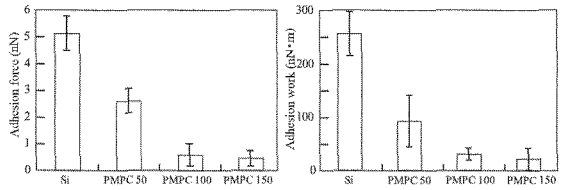

Fig.9 (a) F_{ad} and (b) W_{ad} calculated with *f-d* curves.

reference to this report, the reason for these results was that the elastic deformation of the PMPC brush chains was measured as the friction force under the low sliding velocity. When the sliding velocity became higher than the elastic mobility of the PMPC brush chains, the elastic deformation did not contribute to the friction force and the friction decreased with an increase in the sliding velocity.

3.3 Protein adhesion properties

The representative *f-d* curves measured with the BSA immobilized cantilever are shown in Fig.8, and F_{ad} and W_{ad} are shown in Fig.9. It was confirmed that the adhesion force between the BSA and the PMPC brush layers was markedly reduced and then decreased with an increase in the thickness of the PMPC brush layers. The F_{ad} and W_{ad} of PMPC 150 was nearly measurement limit, so it was believed that PMPC 150 had little or no interaction with BSA. Hydrophobic interaction is a main interaction when a protein adheres a solid surface, reported by Kidoaki *et al.*[21]. The hydrophilic PMPC brush layers resisted hydrophobic interaction with the BSA. In addition to the reduction of hydrophobic interaction, hydration repulsive force caused the resistance of the BSA adhesion force. Hydration repulsive force said to be a short-range force that usually appeared at nanoscale separation distances and arose whenever water molecules bind to strongly hydrophilic materials. Therefore, the required properties for resisting the protein adhesion are not only just hydrophilicity but also the ability to couple many water molecules, which means that making the thick hydrated layer. The thickness of the hydrated layer increased as the thickness of the polymer brush layer increased, considered from the friction measurements. Hydration repulsive force is the reason why the resistance of the BSA adhesion force depended on the thickness of the polymer brush layer.

4. CONCLUSIONS

We prepared the well-controlled PMPC brush layer using an ATRP method. We controlled the thickness of the PMPC brush layers on nanoscale by changing the amount of the MPC monomer in the polymerization solution. From the nanoscale friction measurements by AFM, it was most important for obtaining lubricity to satisfy the condition of noncontact friction interfaces with the hydrated layer. From the *f-d* curves measurements using the BSA-immobilized cantilever, the BSA adhesion force was clearly decreased by the PMPC grafting because of both the reduction of hydrophobic interaction and the increase of hydration repulsive force. The hydrated layer made by the elongated PMPC brush layer in water served a key role in leading to excellent lubricity and biocompatibility.

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