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# Preparation of biointerfaces using molecularly dispersed polymer alloys

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A novel molecular dispersed polymer alloy composed of polyethylene (PE) and poly(vinyl acetate) (PVAc) with a biocompatible surface was developed for fabricating medical devices. The acetyl group on the surface of this polymer alloy was converted to the hydroxyl group following the phosphorylcholine (PC) group having an excellent biocompatibility. After sarface-initiator was immobilized by making the hydroxyl group on the surface reacting with 2-bromoisobutyryl bromide, 2-methacryloyloxyethyl phosphorylcholine (MPC) was polymerized from the surface by the surface-initiated atom transfer radical polymerization method. The chain length of the poly(MPC) grafts was varied via the ratio of MPC to sacrificial initiator. The surfaces were characterized by water contact angle, X-ray photoelectron spectroscopy (XPS), and atomic force microscope (AFM). The effect of poly(MPC) chain length on fluorescence labeled albumin adsorption was studied in phosphate buffered saline buffer at pH 7.1. The adsorption of protein on the poly(MPC)-grafted or introduced PC group surfaces was greatly reduced compared to the unmodified PE. The protein adsorption decreased with increasing chain length of grafted-poly(MPC).

Key words: 2-methacryloyloxyethyl phosphorylcholine, atom transfer radical polymerization, biointerface molecular dispersed polymer alloy,

## 1. INTRODUCTION

Protein adsorption is recognized as the first event following the implantation of biomaterials and has been shown to play an important role in determining subsequent events, including thrombus formation, the foreign body reaction, bacterial infection, and other undesirable responses [1]. There is thus considerable interest in surfaces that might inhibit or prevent protein adsorption [2]. Approach to "protein resistant" surface is the incorporation of phosphorylcholine (PC) group. This concept evolved from the fact that the zwitterionic phospholipids, which are major components of the outer membranes of cells, were shown by Zwaal et al. to be nonthrombogenic [3]. Chapman et al. synthesized PC containing polymers using diacetylenic phosphatidyl-choline. The nonthrombogenic character of the resulting polymers was attributed to the PC group [4].

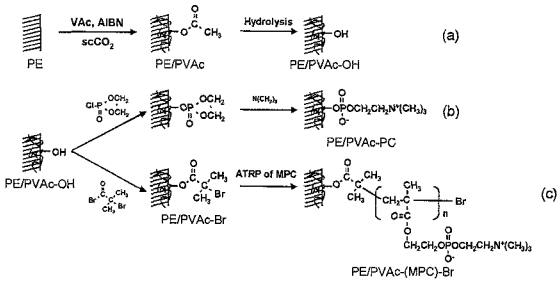
Ishihara et al. reported an improved method for the synthesis of a PC-bearing monomer, 2-methacryloyloxyethyl phosphorylcholine (MPC) [5]. It can also copolymerize with other methacrylate monomers such as n-butyl methacrylate, n-hexyl methacrylate, and n-dodecyl methacrylate [6,7]. MPC-based copolymers have been used extensively as stand-alone biomaterials or as coatings on substrates and, as such, have shown potential in a wide range of medical device applications including biosensors, drug carriers, soft contact lenses, vascular stents, and urological devices [8-10]. However, these materials have several drawbacks including poor mechanical properties caused by the introduction of water-soluble MPC group, and in the case of coatings, weak bonding to the substrate leading to delamination. Surface grafting has been shown to be an effective approach for the surface modification of biomaterials. If the grafts are covalently attached to the substrate, the coating is stable and does not delaminate. Recently, poly(MPC) has been successfully grafted on various substrates through conventional free-radical polymerization initiated by different methods [11,12]. For example, Ishihara et al. reported that poly(MPC)-grafted polyethylene (PE) showed dramatic decreases in protein adsorption and platelet adhesion compared to the unmodified PE [13].

To produce well-defined polymers, controlled "living" radical polymerization has been explored. Atom transfer radical polymerization (ATRP) is particularly useful because of its versatility with respect to monomer type, its tolerance of impurities, and the typically mild reaction conditions under which it is conducted [14]. Recently, Lobb et al. and Ma et al. synthesized poly(MPC) and its copolymers having controlled molecular weight and molecular weight distribution via ATRP [15,16]. Feng et al. described the graft polymerization of MPC by ATRP from silicon surfaces that were functionalized with 10-(2-bromo-2-methyl) propionyloxydecyltrichlorosilane using Cu(I)Br and 2,2'-bipyridyl as catalyst [17]. However, silicon is a hard material, and it is not general as the biomaterial because the main living body organization is the soft tissues except the bone and teeth. Then, the grafting of poly(MPC) onto the surface of a soft polymer material is effective to the preparation of a new biomaterial. It is necessary to introduce the surface-initiator of ATRP onto the surface of the polymer material. The introduction of

the surface-initiator onto chemically stable polymer materials such as polyolefin is difficult.

Recently, we succeeded in the creation of a molecularly dispersed polymer alloy (PE/PVAc) composed of PE and poly(vinyl acetate) (PVAc) [18,19]. PE/PVAc was obtained by the following processes: both the vinyl acetate (VAc) and 2,2'-azobis- isobutyronitrile (AIBN) dissolved supercritical carbon dioxide ($scCO_2$) fluid were impregnated into the PE substrate and subsequently polymerized. The PVAc was generated in the amorphous regions of the PE because the $scCO_2$ could not impregnate into the crystalline regions. The alloy at the nanometer size affected mechanical properties. Further, we converted the acetyl group on the surface of the PE/PVAc polymer alloy to a hydroxyl group following the PC group.

In this study, surface-initiator was immobilized by the reaction of 2-bromoisobutyryl bromide and hydroxyl group that converted by hydrolysis of acetyl group on the PE/PVAc polymer alloy surface. After immobilization of surface-initiator, the graft polymerization of MPC was carried out by the surface-initiated atom transfer radical polymerization method (Scheme 1). Here, we reported on the preparation of poly(MPC)-grafted molecular dispersed polymer alloy and the effect of poly(MPC) chain length to surface properties on hydrophilicity and protein adsorption.




**Scheme 1. Synthetic route of (a) PE/PVAc-OH, (b) PE/PVAc-PC, and (c) poly(MPC)-grafted polymer alloy via ATRP**

## 2.EXPERIMENT

### 2.1 Materials

PE, PE/PVAc, PE/PVAc-OH, and PE/PVAc-PC was synthesized and purified by the previously reported method [18,19] as shown in scheme 1(a),(b). 2-Bromoisobutyryl bromide, 2,2'-bipyridyl (Bpy), ethyl-2-bromoisobutyrate, and copper (I) bromide (CuBr) were purchased from Sigma-Aldrich Co., Saint Louis, USA, and used as-received. Dehydrated pyridine and methanol were purchased from Kanto Chemical Co., Tokyo, Japan, and used without further purification. Tetrahydrofuran (THF) was purchased from Kanto Chemical Co., Tokyo, Japan and used after distillation. MPC was synthesized and purified by the previously reported method [5]. Dulbecco's phosphate-buffered saline (PBS, ×10 concentrate) was purchased from Invitrogen Co., California, USA, and diluted (×1, pH 7.1) by distilled water before use. Fluorescein isothiocyanate conjugate bovine serum albumin (FITC-BSA) was purchased from Sigma-Aldrich Co., Saint Louis, USA, and used as-received.

### 2.2 Immobilization of surface-initiator onto the polymer alloy: Preparation of PE/PVAc-Br

The PE/PVAc-OH sheet was soaked in water overnight and then freeze-dried. It was then refluxed for 5 h in distilled THF (50 mL) containing 2-Bromo-isobutyryl bromide (3.0 mmol) and dehydrated pyridine (3.5 mmol) in order to introduce the bromine for initiator of ATRP. The PE/PVAc-Br sheet obtained was washed five times with methanol and dried *in vacuo* at room temperature.

### 2.3 Preparation of poly(MPC)-grafted polymer alloy: Preparation of PE/PVAc-(MPC)-Br

Argon gas was purged in methanol to eliminate oxygen before the polymerization. MPC (0.01 mol) was added to the Schlenk flask containing a magnetic stir bar, and then was dissolved in 10 mL of methanol bubbled with argon for 15 min to eliminate oxygen. CuBr and Bpy was added in MPC solution with stirring under argon. After being stirred for 30 min under an argon gas atmosphere, the PE/PVAc-Br sheet was submerged into the flask. Then, ethyl-2-bromoisobutyrate was added as a sacrificial initiator ([I]). The grafting polymerization was performed at 25 °C with stirring under an argon gas atmosphere. After 24 h, the poly(MPC)-grafted polymer alloy (PE/PVAc-(MPC)-Br) sheet was removed from the polymerization mixture and rinsed with methanol and water. Subsequently, the PE/PVAc-(MPC)-Br sheet was dried *in vacuo* at room temperature after extracted with methanol for 24 h to remove unreacted reagents and homopolymer by using a Soxhlet-extractor. Four differet [MPC]/[I] ratios, 50, 100, 150, and 200, applied to prepare poly(MPC)-grafted polymer blend with different poly(MPC) chain lengths (MPC monomer units). The polymerization condition is as follows: [MPC] = 1M, [CuBr]:[Bpy]:[I] = 1:2:1.

The molecular weight of free poly(MPC) in solution was measured by gel permeation chromatography (GPC) using a Shodex "SB-804HQ" column (upper limit molecular weight was ~ $1 \times 10^7$ g/mol, the flow rate was 0.40 mL/min). The eluent was distilled water containing 0.20M LiBr. Calibration was based on poly(ethylene oxide) standards ranging from 600 to 107,000 g/min.

### 2.4 Surface characterization

The surface of product sheets was analyzed using highly sensitive X-ray photoelectron spectroscopy (XPS) (AXISHSi, Shimadzu/KRATOS) employing Mg-Kα excitation radiation. The releasing angle of the photoelectron for each atom was fixed at 90°.

The hydrophilicity of the sample surface was characterized on the basis of water dynamic contact angle (DCA) measurements. By a sessile drop method, the contact angle with water was measured at room temperature (20 °C) using a contact angle goniometer (CA-W, Kyowa Interface Science Co., Tokyo, Japan) equipped with a video camera. The advancing ($\theta_A$) and receding ($\theta_R$) contact angles with water were measured for addition to and withdrawal from the drop (0 – 20 μL), respectively. The purified water was dropped on a dry sample using a microsyringe under atmospheric conditions.

The wet condition topology of the poly(MPC)-grafted polymer alloy surfaces was studied by atomic force microscopy (AFM), using a NanoScope IIIa Multimode SPM from the Veeco Instruments. The image were recorded with standard tips in fluid tapping mode at a scan rate of 0.5 Hz.

### 2.5 Protein adsorption test

Polymer alloys with poly(MPC)-grafted surface were

exposed to 4.5 mg/mL FTIC-BSA in PBS at 37 °C for 60 min and then rinsed five times with fresh PBS. The BSA concentration is 10% of the plasma concentration. The sample was dried in an argon stream and observed with a fluorescent microscope. The amounts of adsorbed protein were compared by fluorescent intensity. Fluorescent intensity of the adsorbed protein on the surface of PE was assumed to be 100.

## 3.RESULTS AND DISCUSSION

### 3.1 Preparation of poly(MPC)-grafted polymer alloy

Fig.1 shows the XPS charts of unmodified PE, PE/PVAc-PC, PE/PVAc-Br and PE/PVAc-(MPC)-Br. In the unmodified PE, a strong intensity at 285 eV was observed. This is attributed to carbon atoms in the methylene chain. After addition of PC group, the XPS charts dramatically changed (Fig.1 (b)). In the carbon atom region, XPS peaks became broad. This broad peak was attributed to the ether bond (C-O). In the oxygen atom region, the increase in peak intensity is caused by the increase in amounts of oxygen. The nitrogen peak was observed at 403 eV. In addition, phosphorus peak was observed at 134 eV, and was attributed to the PC group. In the case of the immobilization of ATRP initiator (Fig.1 (c)), XPS peaks became broad in the carbon atom region and the new peak was observed in the oxygen and bromine atom region. This broad peak was attributed to 2-bromoisobutyl group in the carbon atom region. New peaks of the oxygen and bromine atom region were attributed to carbonyl group (C=O) and bromine of 2-bromoisobutyl group. After grafting of poly(MPC) (Fig.1 (d)), the nitrogen and phosphorus peaks were observed and the position of the bromine peak was sifted by ATRP of MPC monomer.

In this study, four [MPC]/[I] ratios, 50,100,150 and 200, were used to prepare poly(MPC) grafts of different chain length. Fig.2 and Fig.3 shows the development of molecular weight of poly(MPC) and the atomic surface composition as a function of [MPC]/[I] ratio. The number average of molecular weight (Mn) of poly(MPC) and the phosphorus composition ($P_{2p}/C_{1s}$ ratio determined by XPS) increased linearly with [MPC]/[I] ratio, suggesting that the ATRP grafting of MPC from the polymer alloy surface was well-controlled process. In addition, the value of $P_{2p}/C_{1s}$ ratio closes in on the theoretical value (0.092) as the chain length of poly(MPC) increases. This fact indicates that the density of PC group on the surface increases with the chain length of grafted poly(MPC).

### 3.2 Contact angle measurements

Dynamic water contact angle measurement has been commonly used to characterize the relative hydrophilicity or hydrophobicity of the surface [20]. For surfaces with comparable structures, a relatively low contact angle value generally implies high hydrophilicity. Fig.4 shows the results of the DCA measurements. The advancing ($\theta_A$) and receding ($\theta_R$) contact angles decreased with an increase in the PC groups on the surface. The hydropilicity of the poly(MPC)-grafted surfaces is greatly improved indicating that poly(MPC) chains cover the surface. Moreover, PE/PVAc-PC and poly(MPC)-grafted surface ([MPC]/[I] = 50) of almost the same surface composition was shown a similar wettability. It is thought that the surface density of the PC group considerably influences the surface function.

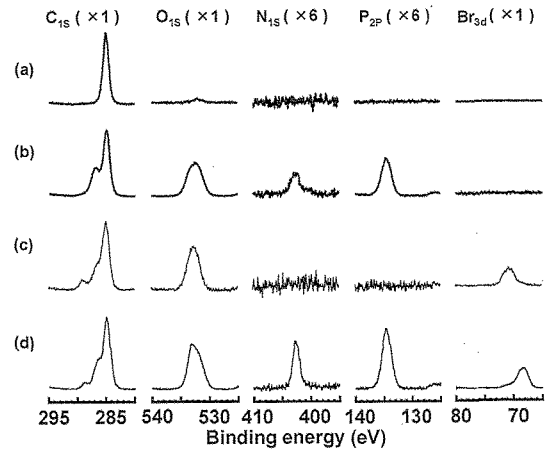



**Fig. 1 XPS charts of (a) unmodified PE, (b) PE/PVAc-PC, (c) PE/PVAc-Br, and (d) PE/PVAc-(MPC)-Br. The intensities of each atom were normalized by that of C1s at 285 eV.**

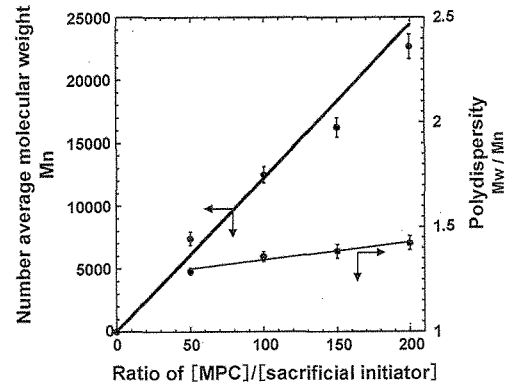



**Fig. 2 Evolution of molecular weight vs ratio of MPC initiator.**

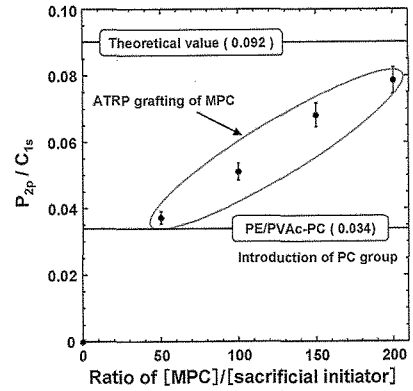



**Fig. 3 XPS elemental ratio ($P_{2p}/C_{1s}$) vs MPC/sacrificial initiator ratio**

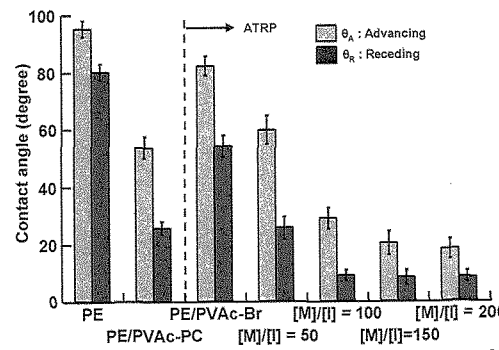



**Fig. 4 Advancing and receding water contact angles of poly(MPC)-grafted surfaces with varying chain length. (Including the results of PE/PVAc-PC, PE/PVAc-Br surfaces.)**

### 3.3 Surface morphology

The wet condition topology of the surfaces was examined by fluid tapping mode AFM in pure water. Fig.5 shows height images of the unmodified PE and grafted-poly(MPC) surface. The unmodified PE surface was smooth with a root means square (RMS) roughness of 1.60 nm. The surface of introduced surface-initiator was RMS roughness of 4.78 nm (Fig.5d). This fact indicates that roughness on the surface has increased by the surface reaction such as addition of PC or 2-bromoisobutyl group. Fig.5e-f indicate that the morphology of poly(MPC)-grafted surface was dependent on the poly(MPC) chain length. The surface of shorter chain length showed greater roughness (Fig.5e) than the surfaces of higher chain length (Fig.5f), as is evident from the RMS data.

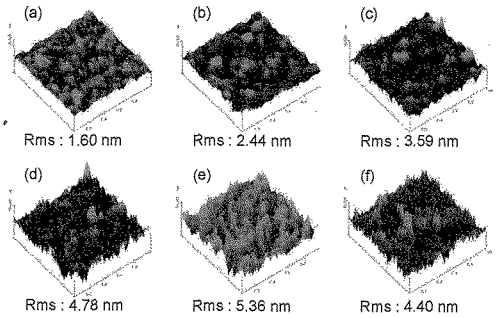



**Fig. 5 AFM 3D height images of (a) PE, (b) PE/PVAc-OH, (c) PE/PVAc-PC, (d) PE/PVAc-Br, and poly(MPC)-grafted surfaces with varying chain length ((e) [MPC]/[I]=50, (f) [MPC]/[I]=100) (Image size: 1 × 1 × 50 μm).**

### 3.4 Protein adsorption test

Fig.6 shows the fluorescent intensity of adsorbed FITC-BSA on the surfaces. Fluorescent intensity is proportional to the amount of adsorbed protein on the surface. The amount of the adsorbed FITC-BSA on the surface was in good agreement with the contact angle with water, as shown in Fig.4. PC-containing surface inhibited the protein adsorption effectively and then the amount of adsorbed protein decreased with chain length of grafted-poly(MPC). Also, surface roughness affected the protein adsorption onto the poly(MPC)-grafted surface. It is thought that the hydrophilicity and surface morphology are significant properties of the protein adsorption resistance.

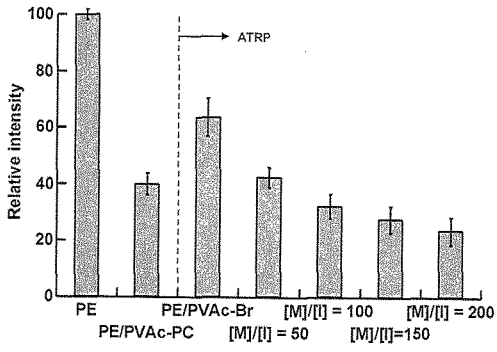



**Fig. 6 Fluorescent intensity of adsorbed FITC-albumin on the surfaces with varying chain length**

## 4.CONCLUSION

We reported a versatile approach to prepare poly(MPC)-grafted substrates based on a novel synthetic method of molecular dispersed polymer blend and surface-initiated atom transfer radical polymerization. The chain length of grafted poly(MPC) was controlled by [MPC]/[sacrificial initiator] ratio. A set of surface samples with various chain lengths from 50 to 200 MPC units was prepared. The density of PC group on the surface increases with the chain length of grafted-poly(MPC). The hydrophilicity of the surfaces was increased with the chain length of grafted-poly(MPC). Roughness of the surfaces with higher poly(MPC) chain lengths is lower than the shorter chain length. The protein repulsion properties of these poly(MPC)-grafted surface were evaluated in fluorescent labeled albumin adsorption experiments. The surfaces with high poly(MPC) chain lengths showed a dramatic reduction in the albumin adsorption.

Preparation of new biomaterials is expected by this creation method of poly(MPC)-grafted polymer alloy.

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